Investigation of the prebiotic synthesis of amino acids and RNA bases from CO₂ using FeS/H₂S as a reducing agent

FERROUS SULFIDE/PYRITE/AMINO ACIDS/PURINES/PYRIMIDINES

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ABSTRACT An autotrophic theory of the origin of metabolism and life has been proposed in which carbon dioxide is reduced by ferrous sulfide and hydrogen sulfide to give amino acids, purines, pyrimidines, and a variety of other products. The proposed reactions proceed entirely on mineral surfaces, so there is no requirement for prebiotic organic compounds. Similar processes have been proposed for purine synthesis. Ferrous sulfide is a strong reducing agent in the presence of hydrogen sulfide and can produce hydrogen as well as reduce alkenes, alkynes, and thiols to saturated hydrocarbons and reduce ketones to thiols. However, the reduction of carbon dioxide has not been demonstrated. We show here that no amino acids, purines, or pyrimidines are produced from carbon dioxide with the ferrous sulfide and hydrogen sulfide system. Furthermore, this system does not produce amino acids from carboxylic acids by reductive amination and carboxylation. Thus, the proposed autotrophic theory, using carbon dioxide, ferrous sulfide, and hydrogen sulfide, lacks the robustness needed to be a geological process and is, therefore, unlikely to have played a role in the origin of metabolism or the origin of life.

EXPERIMENTAL METHODS

Samples of ferrous sulfide (1 mmol) and doubly distilled water (1 cm³) were degassed by three freeze–thaw cycles, and then ammonia (0.5 mmol), hydrogen sulfide (1 mmol), and carbon dioxide (1 mmol) were added, and the samples were heated in a thermostatically controlled heating block. The butyrate experiments were identical, except for the addition of 1 mmol of butyrate. Three types of ferrous sulfide were investigated, one type was synthesized in situ from ferrous sulfate and hydrogen sulfide, another was commercially supplied (Aldrich, -100 mesh) pretreated by heating to 560°C in vacuo to destroy contaminating organic compounds, and a third was also commercially supplied but was not heated. After heating, the solutions were separated from the iron sulfide by centrifugation and then evaporated to dryness, dissolved in a small amount of doubly distilled 1.5 M HCl, and evaporated to dryness. The residue was redissolved in a small volume of doubly distilled water, and a portion of this solution was then treated with a mixture of o-phthalaldehyde and N-acetyl-L-cysteine before injection onto a Beckman UltraspHERE C18 reverse-phase column and elution with a sodium acetate methanol gradient (15). The amino acid concentrations were estimated by excitation at 340 nm and detection at 450 nm. All surfaces coming into contact with the solutions used in this procedure were meticulously cleaned before use, glassware was heated to 560°C in air to oxidize all contaminating organic compounds, and all plasticware was rinsed three times with doubly distilled water and only used once.

Initial experiments in this study showed large yields of amino acids, and these were later shown to be a result of contamination, principally from ion-exchange resins. Meticulous attention to cleanliness and reagent purity was necessary to reduce background contamination to acceptable levels.

RESULTS

Fig. 1 shows an example of an amino acid chromatogram; trace a is the unheated sample; trace b is a sample heated for 122 days at 100°C; and trace c shows the heated sample after it was treated with 50 pmol of each of the indicated amino acids, an amount that would correspond to a yield of 5 × 10⁻⁴% for a

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have been present in any nonreducing atmosphere containing carbon dioxide, and under some conditions substantial carbon monoxide partial pressures are possible (16-18). Carbon monoxide, unlike carbon dioxide, is relatively easily fixed and is a one-carbon industrial reagent used, for example, in the Fischer-Tropsch synthesis (19, 20) and in acetic acid production.

Accordingly, samples of ferrous sulfide, hydrogen sulfide, ammonia, carbon monoxide, and doubly distilled water were mixed and then heated in the absence of oxygen at 100°C for time periods of up to 4 mo. Results are the same as those described with carbon dioxide. No amino acids were detectable above background contamination in the sample heated for 4 mo. Detection was again by the fluorescence of o-phthalaldehyde derivatives separated by HPLC, and the trace was again compared to the sample solution of known amounts of amino acids. Therefore, the yield of amino acids was <10⁻⁴%.

As in the experiments with carbon dioxide, no purines or pyrimidines were detectable after heating at 100°C for 4 mo with carbon monoxide. This result was again established using UV absorbance at 260 nm on samples separated by HPLC, and the trace was compared to known amounts of adenine, guanine, uracil, and cytosine. Therefore, the yield of purines and pyrimidines was also <10⁻⁴%.

