Redox Energy and Sulfur Chemistry in Prebiotic Polymer Synthesis and Replication

Arthur L. Weber, Principal Investigator

NASA Grant NCC 2-784 · NASA Task 344-38-22-14

Progress Report
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Summary: In the past year we have made significant progress in three research areas: 1) Most importantly, we discovered a new pathway of prebiotic amino acid synthesis in which formaldehyde and glycolaldehyde (substrates of the formose reaction) react with ammonia yielding alanine and homoserine in the presence of thiol catalysts. This 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. 2) Finally, in preparation for the analysis of Martian meteorite samples, we upgraded our HPLC system and developed an improved method capable of detecting a 1 femtomole of amino acid enantiomers. 3) We completed our analysis of the energetics of metabolism that revealed that life depends on biosynthetic processes driven by chemical energy made available by the redox disproportionation of carbon groups of sugars. We established that the favorable energy of redox disproportionation is based on the universal reduction potentials of carbon groups. We concluded that it is hard to imagine any other organic molecule besides sugars (formaldehyde oligomers) having the energy and reactivity needed to drive either modern biosynthesis or the chemical processes behind its origin.

New prebiotic synthesis of amino acids via their thioesters from sugar substrates

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. To eliminate the need to synthesize and to deliver labile chemical condensing agents to the site of prebiotic polymer synthesis, we investigated chemical pathways that generate 'activated' amino acid thioesters from aldehyde substrates, ammonia, and thiols without condensing agents. Our early studies at pH 7.8 and 40°C that used N-acetyl-L-cysteine as the thiol catalyst and phosphate as an acid-base catalyst showed that ammonia having reacted 14 days with glycolaldehyde yielded 0.08% homoserine. The synthesis of homoserine was 12-fold less in a control reaction missing thiol. 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. The yields are based on 10 mM aldehyde substrate.

We next explored the possibility that at pH 5 iron oxides like magnetite could substitute for phosphate as an acid-base catalyst. To our surprise we found thiol-dependent amino acid synthesis occurring at pH 5 without magnetite or phosphate. Fig. 1 on the next page describes alanine (ala) and homoserine (hser) synthesis from glycolaldehyde, formaldehyde, and

