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"Prebiotic Polymer Synthesis and the Origin of Glycolytic Metabolism"

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Summary

Our research under NASA Grant NCC 2-784 resulted in several discoveries which contributed to understanding the origin and operation of life. (1) Most importantly, we discovered a new pathway of prebiotic amino acid synthesis in which formaldehyde and glycolaldehyde (formose reaction substrates) react with ammonia to give alanine and homoserine in the presence of thiol catalysts (Weber 1998). The thiol-dependent synthesis of amino acids undoubtedly occurs via amino acid thioester intermediates capable of forming peptides. This 'one-pot' reaction system operates under mild aqueous conditions, and like modern amino acid biosynthesis, uses sugar intermediates which are converted to amino acids by energy-yielding redox disproportionation. Preliminary evidence suggests that this type of process can be "evolved" by a serial transfer methods that lead to enrichment of autocatalytic molecules. (2) We established that prebiotic peptide polymers can be made by condensation of amino acid thioesters (homocysteine thiolactone and S-(N-β-orotidyl-diaminopropionic acid) ethanethiol), and that prebiotic polydisulfide polymers can be generated by oxidation of dithiols with iron(III) in minerals (Weber 1995a). (3) In our analysis of metabolism we discovered the primary energy source of biosynthesis -- chemical energy made available by the redox disproportionation of substrate carbon groups (Weber 1997b). We concluded that the energy and reactivity of sugars make them the optimal substrate for the origin and operation of terrestrial (or extraterrestrial) life. (4) Since it is likely that the use of optimal sugar substrates in biosynthesis sets the average oxidation number of functional biocarbon throughout the Universe near 0.0 (the reduction level of formaldehyde), we proposed that a line(s) in the microwave spectrum of formaldehyde could be rationally selected as a frequency for interstellar communication that symbolizes life (Weber 1995b). (5) Finally, in preparation for the analysis of Martian meteorite samples, we upgraded our HPLC system to one femtomole sensitivity, and developed a new electrophoretic method of sample preparation for HPLC analysis of the meteoritic amino acids. In a sample of the KT boundary layer from Sussex Wyoming, we found about 300 picomoles per gram of meteoritic α-aminoisobutyric acid per gram of KT layer.

Introduction

Modern life can be described as a complex chemical process (metabolism) that is catalytically controlled by its products (proteins-nucleic acids) in a way that enhances the perpetuation of the entire system. Consequently, the origin of life can be described

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as a continuous series of events in which a prebiotic chemical process came increasingly under the control of its catalytic products in a way that facilitated self-perpetuation. From this point of view the problem of the origin of life becomes a search for a plausible prebiotic process that yields products able to take over catalytic control of the reactions of the process. Obviously, this search is restricted to chemical processes (reaction pathways) having a negative free energy change that occur in the presence of water, because only processes with a negative free energy change take place spontaneously, and water is needed to transport substrates to catalytic sites. The requirement for contact between substrates and catalytic products also points to a 'one-pot' process. Moreover, since the simplest survival strategy is replication, the earliest catalytic products most likely acted as autocatalysts that enhanced their own synthesis. In our search for this prebiotic process (earliest metabolism) that yielded catalytic take-over products (such as polypeptides), we have investigated: (1) the chemistry of laboratory models of prebiotic processes that could have been involved in the origin of metabolism and biopolymer synthesis, (2) the thermodynamics of carbon chemistry that governs the operation of modern metabolism and chemical processes involved in its origin, and (3) the abiotic amino acid content of samples of meteorites and KT boundary sediments containing extraterrestrial molecules. Both our experimental and thermodynamic investigations indicate that sugars are the optimal substrate for the origin and operation of life. The results of these studies are reviewed below.

Chemical models of the origin of metabolism and biopolymer synthesis

Prebiotic synthesis of 'activated' amino acid thioesters (Weber 1998): Based on the criteria discussed above, we currently believe that the most attractive process for the origin of life is based on the Prebiotic Sugar Pathway shown on the next page in Figure 1. As shown in Figure 1, the pathway involves the conversion of formaldehyde to sugars which subsequently react with ammonia and hydrogen sulfide to generate a variety of possible autocatalytic products (like peptides from amino acid thioesters, thiols, imidazoles, pyrazines, and pryridines). These transformations leading from formaldehyde to catalytic products -- via sugars -- take place either by anaerobic pathway A or oxidizing pathway B.
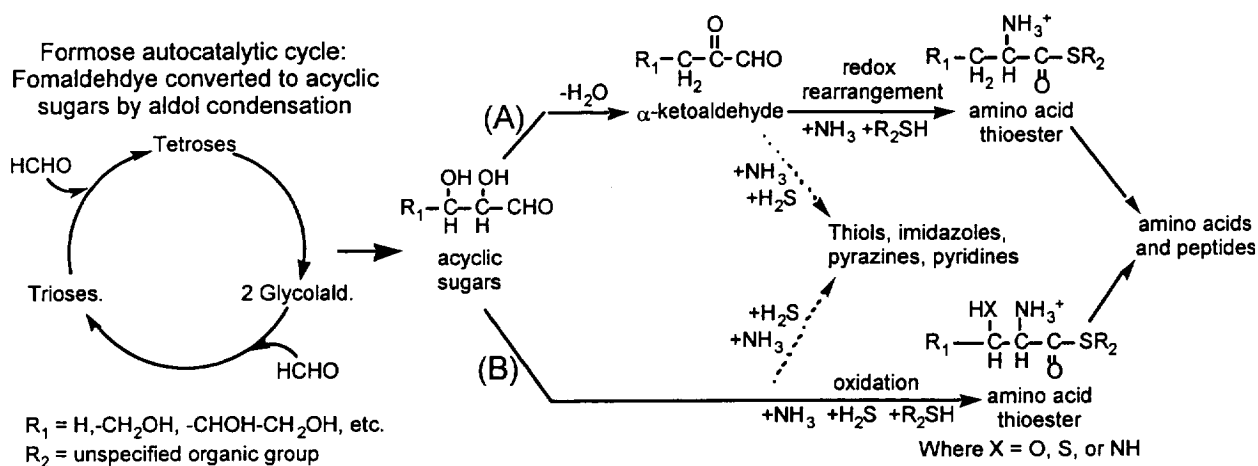
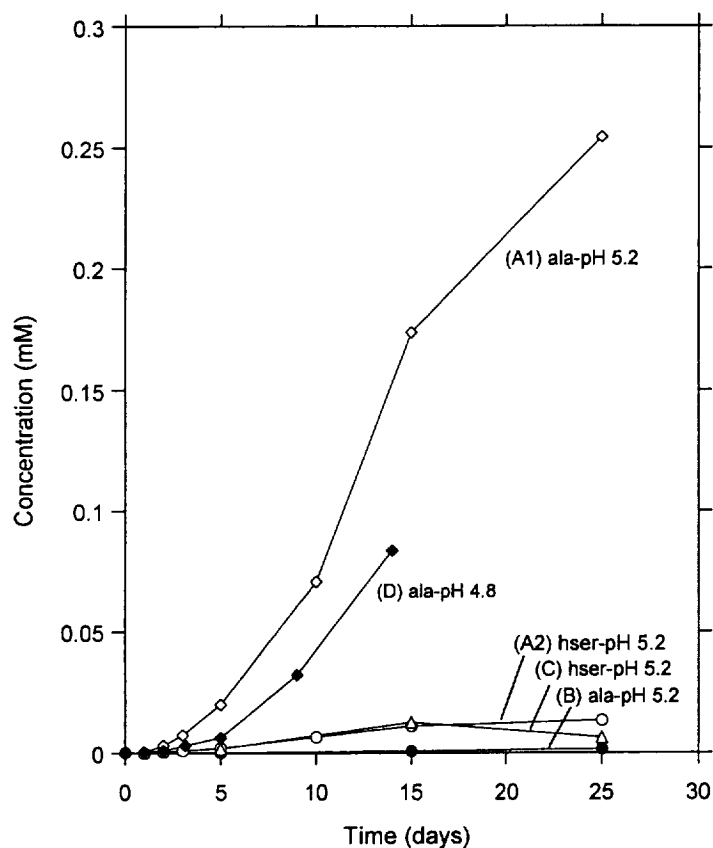Figure 1. Chemistry of the Prebiotic Sugar Pathway.

Figure 2. Time course of formation of alanine (ala) and homoserine (hser) under anaerobic conditions at 40°C. (A1) and (A2) show ala and hser synthesis, respectively, at pH 5.2 from 20 mM glycolaldehyde, 20 mM formaldehyde, 20 mM ammonium chloride, 23 mM sodium acetate, and 23 mM 3-mercaptopropionic acid. (B) shows ala synthesis in a control without 3-mercaptopropionic acid. (C) shows hser synthesis in a control without formaldehyde. (D) shows ala synthesis at pH 4.8.

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In our investigation of anaerobic pathway A, we discovered that formaldehyde and glycolaldehyde (formose substrates) together with ammonia yield alanine and homoserine thioesters in the presence of thiols (1), as depicted earlier in Figure 2. In this anaerobic reaction system the formose reaction is combined with classical redox transformations of sugars to give 'activated' amino acid thioesters that are known to form peptides (2). The formation at pH 5.2 of these three- and four-carbon amino acids from one- and two-carbon aldehyde substrates is surprising, because the required carbon-carbon bond-forming aldol reactions generally require alkaline conditions. This unexpected result is probably caused by ammonia (or amine product) catalyzing the aldol condensation of glycolaldehyde and formaldehyde (3). As shown earlier in Figure 1, the anaerobic synthesis of alanine begins by aldol condensation of formaldehyde with glycolaldehyde to give an acyclic triose sugar that undergoes β-dehydration yielding pyruvaldehyde (an α-ketoaldehyde) which in the presence of ammonia and a thiol forms an imine-hemithioacetal adduct which undergoes an intramolecular redox rearrangement to give an alanine thioester. The 'activated' alanine thioester either hydrolyzes to give alanine or reacts with another amino acid to give an alanine peptide. Related α-hydroxy acid thioesters had been previously synthesized under prebiotic conditions without condensing agents (4. 5).

Formaldehyde, the primary substrate of the sugar transformation process, could have been generated on the early Earth under a variety of conditions. Especially attractive is the photochemical synthesis of formaldehyde in the Earth's early atmosphere (6, 7) or hydrosphere (8, 9). Other aldehyde substrates (e.g. glycolaldehyde, trioses) could have been generated by the same processes, or by subsequent condensation of formaldehyde. Ammonia needed for amino acid synthesis could have been formed by iron (II) reduction of nitrite (10). Thiols could have been synthesized by reaction of sugars with hydrogen sulfide.

The Prebiotic Sugar Pathway has several characteristics that make it a very attractive model of prebiotic chemistry leading to the origin of life. The pathway —

(1) is a one-pot reaction system that uses simple substrates.
(2) yields amino acids as 'activated' thioesters capable of forming peptides without the need for hydrolytically labile condensing agents.
(3) operates in the presence of water under mild conditions allowing its reactions to be acted on by its autocatalytic products (like organic or peptide thiols).

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(4) is energetically very favorable allowing it to function at low substrate concentrations (standard free energy ~-35 kcal/mol for alanine and homoserine synthesis)

(5) has the potential to directly develop into modern biosynthesis, because it chemically resembles modern biosynthesis by using sugars as energized carbon substrates which are converted to 'biochemicals' by energy-yielding redox disproportionation.

Prebiotic peptide synthesis using 'activated' amino acid thioesters:
There is a growing belief among investigators studying the origin of life that polynucleotides did not originate from molecules formed by prebiotic processes, because the synthesis of polynucleotides involves several reactions that are implausible under prebiotic conditions (11). Since polypeptides can act as catalysts and can be made from prebiotically plausible amino acid thioesters (1), we investigated prebiotic peptide synthesis and replication using amino acid thioesters. These studies demonstrated the synthesis of peptides with thiol side chains from homocysteine thiolactone, and peptides with uracil side chains from S-(N-β–orotidyl-diaminopropionic acid) ethanethiol. These particular amino acid thioesters were studied because they yield peptides with side chains that could allow them to act autocatalytically. Presumably, peptides of homocysteine might exhibit autocatalytic behavior if their thiol side chains reacted with homocysteine thiolactone to give homocysteine peptide thioesters that polymerized at a faster rate than homocysteine thiolactone. In a different manner, peptides of β–orotidyl-diaminopropionic acid might show autocatalytic behavior by acting as templates that position β–orotidyl-diaminopropionic acid thioesters in a way that accelerates their polymerization to peptide products. Although DL-homocysteine thiolactone was commercially available, S-(N-β–orotidyl-L-diaminopropionic acid) ethanethiol had to be synthesized from orotic acid, N-α–Boc-L-diaminopropionic acid, and ethanethiol. HPLC analysis of the polymerization of homocysteine thiolactone and S-(N-β–orotidyl-diaminopropionic acid) ethanethiol at pH 7.8 showed the formation of small peptides 2-5 residues long.

Prebiotic oxidative polymer synthesis using dithiols (Weber 1995a):
A major problem in the prebiotic synthesis of anhydride polymers like polypeptides and polynucleotides is the destruction of chemical condensing agents and reactive intermediates by water that prevents the formation of large polymers. As a way to overcome this problem we examined the prebiotic oxidative polymerization of a dithiol (2,3-dimercaptopropanol) by ferric ions on the surface of iron(III) hydroxide oxide

[Fe(OH)O] (12). Figure 3 on the next page shows the oxidative synthesis of the trimer of the polydisulfide polymer. Polydisulfide polymers up to 15 units long were synthesized from low concentrations of dithiol monomer (1mM) under mild conditions (pH 4-6, 40°C, 3 days). Synthetic geothite (α-Fe(OH)O) and synthetic magnetite (Fe3O4) gave similar oligomer yields. The oxidative polymerization of 2,3-dimercaptopropanol is an attractive prebiotic reaction because the reaction needs only a small three-carbon monomer, takes place without interference by water, and occurs readily at low monomer concentrations to give reasonably large oligomers (up to 15-mer) under mild conditions. Ferric ions needed for the formation of Fe(OH)O and Fe3O4 on the primitive Earth could have been continually generated by photooxidation of dissolved ferrous ions (13, 14).
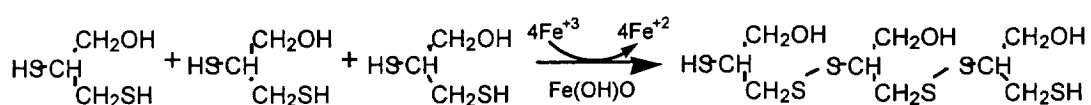

Figure 3. Polymerization of 2,3-dimercaptopropanol

Chemical evolution using serial transfer-enrichment of autocatalytic molecules: We also investigated a method for the *in vitro* selection of autocatalytic molecules generated by synthetic chemical reactions. This procedure, which was previously used to 'evolve' bacteriophage Qβ-RNA (15), provides a way to test synthetic reactions for the presence of autocatalytic products and to increase the yield of autocatalytic products. We call this procedure -- serial transfer-enrichment of autocatalytic molecules (STEAM). The procedure involves initially preparing a series of reaction tubes containing the same reaction solution, which are stored in way that prevents reaction. The first reaction tube is removed from storage and its contents reacted for a specified time. Next, about 5-10% of the contents of the first reaction tube is transferred to a second reaction tube just removed from storage. The reaction in tube 2 is run under the same conditions as tube 1. This serial transfer (seeding) procedure is carried out, one reaction tube at a time, until all of the stored tubes have been reacted. Finally, the reaction products of every other tube are analyzed by HPLC. If autocatalytic molecules are synthesized in reactions subjected to this serial transfer procedure, the HPLC product profile should change going from earlier to later reactions in the series, because the later reactions are expected to contain a larger percentage of autocatalytic products.

We applied the STEAM method to three different reactions: a) the polymerization of homocysteine thiolactone which yields homocysteine peptides, b) the polymerization of

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S-(N-β-orotidyl-diaminopropionic acid) ethanethiol which gives uracil-containing peptides, and c) the reaction of formaldehyde and glycolaldehyde with ammonia and hydrogen sulfide that could yield several products having catalytic properties. In these STEAM-tested reactions we used eight reaction tubes that were stored at -40°C under slightly acidic conditions. The reactions were started by raising the pH and temperature to 40°C. HPLC analysis of reaction series (a) and (b) showed constant product profiles indicating no selection of autocatalytic molecules. However, reaction series (c) showed a small but steady increase in one HPLC peak which co-chromatographed with α-monothioglycerol. This preliminary result suggests 'evolution' of a chemical process.

Thermodynamics of carbon chemistry governing the origin and operation of metabolism

Reduction level of biocarbon (Weber 1995b): We began our studies of biosynthetic metabolism by calculating the average oxidation number of biocarbon of several types of organisms using literature values of their biochemical composition, and the average oxidation number of their molecular constituents (protein, lipid, polysaccharide, and nucleic acid) (16). Figure 4 below shows carbon functional groups placed directly above their oxidation numbers on the formal carbon oxidation number scale. Carbon dioxide with an oxidation number of +4 is the most oxidized form of carbon, and methane with an oxidation number of -4 is the most reduced form of carbon. In the cases studied we found that the average oxidation number of the carbon of all organisms was near 0.0 (when fuel stored as lipid was not used in the estimate). We also established that the average oxidation number of the carbon of the Earth's biosphere was close to 0.0 -- the reduction level of formaldehyde. This chemical characteristic of life most likely applies to extraterrestrial life, and it could be the one thing we know about other carbon-based life in the universe, and the one thing that other intelligent life knows about us. Therefore, we proposed that this common knowledge that biocarbon is at the formaldehyde oxidation level could lead to the selection of a line(s) in formaldehyde's microwave spectrum as a frequency for interstellar communication.

Number of carbon-carbon bonds

$$-\overset{|}{\underset{|}{C}}-$$

$$-\overset{|}{C}OH \qquad -\overset{|}{\underset{|}{C}}H$$

$$>CO \qquad >CHOH \qquad >CH_2$$

$$-COOH \qquad -CHO \qquad -CH_2OH \qquad -CH_3$$

$$CO_2 \qquad HCOOH \qquad HCHO \qquad CH_3OH \qquad CH_4$$

Number of carbon-carbon bonds: 4 3 2 1 0

Oxidation number scale of carbon: +4 +3 +2 +1 0 -1 -2 -3 -4
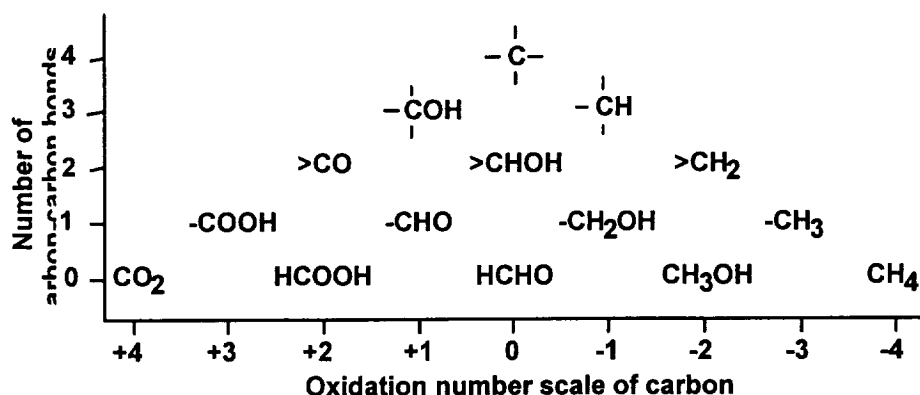
Oxidation number scale of carbon

Figure 4. Oxidation numbers of carbon groups are given directly below each group. Open bonds that link carbons can be saturated or unsaturated (N or S can replace O).

