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# Investigation of the prebiotic synthesis of amino acids and RNA bases from $CO_2$ using $FeS/H_2S$ 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 by means of a reversed citric acid cycle, leading to the production of amino acids. 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.**

An autotrophic theory of the origin of life has been proposed (1, 2), 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 dissolved in the ocean. This theory has generated considerable interest as an alternative to the conventional prebiotic soup theory, but so far there has been no experimental test of whether carbon dioxide can be reduced to compounds presumed to be needed for the origin of life. The ferrous sulfide/hydrogen sulfide system has been shown to produce hydrogen (3) and reduce alkynes, alkenes, and thiols to saturated hydrocarbons (4) and reduce ketones to thiols (5), but these reactions are peripheral to an autotrophic theory, which requires the reduction of carbon dioxide.

The ferrous sulfide/hydrogen sulfide system is a powerful reducing agent (5–7):

$$FeS + H_2S = FeS_2 + H_2 \qquad \Delta G° = -9.23 \text{ kcal/mol},$$

with an $E°$ of −620 mV at pH 7 and 25°C. This reaction corresponds to an equilibrium partial pressure of hydrogen of $6 \times 10^6$ atmosphere. Thus, there is more than adequate free energy available to reduce carbon dioxide to any desired organic compound. However, carbon dioxide is a difficult molecule to reduce efficiently at low temperatures (<300°C) under geological conditions, except in the presence of molecular hydrogen (8) or in biological systems. There are a few prebiotic processes using water as a hydrogen donor, such as with ionizing radiation (9, 10) or ultraviolet light (11–13), but these are inefficient. Under industrial conditions carbon dioxide can be reduced, and much attention has been given to the problem (14), but the catalysts used are mixed metal oxides or transition metal complexes that would not have been abundant on the primitive Earth.

Because of the potential promise of the ferrous sulfide/hydrogen sulfide system as a reducing agent, we have attempted to produce amino acids, purines, and pyrimidines from carbon dioxide. We find that none were produced ($<10^{-4}$% yield) under the conditions examined.

## EXPERIMENTAL METHODS

Samples of ferrous sulfide (1 mmol) and doubly distilled water (1 $cm^3$) 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*-phthaldialdehyde and *N*-acetyl-L-cysteine before injection onto a Beckman Ultrasphere $C_{18}$ 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 $5 \times 10^{-4}$% for a

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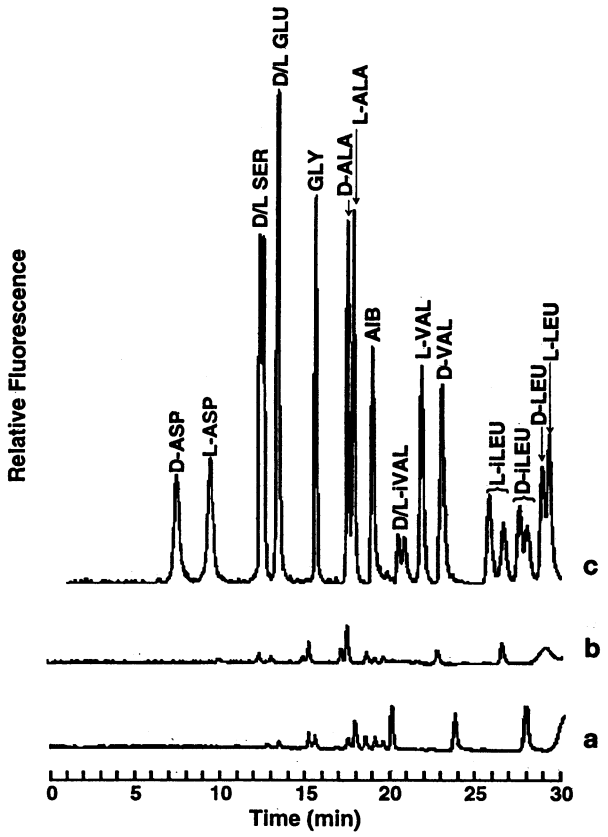