It could be argued that while the proposed archaic reductive citric acid cycle cannot be initiated de novo using carbon dioxide as the sole carbon source, it could have started using organic compounds available from other prebiotic sources or from meteorites (21, 22). To test this hypothesis we attempted a reductive carboxylation and amination using butyrate. This reduction would have given norvaline as a product, a compound that is relatively rare in biology and therefore has a low background. The proposed reaction is shown:

\[
\text{CH}_3-\text{CH}_2-\text{CH}_2-\text{COO}^- + \text{CO}_2 + \text{NH}_4^+ + 2\text{FeS} + 2\text{H}_2\text{S} \rightarrow \text{CH}_3-\text{CH}_2-\text{CH}-\text{COO}^- + 2\text{H}_2\text{O} + 2\text{FeS}_2 + \text{NH}_3^+
\]

Norvaline

This reaction is analogous to the reductive carboxylation of succinate in the reverse Krebs cycle and is used by some bacteria for the synthesis of branched-chain amino acids from carboxylic acids (23, 24).

![Graph](image-url)
DISCUSSION

We have demonstrated that the process proposed by Wächtershäuser of the prebiotic reduction of carbon dioxide with ferrous sulfide/hydrogen sulfide does not work under the conditions we have used, even though the Gibbs free energy is very favorable (25). For glycine synthesis we have the following reaction:

$$2\text{CO}_2(\text{aq}) + \text{NH}_3(\text{aq}) + 3\text{FeS} + 3\text{H}_2\text{S}(g) \rightarrow \text{H}_2\text{N}^-\text{CH}_2\text{COO}^-\text{(aq)} + 2\text{H}_2\text{O}(l) + 3\text{FeS}_2$$

$\Delta G = -38.8 \text{kcal/mol}$

\[H_2N^+\text{-CH}_2\text{COO}^-\text{(aq) + 2H}_2\text{O}(l) + 3\text{FeS}_2\]

For adenine synthesis we have:

$$5\text{CO}_2(\text{aq}) + 5\text{NH}_3(\text{aq}) + 5\text{FeS} + 5\text{H}_2\text{S}(g)$$

$\Delta G = -45.3 \text{kcal/mol}$

\[\text{NH}_2\text{N}^+\text{-CH}_2\text{COO}^-\text{(aq) + 10H}_2\text{O}(l) + 5\text{FeS}_2\]

There were no detectable amino acids, purines, or pyrimidines ($<10^{-4}\%$), even though the Gibbs free energy change is sufficient for quantitative yields. This is another example that demonstrates the difficulty of reducing carbon dioxide at low temperatures under geological conditions. It should not be surprising that no amino acids, purines, or pyrimidines were detected, even though the free energy change is very favorable. The proposed pathway for amino acid synthesis is a reversed Krebs cycle of 5–10 steps that have large kinetic barriers in the absence of enzymes. The whole synthesis is brought to a halt if even one step retains its kinetic barrier on the ferrous sulfide surface. This attribute is in contrast to the prebiotic Strecker synthesis, in which there are no kinetic barriers except for the synthesis of hydrogen cyanide and aldehydes. These syntheses are very efficient under reducing conditions with electric discharges or ultraviolet light as energy sources. The same conclusions apply even if only the carboxylation and reductive amination of carboxylic acids is considered; this is shown below for the formation of DL-alanine.

$$\text{CH}_3\text{-COO}^-\text{(aq) + CO}_2(\text{aq}) + \text{NH}_3^+(\text{aq}) + 2\text{FeS} + 2\text{H}_2\text{S}(g)$$

$\Delta G = -19.1 \text{kcal/mol}$

\[\text{CH}_3\text{-CH-COO}^-\text{(aq) + 2H}_2\text{O}(l) + 2\text{FeS}_2\]

We used a number of different ferrous sulfide preparations, reaction times, and conditions, including those used to reduce thiols, double bonds, etc. (3, 4), and none of them gave products above background. It is possible that there are conditions we did not examine that would give efficient syntheses, but we consider this to be unlikely. Unless the scope of these reactions is very narrow for efficient synthesis, we would have detected substantial yields in our experiments. If a narrow set of reaction conditions are necessary, then this suggests that the proposed reactions are unlikely in most geological environments. Thus, the reduction of carbon dioxide using the ferrous sulfide/hydrogen sulfide system is not a robust process, unlike the synthesis of amino acids and other biomolecules under reducing conditions (26).

This conclusion should not discourage further investigation of the ferrous sulfide/hydrogen sulfide system as a prebiotic reagent. It may well have been important as a reducing agent for reactions of compounds produced by other prebiotic processes.

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