Carbon redox disproportionation drives fermentation and biosynthesis (Weber 1997b):

In contemporary life one-carbon substrates are converted to formaldehyde or its adducts (sugar groups and serine's hydroxymethyl group) before they enter biosynthetic metabolism. This observation that carbon enters biosynthesis at the reduction level of formaldehyde (oxid. # = 0.0), together with our earlier finding that the products of biosynthesis also have an average oxidation number near 0.0, indicates that biosynthesis is primarily a redox disproportionation process where electrons are exchanged between carbons without changing the average reduction level (oxid. #) of the processed carbon. This observation raised the question: why does biosynthesis start with carbon at the sugar (or formaldehyde) reduction level and then proceed by disproportionation? In order to answer this question, we calculated the standard free energy change for 25 carbon fermentation reactions, and for the biosynthesis of E. coli's amino acids, lipids, and nucleotides from glucose (17). We also calculated for each reaction the degree of carbon redox disproportionation -- defined as the sum of the absolute values of the change in the oxidation number of all carbon in a reaction. As shown on the next page in Figure 5, we found that the energy yield (-ΔG/carbon) of the fermentation reactions (numbered 1-25) was directly proportional to the degree of redox disproportionation. This analysis also showed that redox disproportionation is the principal energy source of the biosynthesis of amino acids and lipids from sugars. Redox disproportionation of sugars accounted for 84% and 96% (and ATP only 6% and 1%) of the total energy of amino acid and lipid biosynthesis, respectively. Redox disproportionation of carbon, and not ATP, is the primary energy source driving amino acid and lipid biosynthesis from glucose. In contrast, nucleotide biosynthesis which is not driven by redox disproportionation relies completely on exogenous ATP for energy.

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We also discovered that the standard half-cell potentials of substrate carbon groups, are responsible for redox disproportionation being the energetically favored direction of electron transfer. As seen on the next page in Figure 6, the energetically favored direction of electron transfer is from more oxidized half-cell couples which are stronger reductants (with more negative reduction potentials) to less oxidized half-cell couples (with more positive reduction potentials). This energetically favored transfer of electrons from more oxidized to more reduced carbon results in redox disproportionation.

We concluded that biosynthetic transformations are primarily driven by, and depend on, the chemical energy made available by the redox disproportionation of carbon groups at the sugar (or formaldehyde) level of reduction. Of all the
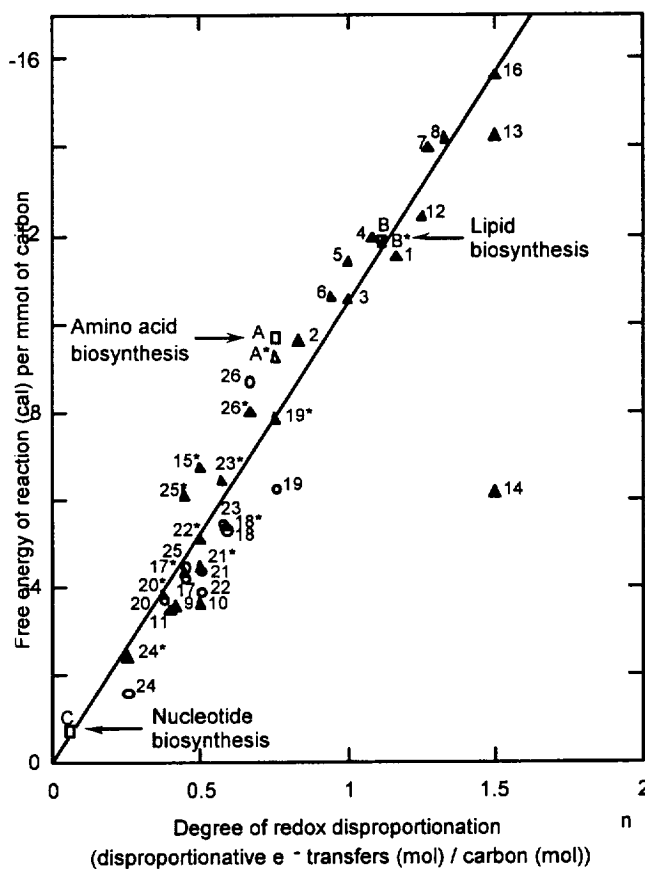

Figure 5. Standard free energy change per carbon of 25 fermentation reactions and 3 biosynthetic processes as a function of the degree of redox disproportionation.
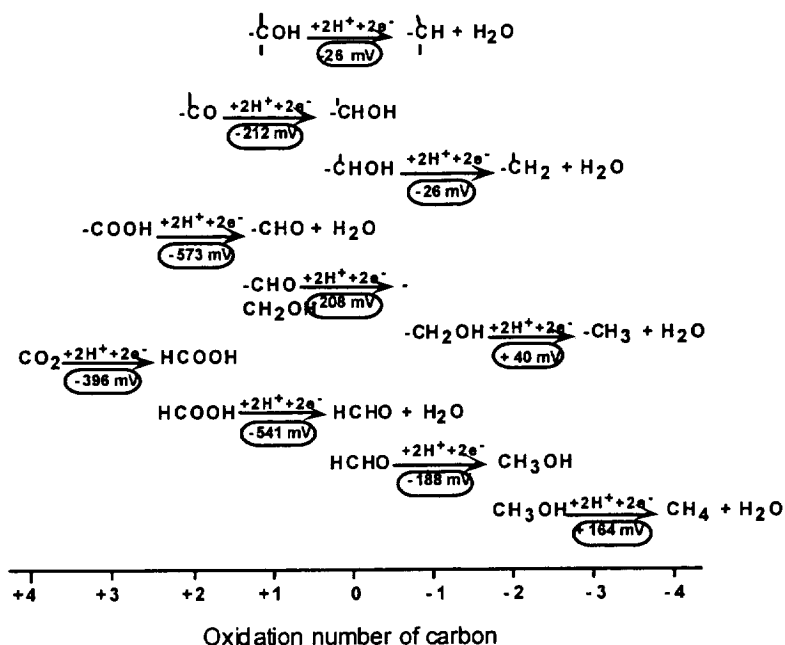
Figure 6. Redox half-cell equations of carbon groups positioned directly above their respective oxidation numbers on the oxidation number scale of carbon. Calculated standard reduction potentials (pH 7, 25°C) are given for each half-cell equation.

multi-carbon fermentation substrates studied glucose yielded the most energy per carbon. This energy content of sugars not only makes sugars good fermentation substrates, but also excellent biosynthetic substrates. Sugars yield more energy, because 1) all the carbon groups of sugars are capable of redox disproportionation compared to only a fraction of the carbons of other substrates, and 2) the strong reducing power of the aldehyde, hydroxymethyl, and hydroxymethylene groups of sugars gives them very favorable redox disproportionation energies. These considerations indicate that sugars are the optimal biosynthetic substrate -- a belief supported by the widespread use of sugars in biosynthesis.

Abiotic amino acid content of extraterrestrial materials

Development of techniques for amino acid analysis of meteorites and KT boundary sediments: In anticipation of analyzing Martian Meteorite ALH84001, we improved the sensitivity of our amino acid analysis methods 10^4-fold from 10 picomole to 1 femtomole of injected fluorescent amino acid derivative. This was accomplished by 1) installing a new fluorescence detector, 2) changing to new narrow bore HPLC columns, 3) replacing the HPLC solvent mixer with a micromixer, 4) modifying the published o-phthaldialdehyde-thiol derivatization procedure (18) to greatly reduce background contamination, 5) obtaining a less contaminated pure water source by acquiring a new

Millipore water system for the Exobiology Branch at Ames, and 6) constructing a vertical laminar flow hood to provide a sterile and particulate-free work space.

Using the improved HPLC system we analyzed a terrestrial KT-boundary sample (provided by Dr. Theodore Bunch) for α-amino isobutyric acid and isovaline. A sample of Cretaceous-Tertiary boundary sediment from Sussex Wyoming was shown to contain about 310 picomoles per gram of the meteoritic amino acid, α-amino-isobutyric acid. Knowledge of the amino acids in extraterrestrial materials, like meteorites, contributes to understanding the processes involved in the delivery of amino acids to the primitive Earth during the origin of life.

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PREBIOTIC AMINO ACID THIOESTER SYNTHESIS: THIOL-DEPENDENT AMINO ACID SYNTHESIS FROM FORMOSE SUBSTRATES (FORMALDEHYDE AND GLYCOLALDEHYDE) AND AMMONIA

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Abstract. Formaldehyde and glycolaldehyde (substrates of the formose autocatalytic cycle) were shown to react with ammonia yielding alanine and homoserine under mild aqueous conditions in the presence of thiol catalysts. Since similar reactions carried out without ammonia yielded α-hydroxy acid thioesters (Weber, 1984a, b), the thiol-dependent synthesis of alanine and homoserine is presumed to occur via amino acid thioesters – intermediates capable of forming peptides (Weber and Orgel 1979). A pH 5.2 solution of 20 mM formaldehyde, 20 mM glycolaldehyde, 20 mM ammonium chloride, 23 mM 3-mercaptopropionic acid, and 23 mM acetic acid that reacted for 35 days at 40°C yielded (based on initial formaldehyde) 1.8% alanine and 0.08% homoserine. In the absence of thiol catalyst, the synthesis of alanine and homoserine was negligible. Alanine synthesis required both formaldehyde and glycolaldehyde, but homoserine synthesis required only glycolaldehyde. At 25 days the efficiency of alanine synthesis calculated from the ratio of alanine synthesized to formaldehyde reacted was 2.1%, and the yield (based on initial formaldehyde) of triose and tetrose intermediates involved in alanine and homoserine synthesis was 0.3 and 2.1%, respectively. Alanine synthesis was also seen in similar reactions containing only 10 mM each of aldehyde substrates, ammonia, and thiol. The prebiotic significance of these reactions that use the formose reaction to generate sugar intermediates that are converted to reactive amino acid thioesters is discussed.

1. Introduction

One of the major obstacles in the aqueous synthesis of prebiotic polymers (like polypeptides) from their respective monomers (amino acids) is the required use of hydrolytically labile chemical condensing agents to generate the obligatory 'activated' monomers needed for polymer synthesis (Hulshof and Ponnamperuma, 1976; Ferris and Hagan, 1984). To eliminate the need to synthesize and deliver hydrolytically labile chemical condensing agents to the site of prebiotic polymer synthesis, we previously investigated chemical pathways that generate 'activated' monomers from carbon substrates without condensing agents. These studies demonstrated the synthesis of 'activated' lactoyl and glyceroyl thioesters using glyceraldehdye as the carbon substrate (Weber, 1984a, b). The same reactions carried out in the presence of ammonia yielded alanine, presumably via alanyl thioester (Weber, 1985). Thioesters of hydroxy acids and amino acids are known to polymerize yielding polyesters and polypeptides, respectively (Weber and Orgel, 1979; Weber,

1987a). We now describe a new 'one-pot' pathway that uses formose aldol reactions to convert formaldehyde and glycolaldehyde to sugar intermediates which subsequently react with ammonia in the presence of thiols to give amino acids, presumably via amino acid thioester intermediates. Unlike our earlier studies, this prebiotic pathway is not limited to glyceraldehyde as the carbon substrate, because aldol condensation of glycolaldehyde with itself, formaldehyde, and other aldehydes can generate a number of different γ-substituted glyceraldehydes from which a variety of amino acid thioesters can be synthesized. In addition to supplying energy needed for prebiotic peptide synthesis, amino acid thioesters also could have provided energy for prebiotic phosphoanhydride synthesis, since thioesters have been shown to drive the chemical synthesis of phosphoanhydrides (Weber, 1981; Hartman, 1975; Buvet, 1977; Weber, 1987b; deDuve, 1991). Earlier studies of prebiotic amino acid synthesis have been reviewed by Miller *et al.* (1976).

Formaldehyde for amino acid synthesis could have been generated on the early Earth under a variety of conditions (Miller, 1957; Hubbard *et al.*, 1971; Bar-Nun and Hartman, 1978; Chittenden and Schwartz, 1981; Miller and Schlesinger, 1984). Especially attractive is the photochemical synthesis of formaldehyde in the Earth's early atmosphere (Pinto *et al.*, 1980; Canuto *et al.*, 1983) or hydrosphere (Chittenden and Schwartz, 1981, and ref. therein; Halmann *et al.*, 1981; Aurian-Blajeni *et al.*, 1981). Other aldehyde substrates (e.g. glycolaldehyde) could have been generated by the same processes, or by formaldehyde condensation and redox reactions (Schwartz and de Graaf, 1993; Weber, 1987b, 1992). Ammonia needed for amino acid synthesis could have been formed by reduction of nitrite by iron (II) (Summers and Chang, 1993). Thiol catalysts have also been synthesized under prebiotic conditions (Sagan and Khare, 1971; Hong and Becker, 1979; Heinen and Lauwers, 1996).

2. Materials and Methods

2.1. MATERIALS

Glycolaldehyde dimer, formaldehyde, o-phthaldialdehyde, glyoxal trimer dihydrate, dihydroxyacetone, erythrose, threose, glyoxylic acid monohydrate, N-acetyl-L-cysteine, 2,4-dinitrophenylhydrazine, acetonitrile (HPLC grade), glycine, DL-protein amino acids, L-protein amino acids, sodium acetate trihydrate, sodium phosphate (dibasic heptahydrate), sodium phosphate (monobasic monohydrate) were obtained from Sigma; DL-glyceraldehyde, DL-homoserine, L-homoserine, 3-mercaptopropionic acid, iron(II,III) oxide, ferrous sulfate (99.999%), sodium tetraborate (99.999%), glacial acetic acid (99.99%) from Aldrich; ammonium phosphate (dibasic) from J. T. Baker; methanol (HPLC grade) from Fisher; sodium azide from Eastman Organic Chemicals; 1.0 ml ampules (vacules) from Thomas Scientific; Centrex microfiltration units (nylon, 0.2 m pore size) from Schleicher and Schuell.

2.2. CHROMATOGRAPHIC ANALYSIS

Amino acid and aldehyde analyses were performed using a Beckman Model 126 HPLC system equipped with a Beckman ultraviolet-visible detector and a JASCO fluorescence detector (except for the pH 7.8 studies that used Perkin-Elmer fluorescence detector). Analysis of the pH 5.2 reactions involved derivatization of the amino acid products with o-phthaldialdehyde (OPA) and the non-chiral thiol, 3-mercaptopropionic acid, as described below (Furst *et al.*, 1990). The fluorescent OPA adducts were separated on an Alltech Alltima C18 column (150 × 3.2 mm) at a flow rate of 0.7 mL min^{-1} with eluent (A) 50 mM sodium acetate (pH 5.5) – acetonitrile (99:1), and eluent (B) 50 mM sodium acetate (pH 5.5) – acetonitrile (40:60). The column was equilibrated with 18% (B) and 0.5 min after injection the solvent mixture was changed linearly to 95% (B) over 11 min. Elution times were DL-asp (2.95 min), DL-ser (4.51 min), DL-hser (5.21 min), glycine (5.55 min), DL-ala (6.23 min), unk-1 (5.82 min), unk-2 (6.00 min), unk-3 (6.54 min), unk-4 (7.67 min) (see Figure 1).

The chirality of the amino acid products was examined by making fluorescent adducts of amino acids with o-phthaldialdehyde (OPA) and the chiral thiol, N-acetyl-L-cysteine (Zhao and Bada 1995). HPLC separation of the fluorescent OPA adducts was performed on a Beckman Ultrasphere C18 column (250 × 4.6 mm) at a flow rate of 1.0 mL min^{-1} using isocratic elution for 60 min with 90% of eluent (A) 50 mM sodium acetate (pH 5.5) – acetonitrile (90:10), and 10% of eluent (B) 50 mM sodium acetate (pH 5.5) – acetonitrile (40:60). Elution times were DL-hser (15.73, 17.92 min), DL-ala (40.40, 41.84 min).

Analysis of the pH 7.8 reactions used a modification of the method of Zhao and Bada (1995) that resolves D- and L- amino acid enantiomers by HPLC of their diastereomeric fluorescent adducts of o-phthaldialdehyde (OPA) and N-acetyl-L-cysteine. HPLC separation of the fluorescent OPA adducts was performed on an Alltech Adsorbosphere C18 column (250 × 4.6 mm) at a flow rate of 1.0 mL min^{-1} with the eluent (A) 50 mM sodium acetate (pH 5.4) – methanol (92:8) and eluent (B) methanol. The column was equilibrated with 0% (B); at 2 min after injection the solvent mixture was changed over 1 min to 12% (B); maintained at 12% for 9 min; changed over 1 min to 45% (B); and maintained at 45% for 10 min. Elution times were DL-asp (5.86, 6.62 min), DL-ser (8.72 min), DL-hser (11.24, 11.74 min), glycine (12.50 min), DL-ala (17.33 min).

HPLC analysis of aldehyde derivatives of 2,4-dinitrophenylhydrazine (DNPH) was performed on a Beckman Ultrasphere C18 column (250 × 4.6 mm) at a flow rate of 1.0 mL min^{-1} with the eluents (A) 50 mM sodium acetate (pH 5.5) – acetonitrile (99:1), and (B) 50 mM sodium acetate (pH 5.5) – acetonitrile (40:60). The column was equilibrated with 70% (B) and 7 min after injection the solvent mixture was changed linearly to 100% (B) over 5 min. Elution times were formaldehyde (12.98 min), DNPH (7.00 min), glycolaldehyde (6.65 min), dihy-

droxyacetone (5.68 min), glyceraldehyde (4.67 min), erythrulose (4.30 min), and tetroses – erythrose and threose (3.91 min).