FIG. 1. Amounts of indicated amino acids detected in mixtures of iron sulfide (−100 mesh, 1.04 mmol, pretreated by heating to 560°C), ammonia (0.52 mmol), hydrogen sulfide (1.03 mmol), carbon dioxide (1.03 mmol), and doubly distilled water (1 $cm^3$), degassed by freezing; trace a is the unheated sample, and trace b is the sample heated for 122 days at 100°C. Amino acid concentrations were estimated by excitation at 340 nm and detection at 450 nm. 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 $5 \times 10^{-4}$% for a four-carbon amino acid.

four-carbon amino acid. In addition to the long-term experiment, samples were heated for 1, 7, and 30 days with the three types of ferrous sulfide. No amino acids were detected above the background of the unheated sample in any of the heated samples, which corresponds to a yield of $<10^{-4}$%. This amount is less than that detected in unheated samples of each mixture. This result suggests that the ferrous sulfide/hydrogen sulfide/carbon dioxide/ammonia mixture, if anything, causes decomposition of the small amount of contaminating amino acids initially present. Even if the peaks were real instead of resulting from contamination, the yields would still be $<10^{-4}$% amino acids.

It is also suggested that the proposed system can synthesize purines from ammonia and carbon dioxide (2). This claim was investigated by using a sample that had been heated for 4 mo with ferrous sulfide pretreated by heating to 560°C *in vacuo*. Fig. 2 shows that no purines or pyrimidines were detectable in this sample. These samples were additionally desalted by using a Dowex 50($H^+$) ion-exchange column. The mixtures were injected onto a YMC-Pack $C_{18}$-AQ reverse-phase column, and detection was made by UV absorption at 260 nm. The trace was compared to solutions treated with known samples of adenine, guanine, uracil, and cytosine. Therefore this system also yields $<10^{-4}$% purines or pyrimidines.

Experiments were also conducted with carbon monoxide as a carbon source in place of carbon dioxide, even though this is not part of the proposed theory. Traces of carbon monoxide would have been present in any nonreducing atmosphere containing

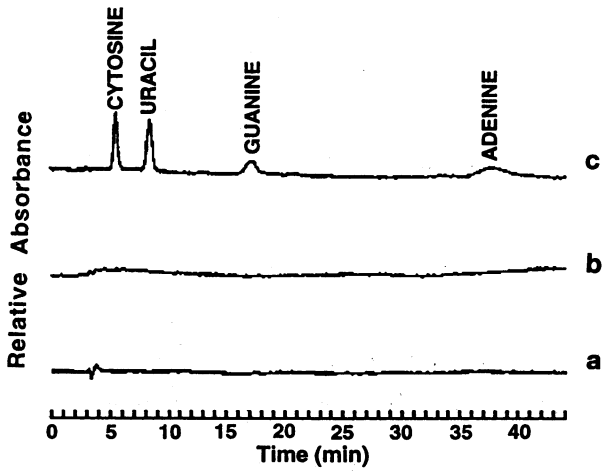



FIG. 2. Amounts of indicated RNA bases in the same mixtures as described for Fig. 1. Trace a is the unheated sample, trace b is the sample heated for 122 days at 100°C, and trace c shows 50 pmol of each of the indicated bases, an amount that would correspond to a yield of $1.25 \times 10^{-3}$% for a five-carbon base.

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*-phthaldialdehyde 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:

$$\underset{\text{Butyrate}}{CH_3{-}CH_2{-}CH_2{-}COO^-} + CO_2 + NH_4^+ + 2FeS + 2H_2S \rightarrow$$

$$CH_3{-}CH_2{-}CH_2{-}\underset{\displaystyle NH_3^+}{\underset{|}{CH}}{-}COO^- + 2H_2O + 2FeS_2$$

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).

Heating degassed aqueous solutions of butyric acid, ammonia, carbon dioxide, hydrogen sulfide, and ferrous sulfide at 100°C for 26 days gave no detectable norvaline as a product, with a yield of $<10^{-6}\%$.