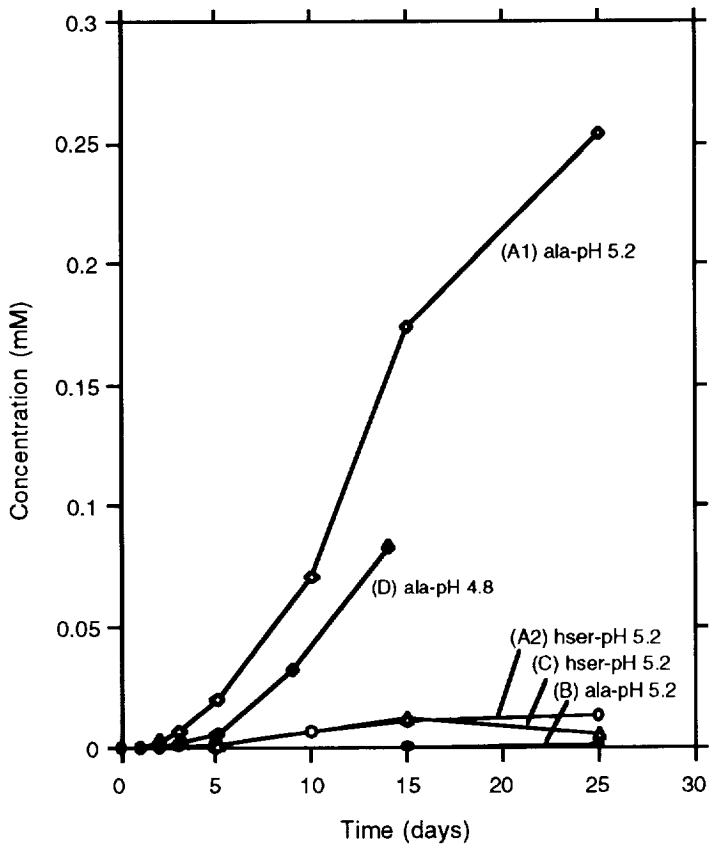

Fig. 1. Time course of formation of alanine (ala) and homoserine (hser) under anaerobic conditions at 40°C. (A1) and (A2) show ala and hser synthesis, respectively, at pH 5.2 from 20 mM glycolaldehyde, 20 mM formaldehyde, 20 mM ammonium chloride, 23 mM sodium acetate, and 23 mM 3-mercaptopropionic acid. (B) shows ala synthesis in a control where 23 mM sodium acetate substituted for 3-mercaptopropionic acid. (C) shows hser synthesis in a control without formaldehyde. (D) shows ala synthesis in a similar reaction but at pH 4.8.

ammonia in the presence and absence of the thiol, 3-mercaptopropionic acid. Three unknown products were also detected. At 35 days alanine synthesis reached 0.36 mM or 1.8% based on the initial formaldehyde concentration. At 25 days the efficiency of alanine synthesis was 2.1% calculated from the ratio of alanine synthesized to formaldehyde reacted (~60%). The yield of homoserine was only 4% that of alanine. Both alanine and homoserine were racemic. Without the thiol the synthesis of both alanine and homoserine was negligible. Without formaldehyde homoserine was synthesized, but not alanine. In other control reactions lacking only glycolaldehyde, or only ammonia, the synthesis of alanine and homoeserine was negligible. In the pH 5.2 reactions, small amounts of the essential triose (<3%) and tetrose (<5%) intermediates accumulated over 25 days. The S-shaped kinetics of alanine synthesis at both pH's indicates that alanine formation is dependent on accumulation or decomposition of a synthetic intermediate. Alanine synthesis was also observed using 10 mM of the aldehyde, ammonia, and thiol reactants.

Fig. 2 below depicts a likely general pathway for the synthesis of amino acids via their thioesters from reaction of glycolaldehyde with itself or another aldehyde, and ammonia in the presence of a thiol catalyst. The pathway proceeds by aldol condensation of glycolaldehyde with itself or another aldehyde to give a γ-substituted glyceraldehyde that undergoes β-dehydration yielding an α-ketoaldehyde which in the presence of ammonia and a thiol forms an imine-hemithioacetal. The imine-hemithioacetal undergoes an intra-molecular redox rearrangement to give an amino acid thioester. The 'activated' amino acid thioester can either hydrolyze liberating an amino acid or react with other amino acids yielding peptides. 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 thioester from glyceraldehyde in the presence of thiols without ammonia. The most surprising finding is that the formose aldol condensation of glycolaldehyde with formaldehyde yielded triose and tetrose intermediates at pH 5.2. Generally, aldol condensations in solution require alkaline conditions. A possible explanation for this result is that ammonia (or amine product) catalyzed 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.

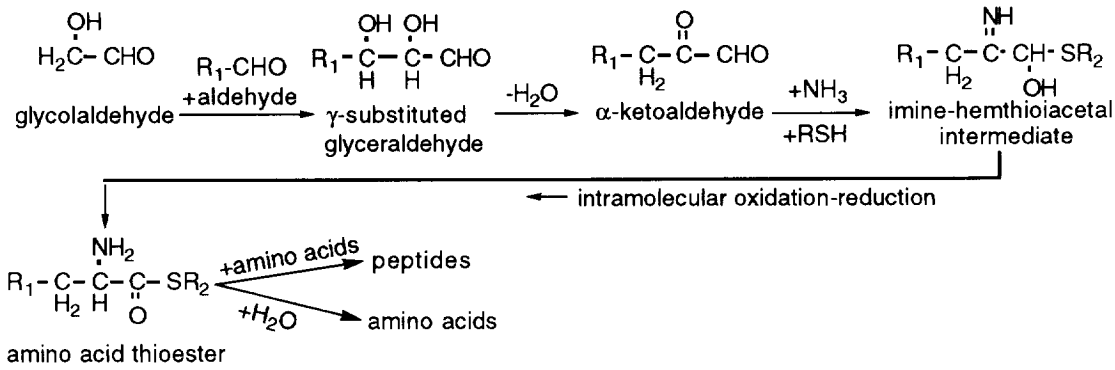

Fig. 2. New general pathway for the prebiotic synthesis of amino acid thioesters.