2.3. Amino acid synthesis and derivatization

In the pH 5.2 reactions a solution containing 22 mM formaldehyde, 22 mM glyco-laldehyde, 22 mM ammonium chloride, and 2.2 mM sodium azide was sterilized using a Centrex microfiltration unit (0.2 μm pore size). Two hundred microliter aliquots of the solution were added to 1.0 ml ampules (vacules) that had been heat sterilized overnight at 550°C. Ten microliters of 500 mM sodium acetate (pH 5.5) and 10 μL of 500 mM sodium 3-mercaptopropionate (pH 5.5) (both solutions sterilized by microfiltration) were added to each ampule. The solution in each ampule was immediately deaerated by cycling five times between a vacuum and nitrogen source while the bottom of the ampule was immersed in an ultrasonic bath; the ampule was flame-sealed in vacuo. Reactions were carried out in the dark at 40°C and stopped by freezing at –40°C. The reactions were analyzed by reacting 1.0 μL aliquots for 1 min with 10 μL of OPA reagent (prepared by dissolving 4 mg of o-phthaldialdehyde in 300 μL of methanol and then adding 325 μL water, 360 μL 0.35 M sodium borate, and 15 μL 1.0 M 3-mercaptopropionic acid (pH 5.6), Furst *et al.* 1990). The reaction was stopped by adding 3.5 μL of 1.0 M acetic acid and 240 μL of water. A 20 μL aliquot was analyzed by HPLC. The pH 4.8 reaction used the same methods.

The pH 7.8 reactions were carried out like the pH 5.2 reactions, but they were analyzed differently. The pH 7.8 reactions were analyzed by adding 10 μL aliquots to 10 μL of 0.4 M sodium borate, and then reacting the resulting solution for 2 min with 10 μL of OPA reagent (prepared by dissolving 4 mg of o-phthaldialdehyde in 300 μL of methanol and then adding 435 μL water, 250 μL 0.35 M sodium borate, and 15 μL 1.0 M N-acetylcysteine (pH 5.6), Zhao and Bada 1995). The reaction was quenched by adding 265 μL of 50 mM sodium acetate (pH 5.5) containing 6 μL of 1.0 N HCl. A 20 μL aliquot was analyzed by HPLC.

2.4. Aldehyde analysis

Aldehydes were measured by HPLC of their 2, 4-dinitrophenylhydrazine (DNPH) derivatives monitored at 360 nm (Steinberg and Kaplan, 1984). The DNPH reagent was prepared the day of use by adding 10% acetonitrile to a saturated solution of DNPH in 2N HCl which had been filtered using a Centrex microfiltration unit (0.2 μm pore size). Five microliter aliquots of the reaction solutions were reacted 30 min with a solution containing 20 μL of DNPH reagent and 325 μL of water. A 20 μL aliquot was analyzed by HPLC.

3. Results and Discussion

Figure 1(A) shows the HPLC separation of the products from reaction of gly-colaldehyde, formaldehyde, and ammonium ion in the presence of the thiol, 3-mercaptopropionate. Chromatogram (B) depicts the products of a control reaction without 3-mercaptopropionate; and chromatogram (C) shows several amino standards. The reactions were carried out under mild aqueous conditions (pH 5.2, 40°C, 15 days). Sterile solutions were used and azide was added to prevent bacterial growth. A comparison of the chromatograms shows that alanine, homoserine, and four unknowns (unk-1,-2,-3,-4) were synthesized from glycolaldehyde, formaldehyde, and ammonium ion in the presence of thiol, but not in the absence of thiol. Alanine and homoserine synthesis occurred steadily under anaerobic conditions and was not affected by the admission of air (oxygen) when the reaction ampules were opened. However the synthesis of three unknown substances (unk-1, unk-2, unk-3) increased dramatically when air was admitted by opening the ampules. These unknown products could be amine-containing acyl thioesters or their hydrolysis products, because previous studies have shown that air oxidation of glyceraldehyde yields glyceroyl thioesters (Weber 1984a,b). The synthesis of the fourth unknown (unk-4) required thiol and formaldehyde, but not glycolaldehyde or ammonia. Therefore, unknown-4 is not an amine or amino acid.

Figure 2 (A1) and (A2) show the time course of alanine and homoserine synthesis from reaction of glycolaldehyde, formaldehyde, and ammonium ion in the presence of the thiol, 3-mercaptopropionic acid. Figure 2 and its caption also describe amino acid synthesis in control reactions lacking various reactants. In the complete system, the time course of alanine (A1) and homoserine (A2) synthesis is S-shaped. Alanine synthesis continued for at least 35 days at which time it reached 0.36 mM or 1.8% based on the initial formaldehyde concentration. The yield of homoserine was only 4% that of alanine. At 25 days the efficiency of alanine synthesis was 2.1% calculated from the ratio of alanine synthesized to formaldehyde reacted ($\sim$60%). Figure 2 (B) shows that alanine was not synthesized in the absence of thiol. Homoserine was also not synthesized in the absence of thiol (see caption). However, Figure 2 (C) shows that homoserine was synthesized in a control reaction lacking formaldehyde. Neither alanine or homoserine were synthesized in control reactions missing either glycolaldehyde or ammonia (not depicted, see caption). For unknown reasons the control reactions containing thiol but missing either formaldehyde, glycolaldehyde, or ammonia had pH values about 0.5 unit higher than the complete system with or without thiol. The figure also shows that alanine synthesis at pH 5.2 was faster than at pH 4.8. Alanine synthesis was also observed in similar reactions (a) using 10 mM of aldehyde, ammonia, and thiol reactants, and (b) carried out in the presence of synthetic magnetite and soluble ferrous ion. HPLC analysis of the pH 5.2 reaction showed that the alanine and homoserine products were racemic.
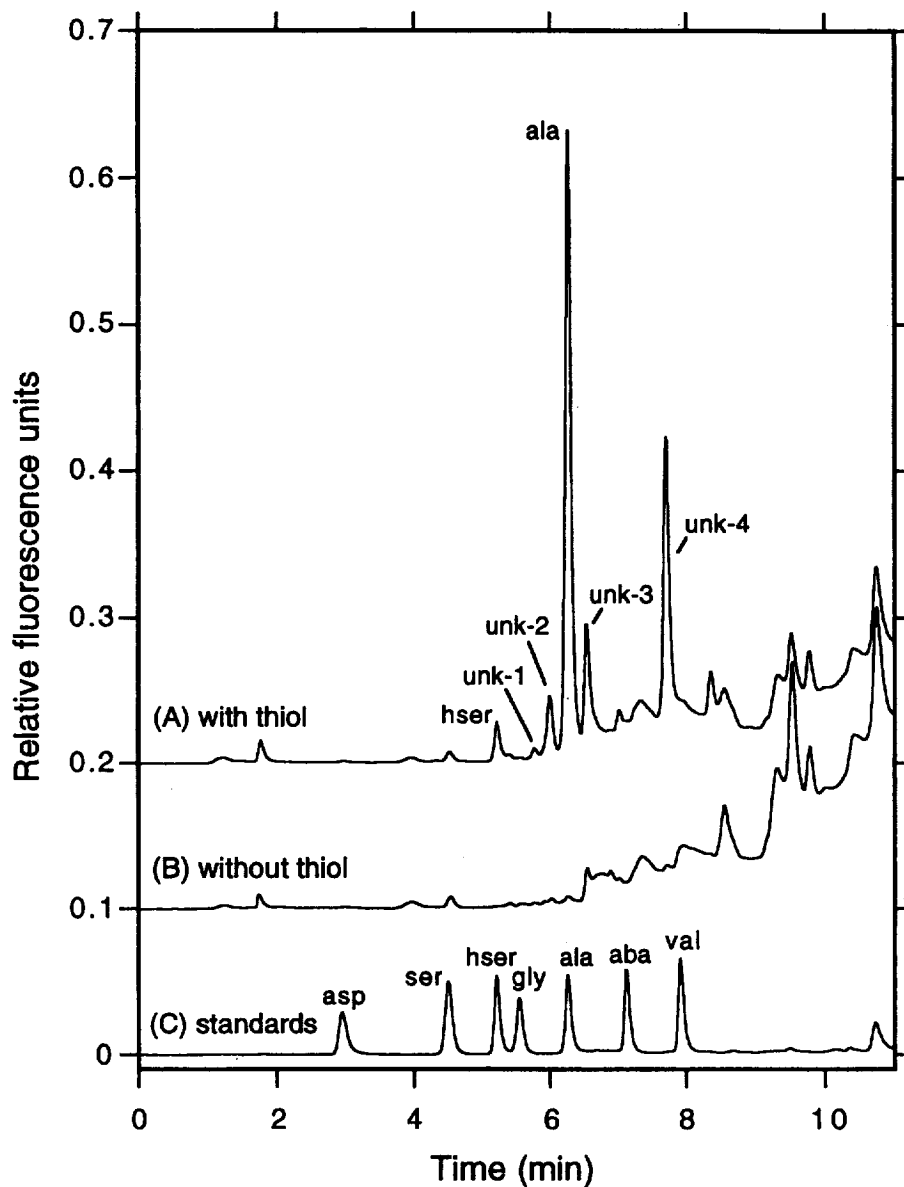
Figure 1. HPLC separation of products from a 15 day reaction at 40°C and pH 5.2 of 20 mM glycolaldehyde, 20 mM formaldehyde, 20 mM ammonium chloride, 23 mM sodium acetate, and 2 mM sodium azide containing (A) 23 mM 3-mercaptopropionic acid and (B) 23 mM sodium acetate substituted for 23 mM 3-mercaptopropionic acid. Chromatogram (C) shows an HPLC separation of DL-amino acid standards. Hser and aba are abbreviations for homoserine and α-amino-n-butyric acid, respectively.
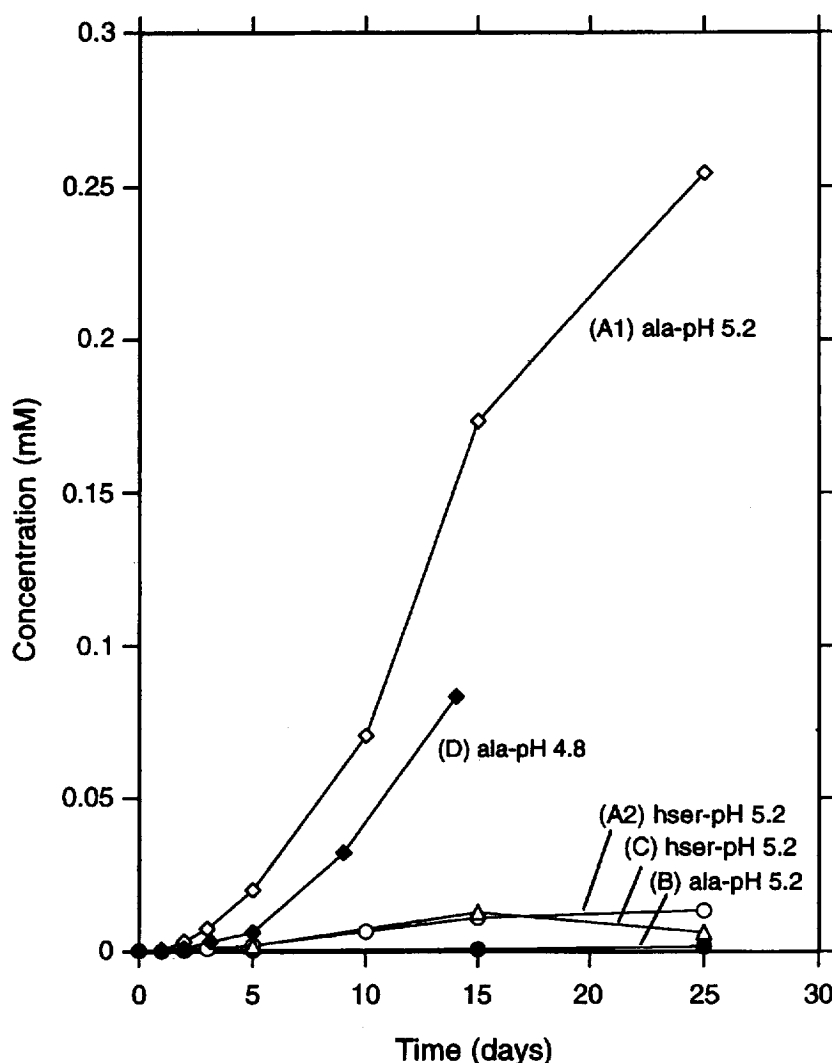
Figure 2. Time course of formation of alanine and homoserine under anaerobic conditions at 40°C in the dark. (A1) and (A2) show alanine and homoserine synthesis, respectively, at pH 5.2 from 20 mM glycolaldehyde, 20 mM formaldehyde, 40 mM ammonium chloride, 23 mM sodium acetate, 23 mM 3-mercaptopropionic acid, and 2 mM sodium azide. (B) shows alanine synthesis in a control reaction where 23 mM sodium acetate substituted for 23 mM 3-mercaptopropionic acid (not shown, the synthesis of homoserine and unknowns -1, -2, -3, -4 was negligible). (C) shows homoserine synthesis in a control reaction without formaldehyde (not shown, a small amount of unknown-1 was detected, but the synthesis of alanine and unknowns-2, -3, -4 was negligible). In other control reactions with thiol minus glycolaldehyde, and with thiol minus ammonia the synthesis of alanine, homoserine and unknowns-1, -2, -3 was negligible (results not shown), but unknown-4 was formed. (D) shows alanine synthesis at pH 4.8 from 20 mM glycolaldehyde, 20 mM formaldehyde, 20 mM ammonium chloride, 23 mM 3-mercaptopropionic acid, and 2 mM sodium azide.

In the pH 5.2 reaction the concentration of aldehyde substrates and sugar intermediates was measured by HPLC of their 2,4-dinitrophenylhydrazine derivatives. In the complete system containing formaldehyde, glycolaldehyde, ammonia and thiol, the aldotriose intermediate, glyceraldehyde, increased for 10 days, and then remained nearly constant at 0.3% of the initial formaldehyde concentration (aldotetrose-2.1%) until the last measurement at 25 days. In control reactions lacking either thiol or ammonia, the glyceraldehyde yield (based on initial formaldehyde) increased continually for 25 days reaching 2.5% (aldotetrose-3.2%) and 0.8% (aldotetrose-2.2%), respectively. In the control reaction missing formaldehyde, the glyceraldehyde accumulation was negligible, but aldotetrose reached 5.3% at 25 days. The synthesis of both triose and tetrose was negligible in the control reaction lacking glycolaldehyde. Only trace amounts of the ketotriose (dihydroxyacetone) and ketotetrose (erythrulose) accumulated in the above reactions. The lower steady state concentration of glyceraldehyde in the complete system, compared to the control lacking only thiol, suggests that the lag in alanine formation (S-shaped kinetics) could be due to a slow rate-limiting formation of triose intermediates from formaldehyde with glycolaldehyde. If triose formation were not rate limiting, then the addition of thiol, which is known to accelerate triose conversion to alanine, would not have decreased the steady-state concentration of glyceraldehyde.

The change in the concentration of formaldehyde and glycolaldehyde in the pH 5.2 reaction was also measured by HPLC of their 2,4-dinitrophenylhydrazine derivatives (accuracy about ± 5 percentage points). In the complete system the formaldehyde concentration over 25 days decreased about 60%, but only 5% in the control missing thiol. In the control reactions lacking either glycolaldehyde or ammonia, the formaldehyde concentration decreased 0 and 15%, respectively. In the complete system the glycolaldehyde concentration in 25 days dropped 40% (or possibly 60%, if the value is calculated by dividing the 25 day measurement by the zero time measurement of the control reactions, instead of dividing by the zero time measurement of the complete system, which was only 67% that of the controls). In control reactions missing thiol or ammonia, the glycolaldehyde concentration decreased 24 and 5%, respectively. These results show that the disappearance of formaldehyde and glycolaldehyde is fastest when both ammonia and thiol are present in the reaction, and that alanine and homoserine account for only a small fraction of the reaction products. Other likely products are the α-hydroxy acid analogs of alanine and homoserine – lactic acid and α,γ-dihydroxybutyric acid – made via their thioesters (Weber, 1984a, b), and nitrogen heterocyclic compounds such as imidazoles and pyrazines (Grimmett, 1965; Kort, 1970).

Preliminary studies at pH 7.8 (done before the pH 5.2 time course study) showed that other aldehydes can act as carbon substrates for amino acid synthesis. These reactions were carried out in 250 mM sodium phosphate buffers (pH 7.8) at 40°C for 14 days using 10 mM aldehyde substrates, 20 mM ammonia, and 5 mM N-acetyl-L-cysteine. Glycolaldehyde reacted alone yielded 0.08% homoserine (based
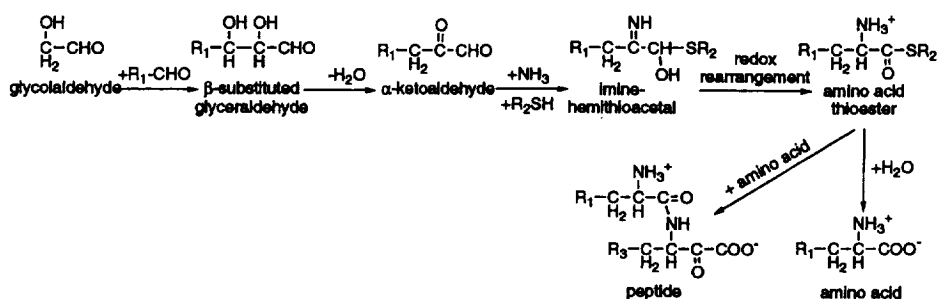
Figure 3. General prebiotic pathway for the synthesis of amino acid thioesters.

on 10 mM aldehyde substrate). Glycolaldehyde and formaldehyde yielded 0.09% alanine and 0.05% homoserine; glycolaldehyde and glyoxylate gave 0.19% alanine, 0.18% glycine, and a trace of homoserine. Glyoxal yielded 0.41% glycine; dihydroxyacetone yielded 0.46% alanine. A reaction of 40 mM glycolaldehyde, 40 mM ammonia in a 400 mM phosphate buffer (pH 7.8) yielded 12-fold more homoserine in the presence of 15 mM N-acetyl-L-cysteine than in its absence.