## 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:

$$2CO_2(aq) + NH_3(aq) + 3FeS + 3H_2S(g)$$

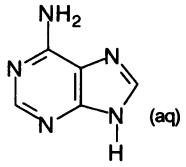
$$\downarrow \Delta G = -38.8 \text{ kcal/mol}$$

$$H_3N^+—CH_2—COO^-(aq) + 2H_2O(l) + 3FeS_2$$

For adenine synthesis we have:

$$5\ CO_2(aq) + 5\ NH_3(aq) + 5\ FeS + 5\ H_2S(g)$$

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




$$+\ 10\ H_2O(l) + 5\ FeS_2$$

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.

$$CH_3–COO^-(aq) + CO_2(aq) + NH_4^+(aq) + 2FeS + 2H_2S(g)$$

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

$$CH_3—\underset{\displaystyle NH_3^+}{\underset{|}{CH}}–COO^-(aq) + 2H_2O(l) + 2FeS_2$$

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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1. Wächtershäuser, G. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 200–204.
2. Wächtershäuser, G. (1988) *Microb. Rev.* **52,** 452–484.
3. Drobner, E., Huber, H., Wächtershäuser, G., Rose, D. & Stetter, K. O. (1990) *Nature (London)* **346,** 742–744.
4. Blochl, E., Keller, M., Wächtershäuser, G. & Stetter, K. O. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8117–8120.
5. Garrels, R. M. & Christ, C. L. (1965) *Solutions, Minerals and Equilibria* (Harper and Row, New York), pp. 216–225.
6. Kaschke, M., Russell, M. J. & Cole, W. J. (1994) *Orig. Life Evol. Biosphere* **24,** 43–56.
7. Wächtershäuser, G. (1988) *Syst. Appl. Microbiol.* **10,** 207–210.
8. Schlesinger, G. & Miller, S. L. (1983) *J. Mol. Evol.* **19,** 376–382.
9. Garrison, W. M., Morrison, D. C., Hamilton, J. G., Benson, A. A. & Calvin, M. (1951) *Science* **114,** 416–418.
10. Getoff, N., Scholes, G. & Weiss, J. (1960) *Tetrahedron Lett.* **18,** 17–23.
11. Groth, W. & Suess, H. (1938) *Naturwissenschaften* **26,** 77.
12. Getoff, N. (1962) *Z. Naturforsch. B.* **17,** 87–90.
13. Åkermark, B., Eklund-Westlin, U., Baeckström, P. & Löf, R. (1980) *Acta Chem. Scand. B* **34,** 27–30.
14. Brändén, C. & Schneider, G., eds. (1994) *Carbon Dioxide Fixation and Reduction in Biological and Model Systems* (Oxford Univ. Press, Oxford).
15. Zhao, M. & Bada, J. L. (1995) *J. Chromatogr. A* **690,** 55–63.
16. Abelson, P. H. (1966) *Proc. Natl. Acad. Sci. USA* **55,** 1365–1372.
17. Kasting, J. F. (1990) *Orig. Life Evol. Biosphere* **20,** 199–231.
18. Holland, H. D. (1962) *Petrologic Studies: A Volume to Honor A. F. Buddington*, eds. Engel, A. E. S., James, H. L. & Leonard, B. F. (Geol. Soc. Am., New York), pp. 447–474.
19. Storch, H. H., Golumbic, N. & Anderson, R. B. (1951) *The Fischer-Tropsch and Related Syntheses* (Wiley, New York).
20. Anderson, R. B., Kölbel, H. & Ralek, M. (1984) *The Fischer-Tropsch Synthesis* (Academic, London).
21. Cronin, J. R. & Pizzarello, S. (1983) *Adv. Space Res.* **3,** 5–18.
22. Lawless, J. G. & Yuen, G. U. (1979) *Nature (London)* **282,** 396–398.
23. Allison, M. J. & Peel, J. L. (1971) *Biochem. J.* **121,** 431–437.
24. Robinson, I. M. & Allison, M. J. (1969) *J. Bacteriol.* **97,** 1220–1226.
25. Miller, S. L. & Smith-Magowan, D. (1990) *J. Phys. Chem. Ref. Data* **19,** 1049–1073.
26. Miller, S. L. & Orgel, L. E. (1974) *The Origins of Life on the Earth* (Prentice-Hall, Englewood Cliffs, NJ).