Amino acid analysis of Martian meteorites and KT boundary sediments

Over the past year 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 to greatly reduce background contamination, 5) obtaining a less contaminated pure water source by managing the purchase and installation of a new Millipore water system for the Exobiology Branch at Ames, 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. We found that detection of these amino acids was not limited by the sensitivity of the HPLC system, but

rather by the presence of other fluorescent compounds in the sample that interfered with measurement of α-amino isobutyric acid and isovaline. We are now looking at sample preparation methods that will remove the contaminants and allow measurement of these amino acids at the 1 femtomole sensitivity limit of our HPLC.

Analysis of the energetics of biotic and abiotic synthesis

We completed our analysis of the energetics of the biosynthesis of small molecules from sugars. This analysis established that redox disproportionation is the principal energy source of the biosynthesis of amino acids and lipids from sugars. This study showed that redox disproportionation of sugar carbon 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. We also discovered that the universal half-cell reduction potentials of carbon groups of substrates sets the direction of electron transfer in favor of redox disproportionation. 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 stronger reductants to more reduced groups that are weaker reductants.

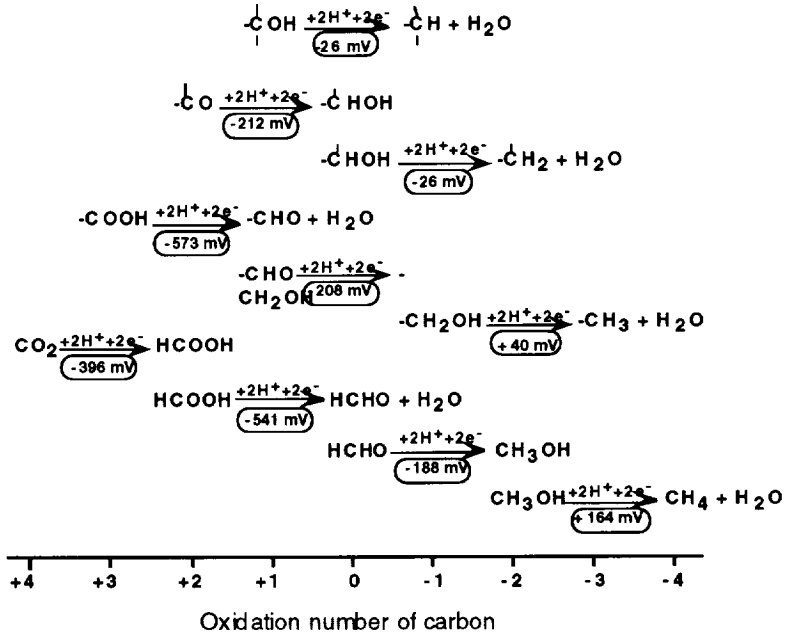

Fig. 3. Redox half-cell equations of carbon groups positioned directly above their respective oxidation numbers on the formal oxidation number scale of carbon. Calculated standard reduction potentials (pH 7, 25°C) are given for each half-cell equation.

Publications and Abstracts:

Weber, A. L. (1996a) The metabolic world: sugars as an energized carbon substrate for prebiotic and biotic synthesis. In: *Abstracts, 8th ISSOL Meeting and 11th International Conference on the Origin of Life,* University of Orleans, Orleans, France, July 5-12,1996, p. 109.

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Weber, A. L. (1997b) Prebiotic Amino Acid Thioester Synthesis: Thiol-Dependent Amino Acid Synthesis from Formose Substrates (Formaldehyde and Glycolaldehyde) and Ammonia. *Origins Life Evol. Biosphere* (submitted).