Figure 3 depicts a general pathway for the synthesis of amino acid thioesters by reaction of glycolaldehyde with either itself or another aldehyde, and ammonia in the presence of a thiol catalyst. The pathway begins by aldol condensation of glycolaldehyde (CH_2OH-CHO) with itself or another aldehyde (R_2-CHO) to give a β-substituted glyceraldehyde (R_1-CH_2OH-CH_2OH-CHO) (Speck, 1958; Gutsche *et al.*, 1967; Harsch *et al.*, 1984). Next, the substituted glyceraldehyde undergoes β-dehydration yielding an α-ketoaldehyde (Speck, 1958; Fedoronko and Konigstein, 1969; Feather and Harris, 1973), which in the presence of ammonia and a thiol forms an imine-hemithioacetal (Sander and Jencks, 1968; Kanchuger and Byers, 1979; Smith, 1965). The imine-hemithioacetal undergoes an intramolecular redox rearrangement to give an amino acid thioester (R-CH_2-$CHNH_2$-$COSR_2$). (Wieland *et al.*, 1955; Franzen, 1957; Weber, 1985). Finally, the 'activated' amino acid thioester hydrolyzes liberating an amino acid or reacts with another amino acid, yielding a peptide (Weber and Orgel, 1979). The pathway is not limited to alanine and homoserine. It could also yield homocysteine, α,γ-diaminobutyric acid, and threonine from formaldehyde, glycolaldehyde, ammonia, and hydrogen sulfide in the presence of a thiol. In addition it is likely that the same substrates in the presence of an aldehyde oxidant (like ferric hydroxide oxide, Weber, 1992), could be converted to glycine, serine, cysteine, α,β-diaminopropionic acid, and aspartic acid via their thioesters, because the related oxidation of glyceraldehyde in the presence of a thiol has been shown to yield glyceroyl thioester (Weber, 1984a, b).

The belief that amino acid synthesis involves amino acid thioester intermediates is supported by the thiol dependency of amino acid synthesis, and by earlier studies showing the synthesis of lactoyl and glyceroyl thioesters from glyceraldehyde

without ammonia in the presence of thiols (Weber, 1984a, b). The most surprising result of our investigation is the formation of carbon-carbon bonds at pH 5.2 by the formose aldol condensation of glycolaldehyde and formaldehyde that yields triose (glyceraldehyde and dihydroxyacetone) needed for alanine synthesis, and tetrose (erythrose, threose, and erythrulose) required for homoserine synthesis. This condensation at pH 5.2 was not expected because generally aldol condensations in solution require catalysis by a base or strong mineral acid (Reeves, 1966). In previous studies, sugar-forming aldolizations have been catalyzed either by organic or inorganic bases in solution reactions (Weber, 1987b, 1992 ref. therein), or by minerals possessing acid-base catalytic sites on their surfaces (Schwartz and de Graaf, 1993 ref. therein). A possible explanation for our results is that ammonia (or amine product) catalyzes the aldol condensation of glycolaldehyde and formaldehyde at pH 5.2, since ammonia and amines have been shown to catalyze the related enolization and retroaldolization of aldehydes (Jencks, 1987).

The pathway in Figure 3 that generates amino acid thioesters has several characteristics that make it a very attractive model of prebiotic chemistry leading to the origin of life. First, the pathway is a one-pot reaction system that uses simple substrates. Second, the system yields amino acids as 'activated' thioesters capable of forming peptides without the need for hydrolytically labile condensing agents (Weber and Orgel, 1979). Third, the pathway operates in the presence of water under mild conditions allowing its reactions to be acted on by any autocatalytic product (e.g. thiolactic acid) it generates. Fourth, the overall process is energetically very favorable allowing it to function at low substrate concentrations. The estimated standard free energy change ($\Delta G^{o\prime}$) is -38 kcal mol^{-1} for alanine synthesis, and -34 kcal mol^{-1} for homoserine formation (Thauer et al., 1977; Mavrovouniotis, 1990, 1991). Finally, the pathway has the potential to directly develop into modern biosynthesis, because it chemically resembles modern biosynthesis by using sugars as energized carbon substrates which are converted to 'biochemicals' by energy-yielding disproportionative redox transformations (Weber, 1997).

Acknowledgements

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PREBIOTIC POLYMERIZATION: OXIDATIVE POLYMERIZATION OF 2,3-DIMERCAPTO-1-PROPANOL ON THE SURFACE OF IRON(III) HYDROXIDE OXIDE

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Abstract. The oxidation of 2,3-dimercapto-1-propanol by ferric ions on the surface of iron(III) hydroxide oxide (Fe(OH)O) yielded polydisulfide oligomers. This polymerization occurred readily at low dithiol concentration under mild aqueous conditions. Polydisulfide polymers up to the 15-mer were synthesized from 1 mM dithiol in 5 ml water reacted with iron(III) hydroxide oxide (20 mg, 160 μmole Fe) for 3 days under anaerobic conditions at 40 °C and pH 4. About 91% of the dithiol was converted to short soluble oligomers and 9% to insoluble larger oligomers that were isolated with the Fe(OH)O phase. Reactions carried out at the same ratio of dithiol to Fe(OH)O but at higher dithiol concentrations gave higher yields of the larger insoluble oligomers. The relationship of these results to prebiotic polymer synthesis is discussed.

1. Introduction

A major problem in the prebiotic synthesis of anhydride polymers like polypeptides and polynucleotides is the destruction of chemical condensing agents and reactive intermediates by water that prevents the formation of large polymers. Studies of prebiotic peptide and oligonucleotide formation in water using chemical condensing reagents have yielded only small amounts of dimers and trimers (Hulshof and Ponnamperuma, 1976; Ferris and Hagan, 1984). As a way to overcome this problem Strehler *et al.* (1982) and Weber (1987a) proposed the oxidative synthesis of peptides and polyesters, respectively. Schwartz and Orgel (1985) also suggested that nucleoside dithiol derivatives might undergo template-directed oxidative polymerization. As a prebiotic model of oxidative polymer synthesis we here describe the oxidative polymerization of 2,3-dimercapto-1-propanol by ferric ions on the surface of iron(III) hydroxide oxide that yields polydisulfide oligomers.

Iron(III) hydroxide oxide was selected as the oxidant because it has been shown to readily oxidize aldehydes to carboxylic acids (Weber, 1992); and ferric ions in solution have been to shown oxidize thiols to disulfides (Wallace, 1966). Ferric ions needed for the formation of Fe(OH)O on the early Earth could have been continually generated by photooxidation of dissolved ferrous ions (Getoff *et al.*, 1960; Braterman *et al.*, 1983; Mauzerall *et al.*, 1993). The prebiotic synthesis of 2,3-dimercapto-1-propanol has not been studied; however, related thiols have been synthesized under prebiotic conditions. In general hydrogen sulfide is readily incorporated into organic compounds synthesized under prebiotic conditions (Raulin, 1978 and ref. therein). The oxidative formation of polydisulfides from dithiols

has been reviewed by Spassky *et al.* (1992). Recently, polydisulfide polymers have been used in batteries as solid redox polymerization electrodes (Liu *et al.*, 1991; Doeff *et al.*, 1992).

2. Materials and Methods

A. MATERIALS

Racemic 2,3-dimercapto-1-propanol was obtained from Fluka; ferric chloride and methanesulfonic acid from Sigma; sodium borohydride and Extract-Clean SCX cation exchange columns from Aldrich; synthetic goethite (iron(III) hydroxide, alpha 99%) from Johnson/Matthey; ampules (1–10 ml vacules) from Scientific Products.

Synthetic iron(III) hydroxide oxide (Fe(OH)O) was prepared by adding 0.5 ml aliquots of 2 M sodium hydroxide (13.8 ml total) to a 5 ml solution of 2 M ferric chloride in a 50 ml centrifuge tube agitated on a vortex mixer. When the pH 6 was reached the precipitate was isolated by centrifugation, washed three times with 10 ml water, and dried overnight in a desiccator in vacuo over phosphorus pentoxide. The yield was 945 mg (Weber, 1992). The elemental analysis described in Weber (1992) indicates that the preparation is primarily Fe(OH)O containing a small amount of Fe(OH)O $\cdot$ Cl$_n$. Electron diffraction of this preparation showed a ring pattern characteristic of a fine grain size polycrystalline specimen; however, we could not identify the crystal type from the ring pattern. The binding isotherm of glycerate on synthetic (Fe(OH)O showed maximal adsorption at 1.0 μmol glycerate per 9.7 μmol Fe(OH)O. If we assume that each glycerate binds to one surface ferric ion, then about 10% of the ferric ions of synthetic Fe(OH)O are on the surface. This conclusion is reinforced by the observation that only 8% of the ferric ions are rapidly reduced by glyceraldehyde (Weber, 1992).

B. CHROMATOGRAPHIC ANALYSIS

The polydisulfide oligomers of 2,3-dimercaptopropanol were separated by high pressure liquid chromatography (HPLC) using an Alltech Carbohydrate OH-100 column (250 × 4.6 mm) eluted with acetonitrile – water (93 : 7 v/v) at a rate of 1.0 ml/min. The absorbance of the eluent at 220 nm and 280 nm was recorded as a function of time. Figure 1 shows a typical chromatogram. The Alltech OH-100 column separates the polydisulfide oligomers by their chain length, because the number of hydroxyl groups increases with the oligomer length, and the affinity of the Alltech column is stronger for substances with a greater number of hydroxyl groups. This hydroxyl group-based separation also applies to alditols and carbohydrates as described in an Alltech technical bulletin on the OH-100 column.

C. OXIDATIVE POLYMERIZATION OF 2,3-DIMERCAPTO-1-PROPANOL BY IRON(III) HYDROXIDE OXIDE

In a typical reaction 20 mg of synthetic iron(III) hydroxide oxide and 4.9 ml of

sterile water was placed in a 10 ml ampule (vacule) that had been sterilized with ethanol. While the ampule was partially immersed in an ultrasonic bath, the air was removed by cycling four times between a vacuum and a source of nitrogen. Then 100 μl of 50 mM 2,3-dimercapto-1-propanol was added to the ampule. The deaeration procedure was repeated and the ampule was sealed in vacuo. The reactions were carried out in the dark at 40 °C at pH 4.0. All reactions used 5 μmoles of dithiol and 160 μmoles of Fe(OH)O (20 mg) of which about 8% reacts rapidly as an oxidant (Weber, 1992). This translates to 2.5 μmoles of active iron (III) oxidant per 1.0 μmole of dithiol reductant.

At the end of the reaction the soluble reaction products were separated by centrifugation from insoluble products including Fe(OH)O. A 60 μl aliquot of the supernatant containing the soluble products was placed on an Extract-Clean cation exchange column. The products were eluted with 1.0 ml of acetonitrile. This column treatment was used to remove iron cations and to prevent further oxidation of thiol groups by acidifying the reaction solution. Finally the eluent was concentrated to 350 μl under a stream of nitrogen, and a 40 μl aliquot was analyzed by HPLC. The insoluble fraction containing Fe(OH)O and insoluble oligomers was extracted with 350 μl of acetonitrile three times, and the extract was passed through an Extract-Clean cation exchange column. The eluent was concentrated to 350 μl under a stream of nitrogen, and a 40 μl aliquot was analyzed by HPLC.

D. Characterizations of polydisulfide products

Several types of evidence support the identification of the Peaks in Figure 1 as an ascending series of oligomers from the 1-mer to the 15-mer. First, a time course study of the oxidation of 2,3-dimercaptopropanol by moleuclar oxygen showed that the disappearance of the dithiol was accompanied by a sequential appearance of peaks identified as the 2-mer, 3-mer, 4-mer and 5-mer. This oxidation was accompanied by an increase in the absorbance at 250 nm where alkyl disulfides have a characteristic absorption peak (Calvin and Barltrop, 1952). Furthermore, chemical reduction of the isolated 3-mer, 4-mer, 5-mer and 6-mer yielded the expected series of shorter oligomres. Polydisulfide oligomers from the Fe(OH)O oxidation reaction with retention times of 3.65 min (3-mer), 3.86 min (4-mer), 4.10 min (5-mer), and 4.25 min (6-mer) were each collected from the HPLC column. The volume of each sample was adjusted to 700 μl. A 350 μl aliquot of each solution was acidified by adding 2.0 μl of methanesulfonic acid and 30 μl was analyzed by HPLC as a reduction control. The remaining 350 μl of each solution was chemically reduced by reacting for 30 min with 1.2 μl of freshly prepared aqueous sodium borohydride (3 mg/ml). This solution was then acidified with 2.0 μl of methanesulfonic acid and 30 μl was analyzed by HPLC. No change was observed in the controls. Sodium borohydride reduction of each oligomer generated only oligomers of smaller size, including the 2-mer and monomer with retention times of 3.47 min and 3.31 min, respectively. The oligomers are presumed to contain both D- and L-enantiomers of 2,3-dimercaptopropanol because racemic 2,3-dimercaptopropanol was used in
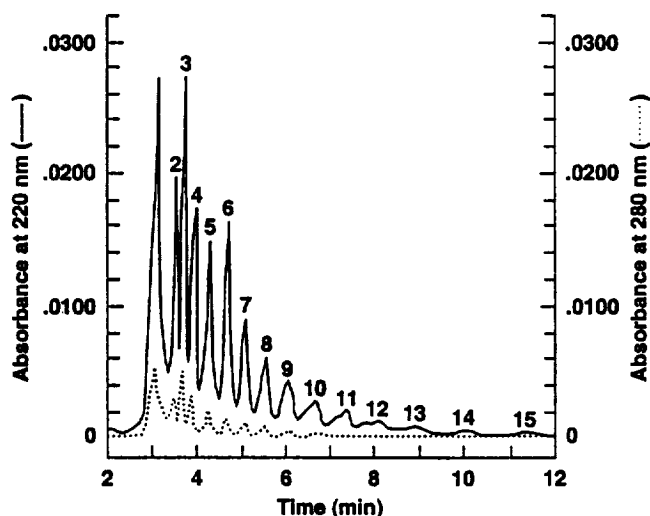
Fig. 1. High pressure liquid chromatographic separation of the insoluble polydisulfide oligomers from the oxidation of 1mM 2,3-dimercaptopropanol in 5 ml water reacted with iron(III) hydroxide oxide (20 mg, 160 μmole Fe) for 3 days under anaerobic conditions at 40 °C and pH 4. The numbers over the peaks indicate the chain length of the oligomers.

the polymerization. The percentage of [2-3]-linked, and [3-3]-linked disulfide isomers present in the oligomeric product was not measured.

3. Results and Discussion

Figure 1 shows the HPLC separation of the insoluble polydisulfide polymers from the oxidation of 1mM 2,3-dimercaptopropanol by ferric ions on the surface of iron(III) hydroxide oxide for 3 days at 40 °C and pH 4. As shown this reaction using 1mM dithiol yielded oligomers up to the 15-mer with the trimer having the highest yield. This insoluble fraction contained about 9% of the total oligomeric products; the remaining 91% of the oligomers were soluble. The soluble fraction was made up of the trimer through hexamer with negligible amounts of shorter or longer oligomers. Controls under nitrogen without Fe(OH)O showed no significant polymer synthesis. Substitution of oxygen for Fe(OH)O yielded oligomers above pH 6, but not at pH 4. This pH effect is consistent with the general stability of thiols towards oxidation under acidic conditions (Capozzi and Modena, 1974).

Figure 2 compares the percent yield of polydisulfide oligomers from 1mM and 10mM dithiol. All reactions were carried out at 40 °C, pH 4, and used 5 μmoles of dithiol and 160 μmoles of Fe(OH)O (20 mg). About 8% of the ferric ions in Fe(OH)O were previously shown to react rapidly as an oxidant (Weber, 1992). This translates to roughly 2.5 μmoles of active iron(III) oxidant per μmole of dithiol reductant – a ratio that is slightly higher than the 2.0 iron(III) ions needed to
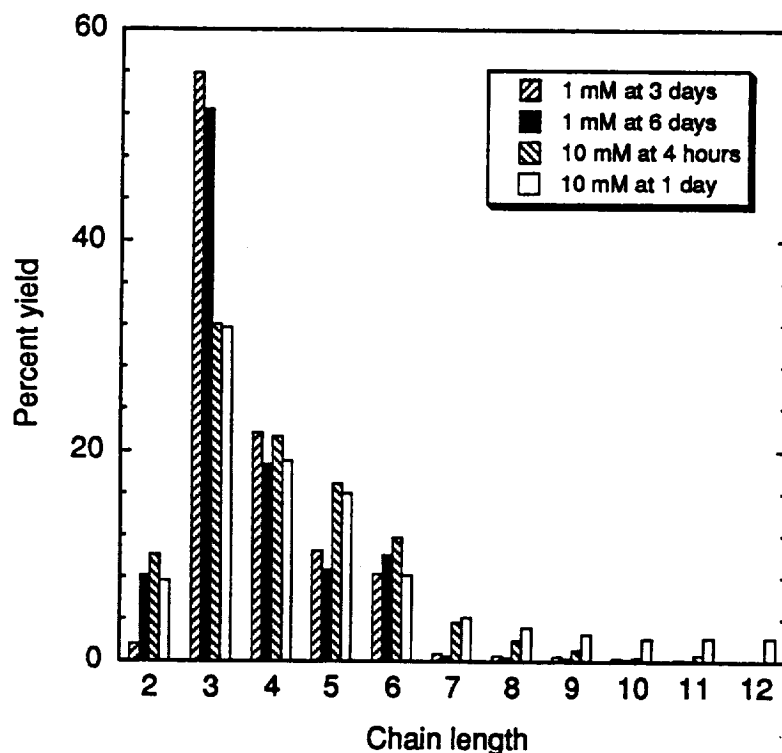
Fig. 2. Percent yield of polydisulfide oligomers from 1 and 10 mM solutions of 2,3-dimercaptopropanol in 5 ml and 0.5 ml of water, respectively, oxidized by iron(III) hydroxide oxide (20 mg, 160 μmole Fe) under anaerobic conditions at 40 °C and pH 4. The percent yields were calculated from the HPLC separation monitored at 220 nm by dividing the area of each oligomer peak by the total of the area of all the soluble and insoluble oligomer peaks. The molar extinction coefficient at 220 nm of the disulfide groups was assumed to be the same for all polydisulfide oligomers.

oxidatively incorporate each dithiol monomer. As shown in Figure 2 the oxidative polymerization of 10 mM dithiol gave a higher yield of the larger oligomers than the 1 mM reaction. The oligomers from the 10 mM reaction were also more insoluble. The yield of insoluble polymer from 1 mM dithiol at 3 days and 6 days, and from 10 mmM dithiol at 4 hours and 1 day were 9%, 7%, 45% and 46%, respectively. Figure 2 also shows that the polymerization of 1 mM dithiol was complete at 3 days. However, the 10 mM reaction was not complete at 4 hours, since the yield of the larger oligomers was greater in the 1 day reaction.

The polymerization of 10 mM dithiol was also examined using synthetic goethite (α-Fe(OH)O) as an oxidant at pH 6 for 2 days. Both synthetic goethite and our Fe(OH)O preparation yielded oligomers up to the 13-mer under these conditions, and gave similar distributions of insoluble oligomers (respective yields as a percent of total insoluble product: 2-mer, 2.6(2.3); 3-mer, 15.6(15.2); 4-mer, 21.9(17.1); 5-mer 25.5(22.7); 6-mer 14.0(14.9); 7-mer 8.3(12.0); 8-mer 4.9(5.0); 9-mer 2.7(4.2);

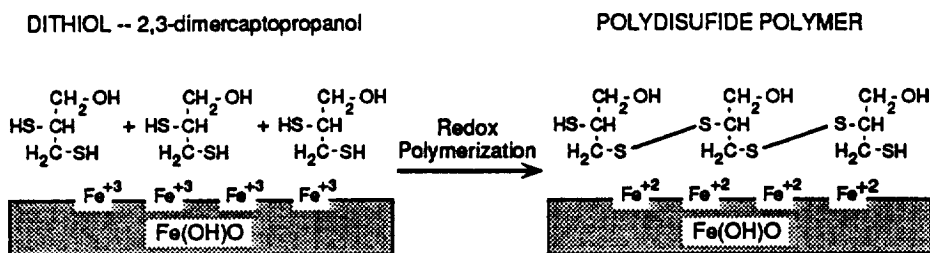DITHIOL -- 2,3-dimercaptopropanol   POLYDISUFIDE POLYMER

Fig. 3. Oxidative polymerization of 2,3-dimercaptopropanol on the surface of iron(III) hydroxide oxide.

10-mer 2.5(3.9); 11-mer 1.3(1.6); 12-mer 0.7(0.9)). HPLC analysis of insoluble products from other reactions above pH 5 frequently showed an anomalous increase in the relative yield and complexity of products in the 10-12-mer region of the chromatogram. This phenomena requires further study. It could be a result of intrastrand disulfide interchange in large oligomers that generates circular polydisulfide polymers (Burns and Whitesides, 1990).

4. Prebiotic Significance

Figure 3 shows the oxidative formation of polydisulfide oligomers from 2,3-dimercaptopropanol on the surface of iron(III) hydroxide oxide. There are several aspects of this oxidative polymerization of dithiols that make it an attractive model of prebiotic polymerization at the earliest stage of the origin of life. First the chemistry seems favorable for a prebiotic reaction. The polymerization uses a simple three carbon monomer that polymerizes readily at low concentration to give reasonably large oligomers under mild conditions. Moreover, water does not interfere chemically in the reaction. Second, the fact that modern life obtains its energy from redox reactions (de Duve, 1991a) suggests that redox reactions could have provided the energy for the earliest type of polymer synthesis involved in the origin of life. In this context it is important to stress that phosphoanhydrides are not a biological energy source, they are simply an energy carrier that is cyclically used to distribute energy from redox reactions to other cellular reactions.

The photocatalytic oxidation of hydrogen sulfide by colloidal semiconductor particles suggests that similar semiconductor particles could have photooxidized dithiols to polydisulfide polymers under prebiotic conditions (Fox and Dulay, 1993; Kamat, 1993; Reber and Meier, 1984). Furthermore, the photoreduction of protons to hydrogen (and carbon dioxide to organic carbon) that accompanies the oxidation of hydrogen sulfide by semiconductor particles suggests that similar substances could have photoreduced carbon dioxide to give organic dithiols under prebiotic conditions using electrons from hydrogen sulfide. Possible photocatalysts that could have been involved in prebiotic dithiol synthesis and polymerization are α-iron(III) hydroxide oxide, partially reduced α-iron(III) hydroxide oxide, iron(III) zinc oxide, zinc oxide, and zinc sulfide – substances that have been shown to photoreduce protons to

hydrogen, and carbon dioxide to organic compounds using electrons from water or hydrogen sulfide (Lu and Li, 1992; Khader *et al.*, 1987; Reber and Meier, 1984; Inoue *et al.*, 1979).

The photocatalytic model described above is similar to earlier proposals describing the photoreduction of carbon dioxide (Granick, 1957, 1965; Halmann *et al.*, 1981), except that it uses hydrogen sulfide as a source of electrons instead of water, and proposes a direct coupling of prebiotic polymer synthesis to the photocatalytic process. The use of hydrogen sulfide instead of water as a source of electrons dramatically lowers energy requirement for the reduction of carbon dioxide to formaldehyde (standard free energy is 118 kcal/mol for water, and 18 kcal/mol for hydrogen sulfide, Thauer *et al.*, 1977). Furthermore, the redox chemistry of the model allows it to be easily adapted to oxidize aldehyde hemithioacetals to thioesters – an advance that would open the door to group transfer reactions required for the synthesis of phosphoanhydrides, polypeptides, and polynucleotides (Weber, 1981, 1984a, b, 1987b; de Duve, 1991b). Since very little is known about the structural requirements of molecular self-replication, there is no reason to exclude the possibility that polydisulfide polymers could have functioned as early autocalysts capable of rudimentary self-replication.

Acknowledgements

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Energy from Redox Disproportionation of Sugar Carbon Drives Biotic and Abiotic Synthesis

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Abstract. To identify the energy source that drives the biosynthesis of amino acids, lipids, and nucleotides from glucose, we calculated the free energy change due to redox disproportionation of the substrate carbon of (1) 26-carbon fermentation reactions and (2) the biosynthesis of amino acids and lipids of *E. coli* from glucose. The free energy (cal/mmol of carbon) of these reactions was plotted as a function of the degree of redox disproportionation of carbon (disproportionative electron transfers (mmol)/mmol of carbon). The zero intercept and proportionality between energy yield and degree of redox disproportionation exhibited by this plot demonstrate that redox disproportionation is the principal energy source of these redox reactions (slope of linear fit = −10.4 cal/mmol of disproportionative electron transfers). The energy and disproportionation values of *E. coli* amino acid and lipid biosynthesis from glucose lie near this linear curve fit with redox disproportionation accounting for 84% and 96% (and ATP only 6% and 1%) of the total energy of amino acid and lipid biosynthesis, respectively. These observations establish that redox disproportionation of carbon, and not ATP, is the primary energy source driving amino acid and lipid biosynthesis from glucose. In contrast, we found that nucleotide biosynthesis involves very little redox disproportionation, and consequently depends almost entirely on ATP for energy. The function of sugar redox disproportionation as the major source of free energy for the biosynthesis of amino acids and lipids suggests that sugar disproportionation played a central role in the origin of metabolism, and probably the origin of life.

Key words: Biosynthesis — Fermentation — Bioenergetics — Origin of metabolism — Prebiotic synthesis — Origin of life — Molecular evolution — Reduction-oxidation — Sugar chemistry — Free energy

Introduction

Figure 1 shows the formal oxidation numbers of carbon atoms in organic groups and one-carbon compounds. These oxidation numbers range from −4 for methane to +4 for carbon dioxide. As shown in the figure, there are only a limited number of oxidation levels available to organisms constructed of organic molecules. In a previous study we estimated the *average* oxidation number of the carbon in the cellular material of a variety of organisms and the biosphere as a whole (Weber 1995). We found that the biosphere and most organisms have average carbon oxidation numbers near zero on the carbon oxidation scale shown in Fig. 1. A few organisms have lower average oxidation numbers because they are composed of a large amount of lipid. However, when we calculated the average oxidation number of organisms and included in the calculation the carbon dioxide generated as a by-product of the biosynthesis of cellular material, we found in all cases that this total biosynthetic product has an average carbon oxidation number close to zero. Since sugars have an average oxidation number of zero and many biosynthetic processes start with sugar substrates, this result suggested to us that biosynthesis operates by redox disproportionation of sugar carbon to give products containing more oxidized and reduced carbon but having the same average carbon oxidation number (zero) as the sugar substrates. Redox disproportionation, as used here, is defined as the transfer of electrons between two substrate carbon groups (molecules) that
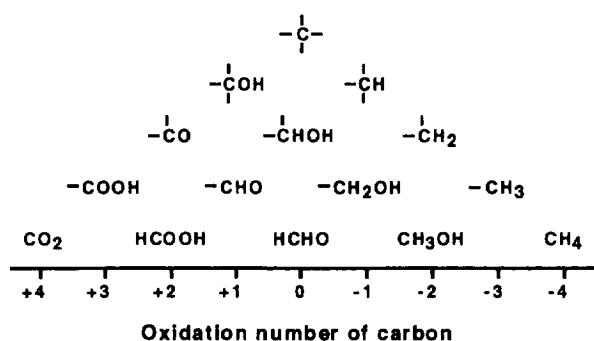
$$-\overset{|}{\underset{|}{C}}-$$

$$-\overset{|}{C}OH \qquad -\overset{|}{C}H$$

$$-\overset{|}{C}O \qquad -\overset{|}{C}HOH \qquad -\overset{|}{C}H_2$$

$$-COOH \qquad -CHO \qquad -CH_2OH \qquad -CH_3$$

CO_2	HCOOH	HCHO	CH_3OH	CH_4

+4	+3	+2	+1	0	-1	-2	-3	-4

Oxidation number of carbon

Fig. 1. Carbon groups and one-carbon molecules positioned directly above their respective oxidation numbers on the formal oxidation number scale of carbon. Open bonds are to other carbons and can be saturated or unsaturated. O can be replaced by N or S.

results in an increase (by two oxidation number units per electron transferred) in the distance separating the two carbon groups (molecules) on the oxidation number scale shown in Fig. 1. An example of redox disproportionation is the transfer of a pair of electrons from formate to methanol that yields carbon dioxide and methane, which are four oxidation numbers further apart than the substrates on the carbon oxidation scale (see Methods for further details).

To better understand the role of redox disproportionation in biosynthesis, especially its role as an energy source, we examined the relationship between carbon redox disproportionation and the free energy change in biosynthetic and fermentative processes. Although previous studies concluded that very little, if any, exogenous energy is required for the biosynthesis of amino acids and lipids from glucose (processes that yield overall almost as much ATP as they consume) these studies did not establish the source or the amount of the free energy driving these biological transformations (Stouthamer 1973, 1979; Battley 1987).

Methods

Biosynthetic Expressions and Energies. The biological reactions we studied are listed in Table 1. They include expressions for the biosynthesis from glucose of *E. coli*'s 20 protein amino acids (reaction A), three lipids (reaction B), and eight nucleotides (reaction C); and also 26 fermentation reactions that use carbon substrates (Neidhardt 1987; Gottschalk 1986). To obtain the biosynthetic expressions we (1) developed reactions describing the synthesis of each of the 20 protein amino acids, eight nucleotides, and three lipids from glucose, ammonia, hydrogen sulfide, and phosphate using pathways in Zubay (1983) and Lehninger (1982), (2) multiplied these individual synthetic reactions by the respective amounts of their products found in *E. coli* (mmol product per dry g of *E. coli*, Neidhardt 1987), and (3) added together the adjusted individual synthetic reactions comprising each of the three biosynthetic processes. The derived expressions give the amount of substrate and product (in mmol) involved in the synthesis of one gram of *E. coli*. Very small amounts of nonglucose substrates (like methyl-FH_4) and extraneous products (like pyruvate) were eliminated from the biosynthetic expressions by adding to them reactions that either gen-

erated the nonglucose substrate from glucose or converted the extraneous products to glucose.

Reactions A*, B*, and C* in Table 1 describe only the carbon redox disproportionation component of their preceding parent reactions noted A, B, and C. The reactions describing disproportionation were developed by substituting oxygen for nitrogen and sulfur in the individual biosynthetic reactions of the 20 protein amino acids, eight nucleotides, and three lipids. Substitution of oxygen for nitrogen in heterocyclic rings changed the rings into unconnected acyclic carbon groups having the same oxidation number as the carbon in the original heterocyclic compound. The reactions were adjusted for *E. coli* composition and summed, and extraneous substrates and products were removed as described above for reactions A, B, and C. To obtain expressions describing only carbon redox chemistry, the NAD(P)H products were eliminated from the expressions by adding to them reactions in which NAD(P)H reduced carbon dioxide to glucose.

The standard free energies (cal/mmol product) for the biosynthesis of each of the 20 protein amino acids, eight nucleotides, and three lipids at 25°C and pH 7 were calculated using the estimation method of Mavrovouniotis (1990, 1991) and the energy values of Thauer et al. (1977) assuming a water activity of 1.0 (Jencks 1976). The free energies of reactions A, B, and C (in cal/g dry wt. of *E. coli*) were calculated by multiplying the standard free energies of formation of products by their respective amounts measured in *E. coli* (mmol per dry g, Neidhardt 1987) and then adding together for each of the three biosynthetic processes the adjusted energies of the individual synthetic reactions. Adjustments were also made for the small energies of reactions used to eliminate nonglucose substrates and extraneous products. Next the energy value of each biosynthetic process was adjusted to 1 mM reactant and product concentrations as described by Jencks (1976). These energy values are listed in Table 1. The free energy of each biosynthetic process was then divided by the total amount of reactant carbon (mmol) to give the free energy of each process in cal/mmol of carbon. The free energies of reactions A*, B*, and C* were calculated in a similar manner with an additional adjustment for energies of reactions used to eliminate NAD(P)H. The energy of reaction C* is not reported because its energy value is small compared to error in the estimation method introduced by substituting oxygen for the nitrogen in heterocyclic rings. The energy values are plotted in Fig. 2.

Fermentation Reactions and Energies. The organic fermentation reactions were obtained from Gottschalk (1986). The free energy (cal/mmol) of each of these reactions at 25°C and pH 7 was calculated using the estimation method of Mavrovouniotis (1990, 1991) and the energy values of Thauer et al. (1977) assuming a water activity of 1.0 (Jencks 1976). The energy values were adjusted to 1 mM reactant and product concentrations as described by Jencks (1976) and then divided by the total amount of reactant carbon (mmol) to give the free energy of each process in cal/mmol of carbon. The fermentation energy values are plotted in Fig. 2.

Redox Disproportionation Calculations. Redox disproportionation is defined as the transfer of electrons between two substrate carbon groups (molecules) that results in an increase (by two oxidation number units per electron transferred) in the distance separating the two carbon groups (molecules) on the oxidation number scale shown in Fig. 1. The reverse of disproportionation is comproportionation, which involves the transfer of electrons between two carbon groups that results in a net decrease in the distance separating the groups.

The number of disproportionative electron transfers occurring in amino acid, lipid, and nucleotide biosynthesis was determined from reactions A*, B*, and C*, respectively, by multiplying the amount of each substrate and product carbon group (mmol/g *E. coli*) by its oxidation number, subtracting the absolute value of the sum of the substrate values from the absolute value of the sum of the product values, and then dividing the result by 2 to give the total number of disproportionative electron transfers (mmol/g *E. coli*). The degree of redox

Table 1. Biosynthetic and fermentative reactions plotted in Fig. 2[a]

A. Amino acid biosynthesis from glucose (mmol/g *E. coli* dry wt.)

    4.522 Glucose + 6.963 NH_3 + 0.233 H_2S + 6.260 NAD^+ + 2.414 NADPH → Protein amino acids (24.353 C, 49.127 H, 6.963 N, 12.176 O, 0.233 S) + 2.778 CO_2 + 9.399 H_2O + 6.26 NADH + 3.846 H^+ + 2.414 $NADP^+$

    [ΔG = −262.9 cal; ΔG from ATP = −15.8 cal; ΔG total = −278.7 cal]

A*. Redox disproportionation component: 4.201 Glucose → Hydroxy acid analogs (24.353 C, 43.906 H, 20.243 O) + 0.855 CO_2 + 3.255 H_2O

    [ΔG redox disprop. = −232.9 cal]

B. Lipid biosynthesis from glucose (mmol/g *E. coli* dry wt).

    1.363 Glucose + 0.097 NH_3 + 0.129 HPO_4^{-2} + 4.870 NAD^+ + 4.318 NADPH → 0.0504 palmitate⁻H^+ + 0.097 phosphatidylethanolamine⁻ + 0.032 phosphatidylglycerol⁻ + 2.564 CO_2 + 2.366 H_2O + 4.87 NADH + 0.3268 H^+ + 4.318 $NADP^+$

    [ΔG = −96.6 cal; ΔG ATP = −1.16 cal; ΔG total = −97.8 cal]

B*. Redox disproportionation component: 1.317 Glucose + 0.129 HPO_4^{-2} + 0.1282 H^+ → 0.0504 palmitate⁻H^+ + 0.097 phosphatidylglycol⁻ + 0.032 phosphatidylglycerol⁻ + 2.288 CO_2 + 2.545 H_2O

    [ΔG redox disprop. = −93.9 cal]

C. Nucleotide biosynthesis from glucose (mmol/g *E. coli* dry wt.)

    1.169 Glucose + 2.865 NH_3 + 0.730 HPO_4^{-2} + 1.794 NAD^+ + 1.335 $NADP^+$ → 0.136 UMP^{-2} + 0.126 CMP^{-2} + 0.165 AMP^{-2} + 0.203 GMP^{-2} + 0.0246 $dTMP^{-2}$ + 0.0254 $dCMP^{-2}$ + 0.0246 $dAMP^{-2}$ + 0.0254 $dGMP^{-2}$ + 4.222 H_2O + 1.794 NADH + 3.13 H^+ + 1.335 NADPH

    [ΔG = −5.12 cal; ΔG ATP = −68.3 cal; ΔG total = −73.4 cal]

Organic fermantation reactions

1. Glucose → 2 ethanol + $2CO_2$
2. Glucose → 2 lactate⁻H^+
3. Glucose → ethanol + lactate⁻H^+ + CO_2
4. 2 glucose → 3 acetate ⁻H^+ + 2 lactate⁻H^+
5. Glucose + H_2O → ethanol + acetate⁻H^+ + 2 formate⁻H^+
6. 3 glucose → 2 butanediol + 2 ethanol + 2 formate⁻H^+ + $4CO_2$
7. 3 glucose → 4 propionate⁻H^+ + 2 acetate⁻H^+ + $2CO_2$ + $2H_2O$
8. Glucose → 3 acetate⁻H^+
9. 6 citrate⁻³H^{+3} + H_2O → lactate⁻H^+ + 7 acetate⁻H^+ + $11CO_2$ + acetoin
10. Acetate⁻H^+ → methane + CO_2
11. Fumarate⁻²H^{+2} + formate⁻H^+ → succinate⁻²H^{+2} + CO_2
12. 4CO + $2H_2O$ → acetate⁻H^+ + $2CO_2$
13. 4CO + $2H_2O$ → methane + $3CO_2$
14. 4 formate⁻H^+ → methane + $3CO_2$ + $2H_2O$
15. 4 methanol + $2CO_2$ → 3 acetate⁻H^+ + $2H_2O$
16. 4 methanol → 3 methane + CO_2 + $2H_2O$
17. 3 alanine + $2H_2O$ → 2 propionate⁻H^+ + acetate⁻H^+ + CO_2 + $3NH_3$
17*. Redox disproportionation component: 3 lactate⁻H^+ → 2 propionate⁻H^+ + acetate⁻H^+ + CO_2 + H_2O
18. 3 aspartate⁻H^+ + $2H_2O$ → 2 propionate⁻H^+ + acetate⁻H^+ + $4CO_2$ + $3NH_3$
18*. Redox disproportionation component: 3 malate⁻²H^{+2} → 2 propionate⁻H^+ + acetate⁻H^+ + $4CO_2$ + H_2O
19. 4 glycine + $2H_2O$ → 3 acetate⁻H^+ + $2CO_2$ + $4NH_3$
19*. Redox disproportionation component: 4 glycolate⁻H^+ → 3 acetate⁻H^+ + $2CO_2$ + $2H_2O$
20. 2 aspartate⁻H^+ + $2H_2O$ → succinate⁻²H^{+2} + acetate⁻H^+ + formate⁻H^+ + CO_2 + $2NH_3$
20*. Redox disproportionation component: 2 malate⁻²H^{+2} → succinate⁻²H^{+2} + acetate⁻H^+ + formate⁻H + CO_2
21. 2 glutamate⁻H^+ + $2H_2O$ → butyrate⁻H^+ + 2 acetate⁻H^+ + $2CO_2$ + $2NH_3$
21*. Redox disproportionation component: 2 2-hydroxyglutarate⁻²H^{+2} → butyrate⁻H^+ + 2 acetate⁻H^+ + $2CO_2$
22.* Lysine + $2H_2O$ → acetate⁻H^+ + butyrate⁻H^+ + $2NH_3$
22*. Redox disproportionation component: 2,6-dihydroxycaproate⁻H^+ → acetate⁻H^+ + butyrate⁻H^+
23. Alanine + 2 glycine + $2H_2O$ → 3 acetate⁻H^+ + CO_2 + $3NH_3$
23*. Redox disproportionation component: lactate⁻H^+ + 2 glycolate⁻H^+ → 3 acetate⁻H^+ + CO_2 + H_2O
24. Leucine + 2 proline + $2H_2O$ → 3-methylbutyrate⁻H^+ + 2 5-aminovalerate⁻H^+ + CO_2 + NH_3
24*. Redox disproportionation component: 2-hydroxy-4-methylvalerate⁻H^+ + 2 2,5-dihydroxyvalerate⁻H^+ → 3-methylbutyrate⁻H^+ + 2 5-hydroxyvalerate⁻H^+ + CO_2 + H_2O
25. Histidine + sarcosine + $4H_2O$ → glutamate⁻H^+ + acetate⁻H^+ + CO_2 + $2NH_3$ + methylamine
25*. Redox disproportionation component: 2,4,5-trihydroxy-4-ene-valerate⁻H^+ + formate⁻H^+ + methanol + glycolate⁻H^+ → 2-hydroxyglutarate⁻²H^{+2} + acetate⁻H^+ + methanol + CO_2 + H_2O
26. Foraminoglycine + $2H_2O$ → acetate⁻H^+ + CO_2 + $2NH_3$
26*. Redox disproportionation component: formate⁻H^+ + glycolate⁻H^+ → acetate⁻H^+ + CO_2 + H_2O

[a] The derivation of the biosynthetic and fermentation reactions and their energy values is described in the Methods section
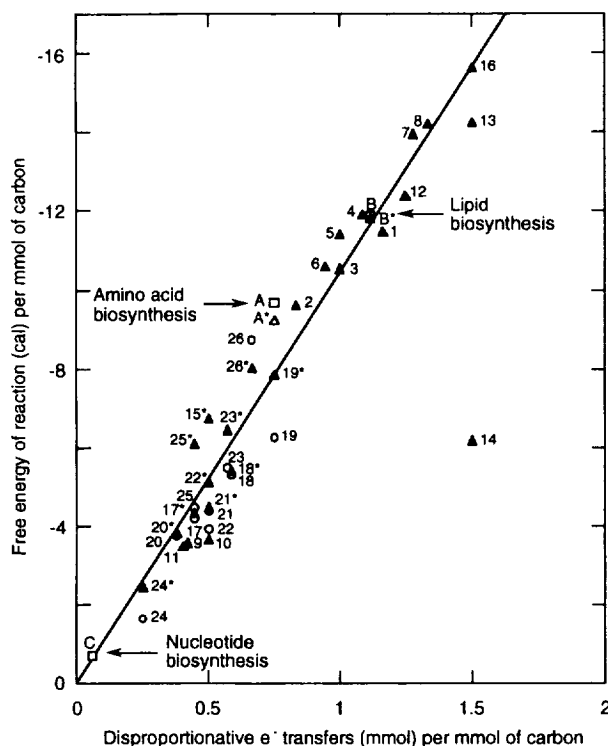
Fig. 2. Free energy (cal/mmol of carbon) at 1 mM concentration of substrates and products of the reactions shown in Table 1 plotted as a function of the degree of redox disproportionation (mmol of disproportionative e^- transfers/mmol of carbon). Energy values were calculated for reactions at pH 7 and 25°C as described in the Methods Section.

by first estimating the standard free energy (kcal/mol) of reactions in which each group (molecule) drives hydrogen formation at pH 7 and 25°C using the estimation method of Mavrovouniotis (1990, 1991) and the energy values of Thauer et al. (1977). The free energy values were then converted to mV units using this relationship—a difference of 100 mV corresponds to a free energy change of –4.6 kcal/mol at pH 7 and 25°C (Zubay 1983b). The reactions were then expressed as two pH 7 half-cell equations (one of which is the formation of hydrogen at pH 7). Knowing the $\Delta E'_0$ of the reaction and the standard reduction potential of the hydrogen half-cell at pH 7 ($E_0' = -421$ mV), the standard reduction potentials of the groups at pH 7 and 25°C relative to the standard hydrogen half-cell were calculated from the relationship: $\Delta E'_0 = E'_0$ (acceptor) $- E'_0$ (donor) (Zubay 1983b). In the example below, this method is used for the calculation of the reduction potential of the aldehyde group [E'_0 (–CHO) at pH 7, 25°C]. This method yields an aldehyde potential of –573 mV, which is close to the –581 mV value of the acetate/acetaldehyde half-cell reported by Loach (1976).

Aldehyde-drive hydrogen formation at pH 7 and 25°C:

$$-CHO + H_2O \rightarrow -COO^-H^+ + H_2$$

$$[\Delta G^{o'} = -7.01 \text{ kcal/mol}, \Delta E'_0 = +152 \text{ mV}]$$

Half-cell equations:

$$2H^+ + 2e^- \rightarrow H_2 \ [E'_0 \text{ (pH 7)} = -421 \text{ mV}]$$
$$-CHO + H_2O \rightarrow -COO^-H^+ + 2H^+ + 2e^-$$

Calculations:

$\Delta E'_0$ (reaction) $= E'_0$ (acceptor, $2H^+$) $- E'_0$ (donor, –CHO)
or E'_0 (donor, –CHO) $= E'_0$ (acceptor, $2H^+$) $- \Delta E'_0$ (reaction)
E'_0 (–CHO) $= E'_0$ (–421 mV at pH 7) $- \Delta E'_0$ (+152 mV at pH 7)
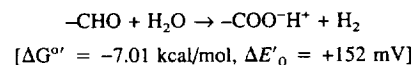E'_0 (–CHO) $= -573$ mV

disproportionation of each process, defined as the number of disproportionative electron transfers (mmol) per mmol of carbon, was then calculated by dividing the total number of disproportionative electron transfers (mmol) by the total amount of carbon (mmol). This calculation method can only be applied to reactions in which the substrates and products have average oxidation numbers of zero.

The number of disproportionative electron transfers of each fermentation reaction was graphically determined by (1) plotting on the carbon oxidation scale each substrate and product group, (2) drawing vectors from substrate groups (beginning with the most oxidized and reduced groups) toward more oxidized and reduced product groups, respectively, (3) summing the absolute value of the lengths (number of oxidation numbers traversed) of all the vectors in the reaction, excluding the length of any two vectors of opposite direction in the same region of the oxidation scale because in this circumstance disproportionation is counterbalanced by comproportionation, and (4) dividing the result by 2 to give the total number of disproportionative electron transfers for all the carbon atoms in the reaction. The degree of redox disproportionation of each reaction, defined as the number of disproportionative electron transfers (mmol) per mmol of carbon (which is equivalent to the number of disproportionative electron transfers per carbon atom), was then calculated by dividing the total number of disproportionative electron transfers by the total number of carbon atoms in the reaction. All reactions involved net carbon redox disproportionation. In reaction 15 the comproportionative reduction of carbon dioxide to a carboxylic acid was corrected for by subtracting the number of electrons transfers involved in carbon dioxide reduction from the total number of disproportionative electron transfers.
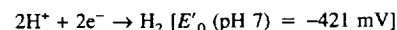
Reduction Potential Calculations. As shown below for the aldehyde group, the standard reduction potentials in Fig. 3 were determined

Results and Discussion

The biological reactions we studied are listed in Table 1. They include the biosynthesis of *E. coli*'s protein amino acids (A), lipids (B), and nucleotides (C) from glucose, and 26 fermentation reactions that use carbon substrates (Neidhardt 1987; Gottschalk 1986). Reactions marked with an asterisk describe only the redox disproportionation chemistry of the preceding parent reaction with the same letter or number designation. These asterisk-marked disproportionation reactions were obtained by adjusting the parent reactions to remove ammonia, hydrogen sulfide, NAD(P)$^+$, and NAD(P)H from the synthetic equations as described in Methods. The free energy change of each these reactions was calculated at the approximate microbiological substrate and product concentrations of 1 mM (Battley 1987; Wheatley et al. 1986).

In Fig. 2 the free energies per mmol of carbon of the reactions listed in Table 1 are plotted as a function of degree of redox disproportionation (mmol of disproportionative e^- transfers/mmol of carbon). The linear curve fit in Fig. 2 uses values of reactions describing only the redox disproportionation chemistry of the reactions in Table 1 (reactions A*, B,*,1–16, and 17*–26* asterisks only, except reaction 14, which was omitted because it
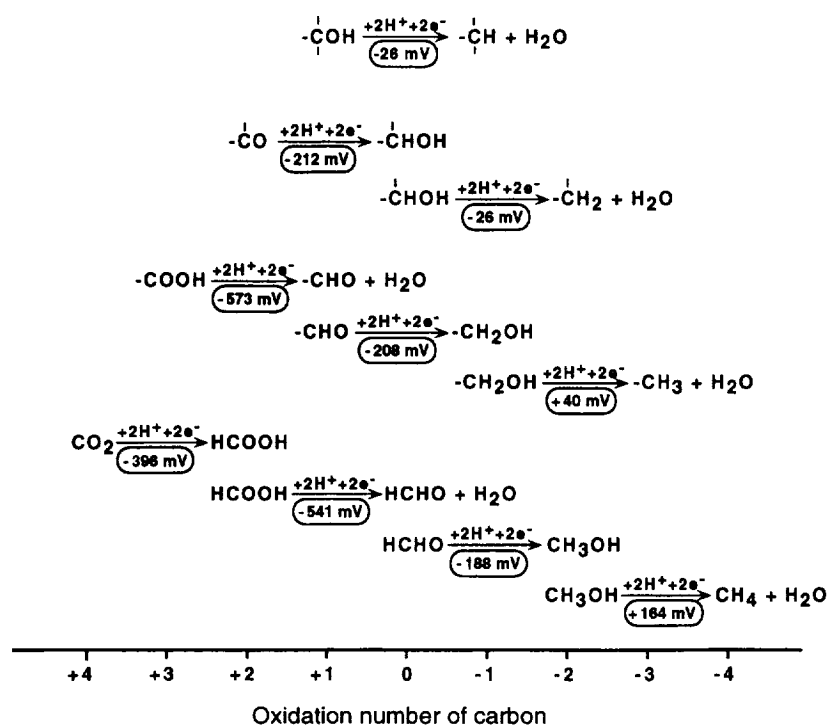
358

$$-\overset{|}{C}OH \xrightarrow[\boxed{-26\ mV}]{+2H^++2e^-} -\overset{|}{C}H + H_2O$$

$$-\overset{|}{C}O \xrightarrow[\boxed{-212\ mV}]{+2H^++2e^-} -\overset{|}{C}HOH$$

$$-\overset{|}{C}HOH \xrightarrow[\boxed{-26\ mV}]{+2H^++2e^-} -\overset{|}{C}H_2 + H_2O$$

$$-COOH \xrightarrow[\boxed{-573\ mV}]{+2H^++2e^-} -CHO + H_2O$$

$$-CHO \xrightarrow[\boxed{-208\ mV}]{+2H^++2e^-} -CH_2OH$$

$$-CH_2OH \xrightarrow[\boxed{+40\ mV}]{+2H^++2e^-} -CH_3 + H_2O$$

$$CO_2 \xrightarrow[\boxed{-396\ mV}]{+2H^++2e^-} HCOOH$$

$$HCOOH \xrightarrow[\boxed{-541\ mV}]{+2H^++2e^-} HCHO + H_2O$$

$$HCHO \xrightarrow[\boxed{-188\ mV}]{+2H^++2e^-} CH_3OH$$

$$CH_3OH \xrightarrow[\boxed{+164\ mV}]{+2H^++2e^-} CH_4 + H_2O$$

+4	+3	+2	+1	0	-1	-2	-3	-4

Oxidation number of carbon

Fig. 3. Redox half-cell equations of carbon groups positioned directly above their respective oxidation numbers on the formal oxidation number scale of carbon. Standard reduction potentials at pH 7 and 25°C given for each half-cell equation were calculated as described in the Methods section.

had an unusually low energy value due mainly to formate ionization at pH 7). The linear fit intercepts the origin and has a slope of -10.4 cal/mmol of disproportionative electron transfers. The zero intercept and proportionality between energy yield and degree of redox disproportionation demonstrate that disproportionation is the principal energy source of the reactions that describe only redox disproportionation. The fact that the values for amino acid biosynthesis from glucose (reaction A), lipid biosynthesis from glucose (reaction B), and fermentation reactions 1–13 and 15–26 without asterisks lie near the linear fit indicates that redox disproportionation is the major energy source of these biological processes. From the energy of the biosynthetic reactions and the reactions describing their disproportionation chemistry listed in Table 1, we estimate that redox disproportionation contributes 89% and 97% of the substrate transformation energy of amino acid and lipid biosynthesis, respectively. Since substrate transformations account for 94% and 99% (and ATP only 6% and 1%) of the total energy of amino acid and lipid biosynthesis, we conclude that redox disproportionation contributes 84% and 96% of the total energy of amino acid and lipid biosynthesis from glucose, respectively. In organisms where excess NADH generated by biosynthesis is used to reduce a fermentation product, such as fumarate, carbon redox disproportionation would contribute an even larger percentage of the total energy (Lin and Kuritzkes 1987). Even if all the NAD(P)H needed for biosynthesis was generated from NADH in an energy-linked process driven by ATP (Rydstrom et al. 1976), redox disproportionation would still provide about 77% of the energy of

amino acid biosynthesis and 66% of the energy of lipid biosynthesis. Because amino acids and lipids are the major products of intermediary metabolism (Weber 1995) redox disproportionation of glucose carbon is clearly the dominant energy source for the biosynthesis of small molecules.

As shown in Fig. 2 nucleotide biosynthesis from glucose involves negligible redox disproportionation of carbon. As a consequence nucleotide biosynthesis derives 93% of its energy from ATP and only 7% of its energy from substrate transformations (see energy values in Table 1). In organisms that live by fermenting carbon substrates, redox disproportionation provides the energy used to generate ATP for nucleotide biosynthesis and other energy-requiring processes.

As shown in Fig. 2 glucose fermentation (reactions 1–8) yields more energy (9.5–14 cal/mmol of carbon) than the other fermentations of multicarbon substrates. Only three fermentations of one-carbon substrates yield as much energy as glucose. The high energy yield of glucose and other sugars not only makes sugars good fermentation substrates but also excellent biosynthetic substrates. Sugars yield more energy per carbon atom than other multicarbon substrates for two reasons. First, all the carbon groups of sugars are capable of redox disproportionation compared to only a fraction of the carbons of other substrates. For example, in the fermentation of glycine and alanine (reaction 23) only four of the seven substrate carbons undergo disproportionation. Second, as discussed later, the strong reducing power (indicated by the large negative reduction potentials shown in Fig. 3) of the aldehyde, hydroxymethyl, and

hydroxymethylene groups of sugars gives these groups very favorable redox disproportionation energies. For these reasons it is hard for other multicarbon substrates to have a significantly larger disproportionation energy than sugars. Therefore, sugars appear to be the optimal biosynthetic substrate—a view supported by the widespread use of sugars as biosynthetic substrates, and by the conversion of one-carbon molecules to sugars before their carbon is used for biosynthesis (Large 1983; Gottschalk 1986).

The property of organic substrates that establishes the direction of electron transfer in favor of redox disproportionation is the relative half-cell reduction potentials of their carbon groups. As shown in Fig. 3, electron donor groups (positioned just to the right of their reduction potentials) with higher formal oxidation numbers are stronger reductants having larger negative reduction potentials than those groups with lower formal oxidation numbers. This systematic difference in potential results in disproportionation, because it drives electron transfer from more oxidized carbon groups that are strong reductants to more reduced groups that are weaker reductants. In other words, the reduction potentials of carbon groups favor the oxidation of more oxidized carbon and the reduction of more reduced carbon. The only exception is formate, which, being a slightly weaker reductant than formaldehyde and the aldehyde group, cannot disproportionate to formaldehyde and carbon dioxide, and cannot chemically reduce a carboxylic acid to an aldehyde. However, formate can participate in disproportionation reactions by donating electrons to molecules and groups more reduced than itself or carboxylic acids.
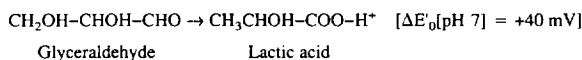
The role of reduction potentials of carbon groups in bringing about redox disproportionation is seen below in the calculation of the free energy of the disproportionation of glyceraldehyde to lactic acid using reduction potentials of the half-cell equations comprising the reaction: (a) the oxidation of the glyceraldehyde's aldehyde group to lactic acid's carboxylic acid group and (b) the reduction of the glyceraldehyde's hydroxymethyl group to lactic acid's methyl group. As shown below, the calculated reduction potential difference of this redox disproportionation reaction ($\Delta E'_0$) is +610 mV, which is equivalent to a favorable free energy of −28 kcal/mol. This example illustrates the energy source driving redox

CH$_2$OH–CHOH–CHO → CH$_3$CHOH–COO–H$^+$ [$\Delta E'_0$[pH 7] = +40 mV]
   Glyceraldehyde   Lactic acid

Half-cell equations written in the direction of reduction:

(a) –COO$^-$H$^+$ + 2H$^+$ + 2e$^-$ → –CHO + H$_2$O   [E_0 (pH 7) = −570 mV]
(b) –CH$_2$OH + 2H$^+$ + 2e$^-$ → –CH$_3$ + H$_2$O   [E_0 (pH 7) = +40 mV]

ΔE_0 (pH 7) = E_0 (acceptor) − E_0 (donor)

ΔE_0 (pH 7) = +40 mV − (−570 mV) = +610 mV ($\Delta G^{\circ\prime}$ = −28 kcal/mol)

disproportionation reactions—the stronger reducing power (more negative reduction potential) of the more oxidized carbon groups (in this case glyceraldehyde's aldehyde group, E'_0 = −570 mV), compared to the weaker reducing power (more positive reduction potential) of the more reduced carbon groups (like lactic acid's methyl group, E'_0 = +40 mV). From this energy perspective *biosynthesis is seen as a substrate-level electron transfer chain that leads to redox disproportionation as electrons flow energetically downhill from the carbon groups of sugars to the more reduced carbon groups of products.*
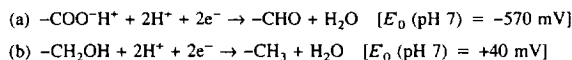
Most carbon–carbon bond-breaking reactions also entail redox disproportionation as indicated by the increase in the separation on the oxidation number scale of two bonded carbons as they undergo scission. Examples are the decarboxylation of pyruvate to acetaldehyde and carbon dioxide, the thiolytic cleavage of β-keto acids, the retroaldol reactions of sugars, and the cleavage of carboxylated ribulose-1,5-diphosphate. In these reactions an electron pair shared by two carbons is transferred to the more reduced carbon, resulting in disproportionation. An exception is the pyruvate formate lyase reaction that occurs without disproportionation or comproportionation (Gottschalk 1986). Overall redox disproportionation by a combination of hydride transfers and carbon–carbon bond-breaking reactions converts carbon substrates like glucose to essential biochemicals and ultimately to methane and carbon dioxide—the thermodynamically most stable products.

Prebiotic Significance

The crucial role of sugars as an indispensable energy source for contemporary biosynthesis suggests that sugars were involved in the origin of metabolism. The prebiotic synthesis of sugars from formaldehyde (Decker et al. 1982), together with the nonenzymatic conversion of acyclic sugars to amino acids, thioesters, and imidazoles by redox disproportionation (Weber 1984, 1985; Grimmett 1965), suggests that sugar transformations could have provided the chemical foundation for the origin of primitive anabolic metabolism. An attractive property of this pathway from formaldehyde to biochemicals is its extremely favorable energetics, which would have allowed it operate at low prebiotic formaldehyde concentrations without assistance from any other energy source. For example, the synthesis of alanine from formaldehyde and ammonia occurs with a standard free energy of −44.9 kcal/mol (Mavrovouniotis 1990, 1991; Thauer et al. 1977). This energy value is so favorable that the formation of 1 mM alanine would have been thermodynamically favorable (energy = −10 kcal/mol) when the concentration of formaldehyde and ammonia in the prebiotic oceans was a plausible 10^{-7} M (Chang 1993). The ener-

getics of fatty acid formation from formaldehyde would have been even more favorable (Weber 1991).

From an energy perspective it is hard to imagine any other organic molecule besides sugars (or formaldehyde) having the energy and reactivity needed to drive either modern anabolic metabolism or the chemical processes behind its origin. Although modern biosynthesis converts some of the energy of sugar redox disproportionation into the hydrolytic energy of ATP to drive group transfer reactions, ATP and related phosphoanhydrides would not have been required for the origin of the earliest metabolism which directly used the energy of sugar disproportionation to generate peptide-forming amino acid thioesters, hydroxy acid thioesters, and possibly fatty acids (Weber and Orgel 1979; Weber 1984, 1985, 1987, 1991; de Duve 1991).

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A BIOCHEMICAL MAGIC FREQUENCY BASED ON THE REDUCTION LEVEL OF BIOLOGICAL CARBON

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ABSTRACT   We have calculated the average number of electron pairs required for the chemical reduction of carbon dioxide to biological carbon using (a) estimates of the reducing equivalents (electron pairs) needed to synthesize biomolecules from carbon dioxide, and (b) measurements of the molecular composition of different organisms. These calculations showed that the carbon of the Earth's biosphere is at the reduction level of formaldehyde that requires two electron pairs per carbon atom to be synthesized from carbon dioxide. This was also the reduction level of cellular carbon when fuel stored as lipid was not used in the estimate. Since this chemical characteristic of life is probably universal, it could be the one thing we know about other carbon-based life in the universe, and the one thing that other intelligent life knows about us. We believe that this common knowledge that biological carbon throughout the universe is at the reduction level of formaldehyde could lead to the selection of the 72.83814 GHz line of the $0,0,0 \rightarrow 1,1,1$ rotational transition of formaldehyde as a frequency for interstellar communication.

INTRODUCTION

Beginning with Cocconi and Morrison (1959), proposed interstellar contact channels have generally involved natural lines of inorganic substances (Kardashev 1978, Morimoto *et al.* 1978, Papagiannis 1985, Bania 1993, Blair *et al.* 1991, 1993). SETI searches to date at these and other frequencies have been reviewed by Tarter (1992). Here we propose an interstellar contact channel based on a property of life that is most likely universal – that the average carbon of life is at the reduction level of formaldehyde that requires two electron pairs per carbon atom to be synthesized from carbon dioxide. Since other intelligent carbon-based life in the universe would also be aware of this shared property and our knowledge of it, we believe this common knowledge could lead to the selection of the 72.83814 GHz line of the rotational transition of formaldehyde as an interstellar contact channel. The average reduction level of the biological carbon was calculated using the

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dry weight composition of organisms and the reduction level of each of their molecular components.

METHODS

The standard free energies of synthetic reactions were calculated from balanced chemical equations using estimates of the standard free energy of formation reactants and products. These calculations used energy values of reactants (Thauer et al. 1977) and products (Mavrovouniotis 1990, 1991) in aqueous solution at pH 7 and 25° C. The reduction level – defined as the number of electron pairs per carbon atom (e^{-1} pairs/C) needed to synthesize a substance from carbon dioxide and ammonia – was determined from the coefficient of molecular oxygen in chemical equations describing the synthesis that used water as the reductant. The average reduction level of the carbon of organisms listed in Table 1 was calculated from the dry weight composition of the organisms and the reduction level of the molecular components. Proteins, nucleic acids, and polysaccharides were assumed to be at the reduction level of their constituent monomers.

Unless the composition data listed the amount of each molecular component, the reduction level of (a) lipid was assumed to be 2.84 e^{-1} pairs/C – the value of tripalmityl glycerol, (b) protein was estimated at 1.98 e^{-1} pairs/C using the average amino acid composition data of Dayhoff et al. (1978), (c) polysaccharide was taken to be 2.00 e^{-1} pairs/C – the value of hexose, and (d) nucleic acid was estimated at 1.56 e^{-1} pairs/C by assuming equal amounts of the four ribonucleosides. The reduction value of protein was remarkably constant: E. coli. (1.99 e^{-1} pairs/C), Avian PPLO (2.00 e^{-1} pairs/C), C. methanophilium (2.04 e^{-1} pairs/C), and the average protein (1.98 e^{-1} pairs/C). The values in Table 1 are probably slightly high because polar lipids with values below 2.84 e^{-1} pairs/C are present in the lipid fraction, and nucleic acids with low reduction values were not reported for plants and animals.

REDUCTION LEVEL OF BIOLOGICAL CARBON

Life is composed primarily of four biomolecules – protein, nucleic acids, carbohydrates, and lipids. A typical bacterial cell contains roughly 50% protein, 10-20% nucleic acid, 10-20% carbohydrate, and 10-15% lipid on a dry weight basis (Luria 1960). The synthesis of each of the substances from carbon and ammonia requires donation electron pairs from a reductant. Our estimates showed that the synthesis of average protein required 1.98, nucleic acid 1.56, carbohydrate 2.00, and lipid 2.84 electron pairs per carbon atom (e^{-1} pairs/C). From reports of the molecular composition of individual organisms and these reduction values, we calculated the average reduction level of biological carbon of the organisms listed in Table 1.

Table 1 shows that the reduction level of the biocarbon of most plants and microbes is between 1.98 and 2.11 e^{-1} pairs/C. Since over 99% of the

carbon of the biosphere resides in land plants of which 90% is found in forests (Bowen 1979, Woodwell et al. 1978), the average reduction level of the carbon in the biosphere is most likely between 2.0 and 2.1 e^{-1} pairs/C. This leads to the conclusion that *the carbon of the biosphere is at the reduction level of formaldehyde* that requires 2.0 e^{-1} pairs/C to be synthesized from carbon dioxide.

Not all organisms have average reduction levels near 2.0 e^{-1} pairs/C. As seen in Table 1 several animals, one plant (*B. braunii*), and one microbe (*R. gracilis* - under low nitrogen) have reduction values above 2.2 e^{-1} pairs/C. These organisms store large amounts of lipid in intra- and extra-cellular droplets that are separate from the cytoplasm (Berkaloff et al. 1984, Park et al. 1990, Lehninger 1982). The amount of lipid found in these droplets can increase and decrease depending on the environmental conditions, like the nutrient availability (Luria 1960, Park et al. 1990). This environmental dependency and the general use of lipids as an energy source indicates that these lipid droplets are primarily stored fuel that secondarily might also provide thermal insulation and buoyancy. If we calculate the reduction level of the carbon of the organisms in Table 1 without including the fuel stored in lipid droplets, all the organisms would probably have values near 2.0 e^{-1} pairs/C. Therefore we conclude that *the cellular material of organisms, not including fuel stored in lipid droplets, is at the reduction level of formaldehyde.*

CHEMISTRY AND THE REDUCTION LEVEL OF BIOLOGICAL CARBON

The observation that the carbon of organisms throughout the biosphere is at the formaldehyde reduction level suggests that this is a universal property of life. We believe that this level is fixed by the chemistry of carbon and is not a product of life's evolutionary history. Only at this level do carbon compounds and carbon functional groups exhibit a reactivity and favorable energetics that allows them to be transformed into substances of higher and lower reduction states. Therefore, only at this level can a complex carbon metabolism develop that provides the foundation for the synthesis of versatile and powerful macromolecular catalysts of life.

As depicted in Figure 1, this view is supported by the conversion of 1-carbon substrates (methane, methanol, formic acid, carbon dioxide) to sugars at the formaldehyde level before their carbon is used in biosynthetic reactions (Large 1983). Sugars, oligomers of formaldehyde, are the prime biosynthetic metabolite for three reasons. First, since they contain both hydrogen and oxygen, they can be converted to molecules of higher and lower reduction levels without the need for an exogenous reductant or oxidant. Second their reducing power, equal to molecular hydrogen, is strong enough to drive biosynthetic reductions to completion. Internal alcohol groups of sugars have a reducing power that is about -7 kcal/electron pair more favorable than the methylene group of fatty acids or the average amino acid side chain. This reducing power is shown by the favorable energy (-28 kcal/mol)

Table 1. Number of Electron Pairs Required to Reduce Carbon
Dioxide to the Biological Carbon of Various Organisms

Organism	Number of Pairs per Carbon Atom	Reference
PLANTS		
Aver. of 7 hardwoods and 6 softwoods	2.11	Braunstein et al. (1981)
Corn, husk	2.05	Braunstein et al. (1981)
cob	2.04	
grain	2.07	
leaf	2.07	
stalk	2.08	
Aver. of 4 duckweeds	2.09	Braunstein et al. (1981)
Giant alga (kelp)	2.01	Braunstein et al. (1981)
Microalga, *B. brauni*	2.60	Vazquez-Duhalt and Arredondo-Vega (1991)
MICROBES		
E. coli, rapidly growing	1.98	Watson (1970)
Avian PPLO, *M. gallisepticum*	2.06	Morowitz et al. (1962)
Yeast, *S. cerevisiae*	2.09	Grylls (1961)
T. utilis	2.01	
R. gracilis	2.09	
R. gracilis, low nitrogen	2.31	
C. methanophilium	2.07	Mimura et al. (1978)
ANIMALS		
Lobster	2.11	Lapedes (1977)
Chicken	2.13	
Oyster	2.15	
Halibut	2.25	
Rabbit	2.26	
Herring	2.40	
Eel	2.45	

of the disproportionation of two sugar hydroxyaldehyde groups to give an
ethyl alcohol group at a higher reduction level and a ketoacid group at a
lower reduction level as depicted in Figure 1. This is also shown in fatty
acid biosynthesis where glyceraldehyde is converted to an ethylene group of
a fatty acid, carbon dioxide, and water with a favorable free energy of -41
kcal/mol. Third, sugars and other functional groups at the formaldehyde

level are chemically reactive. They undergo dehydration, isomerization, eno-
lization, decarboxylation, redox reactions, aldol condensations, rearrange-
ments, and equilibrium additions. (Weber 1985, 1987, 1991 and references
therein). This chemical reactivity at the formaldehyde level underlies bio-
chemical enzyme catalyzed reactions. It could be responsible for the univer-
sal nature of glycolysis, and probably played an important role in the origin
of life. From these observations we conclude that *carbon metabolism is based
on the reactivity and energetics of compounds and functional groups at the
formaldehyde level of reduction.*
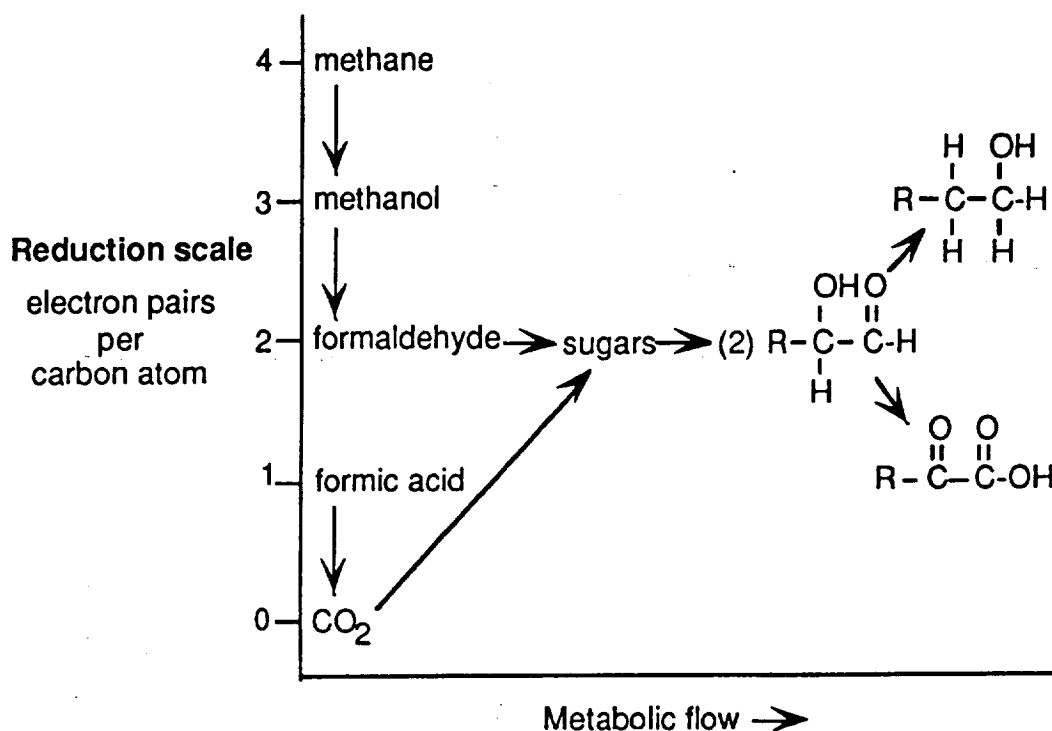
Figure 1. Biological conversion of one carbon substrates to sugars at
the formaldehyde level that are subsequently used for the synthesis of
metabolites of higher and lower reduction level.

Although the chemistry of carbon fixes metabolism near the formalde-
hyde reduction level, it is still difficult to explain why protein, the dominant
cellular material, lies precisely at formaldehyde level. This could be due to
other unknown chemical constrains on metabolism. Alternatively, the oper-
ation of proteins in water could require that they possess hydrophobic cores
composed of hydrocarbon groups and hydrophilic surfaces made up of more
oxidized groups like carboxylic acids. This would cause their average reduc-
tion state to be of intermediate value – near that of formaldehyde.

DEFINING A BIOLOGICALLY BASED CONTACT CHANNEL

Our examination of the molecular composition of life indicates that bio-carbon is at the reduction level of formaldehyde that requires two electron pairs per carbon atom to be synthesized from carbon dioxide. This reduction level was found for both the total carbon of the biosphere and the cellular material of individual organisms when fuel stored as lipid was not used in the estimate. Our analysis suggests that this property is predetermined by the chemical reactivity of carbon at the formaldehyde level that underlies metabolic processes. This chemical determinism and the fact that this property is exhibited by different types of organisms throughout the biosphere indicate that this is a universal characteristic of life. If this is the case, then other intelligent life in the universe will most likely be aware of this shared property and our knowledge of it. This common knowledge could lead to the selection of the 72.83814 GHz line of the rotational transition of formaldehyde (Cord *et al.* 1968) as an interstellar contact channel. An attractive aspect of this biological contact channel is that the signal's frequency indicates life. Due to atmospheric attenuation and noise above 10 GHz, a thorough search at this frequency may require a space-based receiver (Oliver 1981). It is also possible that another formaldehyde line below 10 GHz that is free of interference could be used as an interstellar contact channel.

ACKNOWLEDGEMENTS

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CO-ORIGIN OF METABOLISM AND BIOPOLYMER SYNTHESIS USING FORMOSE SUGAR SUBSTRATES

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Modern life can be described as a complex chemical process (metabolism) that is catalytically controlled by its products (proteins-nucleic acids) in a way that enhances the perpetuation of the entire system. Consequently, the origin of life can be described as a continuous series of events in which a prebiotic chemical process came increasingly under the control of its catalytic products. In our search for this prebiotic process (earliest metabolism) that yielded catalytic take-over products (such as polypeptides), we have investigated – (a) the thermodynamics of the reactions of carbon groups in order to establish the general principles (a historical thermodynamic constraints) that govern modern metabolism and chemical processes involved in its origin, and (b) the chemistry of laboratory models of prebiotic processes that could have been involved in the origin of metabolism and biopolymer synthesis. These studies of the chemical transformations of carbon point to sugars as the optimal substrates for the co-origin of metabolism and biopolymer synthesis.

To better understand the energetics of prebiotic and biotic carbon chemistry, we examined the relationship between redox disproportionation of carbon substrates and the free energy change of biosynthetic and fermentative pathways. Redox disproportionation is defined as the transfer of electrons between two carbon groups that results in an increase (by 4 oxidation number units per 2 electrons transferred) in the distance separating the two carbon groups on the oxidation number scale of carbon. This study showed that in *E. coli*, redox disproportionation of sugar carbon accounted for 84% and 96% (and ATP only 6% and 1%) of the total energy amino acid and lipid biosynthesis, respectively. Redox disproportionation of carbon, and not ATP, is the primary energy source driving amino acid and lipid biosynthesis from glucose. Because disproportionation energy is determined by the invariant half-cell reduction potentials of carbon groups, and since sugars have the highest energy yield of multi-carbon substrates, sugars can be considered the universal optimal biosynthetic substrate.

Since thermodynamic instability of sugars also makes them attractive prebiotic substrates, we are currently investigating the prebiotic synthesis of "activated" amino acid thioesters and peptides from formaldehyde-derived sugars and ammonia in the presence of thiols under mild aqueous conditions. We recently demonstrated that alanine and homoserine are synthesized from formaldehyde, glycolaldehyde (substrates of the formose autocatalytic cycle) and ammonia in the presence of thiol catalysts. This 'one-pot' thiol-dependent prebiotic synthesis presumably yields amino acids via amino acid thioester intermediates that can form peptides. This prebiotic synthesis, which models both metabolism (glycolysis) and biopolymer synthesis (protein synthesis) uses the same redox engine as modern glycolysis and amino acid biosynthesis. We are also the examining possibility that this chemical process generates autocatalytic products by using selection techniques to evolve the system in a way that increases its production of autocatalytic products (rudimentary molecular replicators).

Abstract:

Redox Disproportionation of Glucose as a Major Biosynthetic Energy Source

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Previous studies have concluded that very little if any energy is required for the microbial biosynthesis of amino acids and lipids from glucose -- processes that yield almost as much ATP as they consume. However, these studies did not establish the strength nor the nature of the energy source driving these biological transformations. To identify and estimate the strength of the energy source behind these processes, we calculated the free energy change due to the redox disproportionation of substrate carbon of (a) 26 redox-balanced fermentation reactions, and (b) the biosynthesis of amino acids, lipids, and nucleotides of E. coli from glucose (5-11). A plot of the negative free energy of these reactions per mmole of carbon as a function of the number of disproportionative electron transfers per mmol of carbon showed that the energy yields of these fermentations and biosyntheses were directly proportional to the degree of redox disproportionation of carbon. Since this linear relationship showed that redox disproportionation was the dominant energy source of these reactions, we were able to establish that amino acid and lipid biosynthesis obtained most of their energy from redox disproportionation (>94%). In contrast nucleotide biosynthesis was not driven by redox disproportionation of carbon, and consequently depended completely on ATP for energy. This crucial and previously unrecognized role of sugars as an energy source of biosynthesis suggests that sugars were involved at the earliest stage in the origin of anabolic metabolism.

Abstract:
Weber, A. L. (1996a) The metabolic world: sugars as an energized carbon substrate for prebiotic and biotic synthesis. Abstracts, 8th ISSOL Meeting and 11th International Conference on the Origin of Life, Orleans, France, July 5-12, 1996, *Origins of Life and Evolution of the Biosphere* **26**: 466-467.
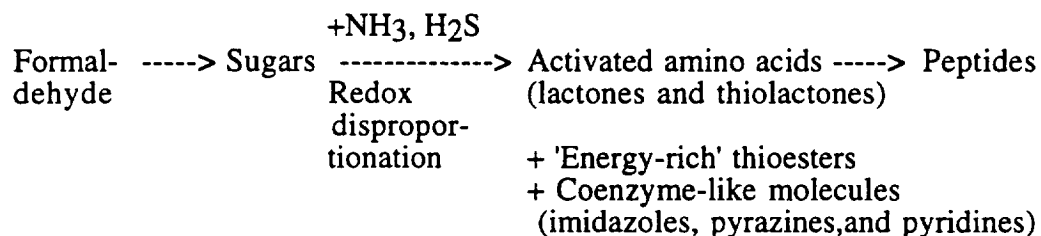
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THE METABOLIC WORLD; SUGARS AS AN ENERGIZED CARBON SUBSTRATE FOR PREBIOTIC AND BIOTIC SYNTHESIS.

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To understand the origin of metabolism and biopolymer synthesis, we investigated the energy sources that drive anabolic metabolism. We found that biosynthesis of amino acids and lipids from sugars is driven by the free energy of redox disproportionation of carbon (see discussion on next page). The indispensable role of sugar disproportionation in the biosynthesis of amino acids and lipids suggests that the origin of life used the same chemical engine, and was therefore based on <u>nonenzymatic</u> redox disproportionation reactions of sugars that occurred in the presence of ammonia and hydrogen sulfide. The chemistry of this 'metabolic' model of the origin of life is depicted below. The model is supported by studies showing the synthesis of sugars from formaldehyde under prebiotic

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                            +NH3, H2S
Formal-  -----> Sugars  -------------> Activated amino acids -----> Peptides
dehyde                  Redox          (lactones and thiolactones)
                        dispropor-
                        tionation      + 'Energy-rich' thioesters
                                       + Coenzyme-like molecules
                                         (imidazoles, pyrazines,and pyridines)
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conditions (Decker et al., 1982); and the nonenzymatic redox transformations of sugars that yield amino acids, energy-rich thioesters, and coenzyme-like molecules such as imidazoles, thiazoles, pyrazoles and pyridines (Yanagawa,, 1980; Weber, 1984, 1985; Grimmett, 1965). The three most attractive aspects of the model are -- (a) its simplicity and similarity to biosynthetic processes, (b) its generation of <u>activated</u> amino acids by redox transformation of sugar substrates, and (c) its extremely favorable energetics that would have allowed it to function at very low substrate concentrations (~1μM formaldehyde). To evaluate the feasibility of this model we are currently studying the formation of peptides from homoserine lactone and homocysteine thiolactone, and the formation of these activated amino acids from tetroses (thiotetroses) by β-dehydration followed by intramolecular redox rearrangement of the ammonia adduct of the resulting α–ketoaldehyde hemiacetal (hemithioacetal) (Weber, 1985).

Biosynthetic energy from redox disproportionation. Although previous studies concluded that very little, if any, energy is required for the biosynthesis of amino acids and lipids from glucose, they did not establish the source nor the amount of free energy driving these biosynthetic processes (Stouthamer, 1973; Battley, 1987). To determine the energy source behind these processes, we calculated the free energy change due to the redox disproportionation of the substrate carbon of (a) 26 redox-balanced fermentation reactions, and (b) the biosynthesis of amino acids, lipids, and nucleotides of E. coli from glucose ((Gottschalk, 1986; Neidhardt, 1987; Mavrovouniotis, 1991)). A plot of the negative free energy of these reactions per mmol of carbon as a function of the number of disproportionative electron transfers per mmol of carbon shows that the energy yields of these reactions are directly proportional to the degree of redox disproportionation of carbon. The zero intercept and linearity of this plot (slope = -10.4 cal/mmol of disproportionative electron transfer) demonstrates that the energy yields of these reactions come mainly from redox disproportionation of carbon. Knowing this, we were able to estimate that redox disproportionation accounted for 84% and 96% (and ATP only 6% and 1%) of the total free energy change of amino acid and lipid biosynthesis from glucose, respectively. We also found that the biosynthesis of nucleotides from sugars depends entirely on ATP for energy occurring without appreciable redox disproportionation of sugar substrates.

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REDOX ENERGY SOURCES FOR THE ORIGIN OF METABOLISM AND PREBIOTIC POLYMERIZATION

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One of the most important questions concerning the origin of life is the nature of the primitive process that provided the chemical energy necessary for the emergence of biological complexity. In contemporary life the primary energy source is electrons of high energy level. These electrons are used in biological reductions, and to drive redox reactions that yield ATP - a recyclable energy carrier that distributes redox energy to other cellular processes. The nearly universal use of redox energy sources by modern life indicates that related redox reactions probably provided the energy for the origin of life. It also seems likely that the earliest type of polymer synthesis was a redox process that could be directly coupled to the redox energy sources. The alternative of converting redox energy to ATP, and then using ATP to drive early polymer formation seems implausible, because it requires powerful enzyme catalysts that were not present early in biogenesis. The other option of using chemical condensing agents from the environment to drive early polymer synthesis seems equally implausible, because under ideal conditions such polymerizations in water have yielded only small amounts of dimers and trimers. Given these observations that support the belief that prebiotic polymerizations were redox processes, we have begun to investigate redox polymerization reactions that could have generated prebiotic polymers (described below).

As a model of redox polymer synthesis we have examined the oxidative polymerization of 2,3-dimercapto-1-propanol by ferric ions on the surface of iron(III) hydroxide oxide (Fe(OH)O) that yields polydisulfide oligomers. This polymerization occurred readily at low dithiol concentration under mild aqueous conditions. Polydisulfide polymers up to the 15-mer were synthesized from 1 mM dithiol in 5 ml water reacted with iron(III) hydroxide oxide (20 mg, 160 μmole Fe) for 3 days under anaerobic conditions at 40°C and pH 4. About 91% of the dithiol was converted to short soluble oligomers and 9% to insoluble larger oligomers that were isolated with the Fe(OH)O phase. Reactions carried out at the same ratio of dithiol to Fe(OH)O but at higher dithiol concentrations gave higher yields of the larger insoluble oligomers. Presumably, ferric ions needed for the formation of Fe(OH)O on the early Earth could have been continually generated by photooxidation of ferrous ions (in minerals or solution). Oxidation power could also have been photochemically generated on the surface of semiconductor particles, like partially reduced α-iron(III) hydroxide oxide.

In addition to the above experimental study we have also analyzed in detail the energy generated by redox reactions of biosynthetic metabolism. This analysis established that most of the energy of intermediary metabolism comes directly from the redox disproportionation of carbon in substrates and not from ATP. Essentially, intermediary metabolism is a redox process (fermentation) that could have originated before energy was distributed in an anhydride form, like ATP. This knowledge further supports a central role of redox processes in the origin of life; it also suggests that the earliest life may have had metabolism based entirely on redox reactions that functioned without ATP.

Abstract:

Weber, A. L. (1994a) Prebiotic oxidative polymerization of 2,3-dimercaptopropanol on the surface of iron(III) hydroxide oxide. In: *Abstracts, 207th American Chemical Society National Meeting*, San Diego, Calif., March 13-17,1994, Division of Geochemistry, abstract no. 43.

43. **PREBIOTIC OXIDATIVE POLYMERIZATION OF 2,3-DIMERCAPTOPROPANOL ON THE SURFACE OF IRON(III) HYDROXIDE OXIDE. A. L. Weber, M. S. 239-4, NASA Ames Research Center, Moffett Field, CA 94035-1000**

The oxidation of 2,3-Dimercapto-1-propanol by ferric ions on the surface of iron (III) hydroxide oxide yielded polydisulfide polymers. This polymerization occured readily at low dithiol concentration under mild aqueous conditions. Polydisulfide polymers up to the 15-mer were synthesized from 1 mM dithiol in 5 ml water reacted with iron(III) hydroxide oxide (20 mg, 160 µmole Fe) for 3 days under anaerobic conditions at 40°C and pH 4. About 91% of the dithiol was converted to short soluble oligomers and 9% to insoluble larger oligomers that were isolated with the mineral phase. Reactions at higher dithiol concentrations with the same ratio of dithiol to mineral gave a higher yield of the larger insoluble oligomers. The relationship of these results to prebiotic polymer synthesis will be discussed.

A Biochemical Magic Frequency

ARTHUR L. WEBER

Life is composed principally of four classes of biomolecules - protein, nucleic acid, polysaccharide and lipid. Using *1)* estimates of the reducing equivalents (electron pairs) needed to synthesize these biomolecules from carbon dioxide, and *2)* measurements of the molecular composition of different organisms, we calculated the average number of electron pairs required for the reduction of carbon dioxide to biological carbon (electron pairs/carbon atom). These calculations showed that the carbon of the Earths biosphere is at the reduction level of formaldehyde that requires 2 electron pairs/carbon atom to be synthesized from carbon dioxide. This was also the reduction level of carbon of individual organisms, except for those that stored large amounts of fuel as lipid. Since this chemical property of life is easily discovered and probably universal, it's most likely known by other intelligent life in the universe. It could be the one thing we know about other carbon-based life in the universe, and the one thing that other intelligent life knows about us. We believe this common knowledge that formaldehyde represents the reduction level of life's carbon could lead to the selection of the 72.83814 GHz line of the 0,0,0 - 1,0,1 ground-state rotational transition of formaldehyde as a frequency for interstellar communication.

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A REDOX BEGINNING: WHICH CAME FIRST PHOSPHORYL, ACYL, OR ELECTRON TRANSFER ?
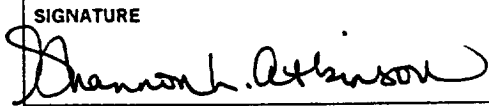
Arthur L. Weber
SETI Institute, NASA Ames Research Center
Moffett Field, CA 94035

Thermodynamic and kinetic information available on the synthesis of prebiotic monomers and polymers will be examined in order to illuminate the prebiotic plausibility of polymer syntheses based on a) phosphoryl transfer that yields phosphodiester polymers, b) acyl transfer that gives polyamides, and c) electron transfer that produces polydisulfide or poly(thio)ester polymers. New experimental results on the oxidative polymerization of 2,3-dimercaptopropanol by ferric ions on the surface of ferric hydroxide oxide will be discussed as a chemical model of polymerization by electron transfer. This redox polymerization that yields polymers with a polydisulfide backbone was found to give oligomers up to the 15-mer from 1 mM of 2,3-dimercaptopropanol after one day at 25°C. HPLC analysis of the oligomers was carried out on an Alltech OH-100 column eluted with acetonitrile-water.

FEDERAL CASH TRANSACTIONS REPORT

(See instructions on the back. If report is for more than one grant or assistance agreement, attach completed Standard Form 272-A.)

OMB APPROVAL NO. 0348–0003

1. Federal sponsoring agency and organizational element to which this report is submitted
NASA Ames Research Center

2. RECIPIENT ORGANIZATION

4. Federal grant or other identification number	5. Recipient's account number or identifying number
NCC 2-784	N/A

Name : SETI Institute

6. Letter of credit number	7. Last payment voucher number
80-00-2124	N/A

Number and Street : 2035 Landings Drive

Give total number for this period	
8. Payment Vouchers credited to your account N/A	9. Treasury checks received (*whether or not deposited*) N/A

City, State and ZIP Code : Mountain View, CA 94043

10. PERIOD COVERED BY THIS REPORT

FROM (*month, day, year*)	To (*month, day, year*)
02/01/93	11/30/98

3. FEDERAL EMPLOYER IDENTIFICATION NO. ▶ 94-2951356

11. STATUS OF FEDERAL CASH *(See specific instructions on the back)*		
	a. Cash on hand beginning of reporting period	$ -0-
	b. Letter of credit withdrawals	623,834
	c. Treasury check payments	-0-
	d. Total receipts *(Sum of lines b and c)*	623,834
	e. Total cash available *(Sum of lines a and d)*	623,834
	f. Gross disbursements	623,834
	g. Federal share of program income	-0-
	h. Net disbursements *(Line f minus line g)*	623,834
	i. Adjustments of prior periods	-0-
	j. Cash on hand end of period	$ -0-

12. THE AMOUNT SHOWN ON LINE 11j, ABOVE, REPRESENTS CASH REQUIREMENTS FOR THE ENSUING ___ Days

13. OTHER INFORMATION	
a. Interest income	$ -0-
b. Advances to subgrantees or subcontractors	$ -0-

14. REMARKS *(Attach additional sheets of plain paper, if more space is required)*

FINAL REPORT - to close out award.

15. CERTIFICATION

I certify to the best of my knowledge and belief that this report is true in all respects and that all disbursements have been made for the purpose and conditions of the grant or agreement

AUTHORIZED CERTIFYING OFFICIAL

SIGNATURE	DATE REPORT SUBMITTED
Shannon L. Atkinson	4/15/99
TYPED OR PRINTED NAME AND TITLE	TELEPHONE (*Area Code, Number, Extension*)
Shannon L. Atkinson, CPA Chief Fiscal Officer	(650) 961-6633

THIS SPACE FOR AGENCY USE
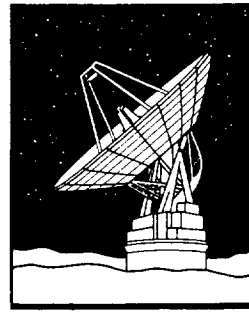
April 12, 1999

Ms. Venoncia Braxton
Grants Office
Mail Stop 241-1
NASA Ames Research Center
Moffett Field, CA 94035-1000

Re: Close-out of Cooperative Agreement NCC 2-784

Dear Nonnie,

We have been advised by the Principal Investigator, Dr. Arthur Weber, and hereby confirm, that no patent was issued or applied for, nor have any inventions resulted from the research performed under the above referenced Cooperative Agreement entitled "Prebiotic Polymer Synthesis and the Origin of Glycolytic Metabolism".

Sincerely,

Thomas Pierson
Executive Director

cc: Sue Palk, NASA Ames Acting Patent Counsel

FINAL INVENTORY REPORT -GRANTEE ACQUIRED EQUIPMENT(NCC 2-784)

Nonexpendable personal property: having a useful life of more than 2 years and an acquisition cost of $500 or more per unit.

Item #	Description	Model #	Serial #	Asset #	Grant #	Title Vests	Acquisition Date (received date)	Location	Unit cost (dollars)
1	**Beckman HPLC System Gold-----** includes items 1-5. Programmable solvent module	126	514-1053	1484	784	Grantee	January 6, 1994	NASA Ames Research Center Building 239, Arthur Weber	$25,000
2	Variable wavelength detector	166	504-1130	1485	784	Grantee	January 6, 1994	Same as above	Included in above price
3	Analog interface module	406A	442-3151	1486	784	Grantee	January 6, 1994	Same as above	Included in above price
4	IBM Computer System	350-466 DX2	23PBT87	1482	784	Grantee	January 6, 1994	Same as above	Included in above price
5	IBM 15" Monitor	IBM	2327969	1481	784	Grantee	January 6, 1994	Same as above	Included in above price
6	HP Laserjet Printer	4ML	USCB010218	1487	784	Grantee	January 6, 1994	Same as above	$1,300
7	Mettler Balance	AE100	N71680	1403	784	Grantee	May 17, 1993	Same as above	$ 2,700
8	Macintosh Computer	Centris 650	F325AJQCA8	1400	784	Grantee	June 4, 1993	Same as above	$ 4,650
9	Macintosh 16" Computer Monitor	Macintosh	S4317153D0 7	1402	784	Grantee	June 4, 1993	Same as above	Included in above price
10	So-Low ultra-low temperature freezer	U85-13	9798909	1612	784	Grantee	July 31, 1998	Same as above	$ 4,250

*****PLEASE NOTE: The property listed in the Final Property Report will still be in use by the Principal Investigator, Dr. Weber, under his new Cooperative Agreement, number NCC 2-1075, and we hereby request that the property be allocated to him under that Agreement.