

Thermal Synthesis of Amino Acids Under Assumed Prebiotic Conditions

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Abstract

A mixture of methane, ammonia, hydrogen sulfide and water vapor passed through a reaction tube heated to 950° Centigrade produced alanine, phenylalanine, and four atypical ninhydrin-positive compounds. A comparison of the relative percentages of alanine and phenylalanine produced in these experiments with those produced in the absence of hydrogen sulfide indicates that the addition of hydrogen sulfide to a methane, ammonia and water vapor mixture significantly alters the composition of the amino acids produced.

Introduction

Many biologically important compounds, including amino acids (4), the nitrogen bases of nucleic acid (11), monosaccharides (2), porphyrins (7), fatty acids (13), and adenosine triphosphate (9) have been synthesized under assumed prebiotic conditions. Amino acids, both naturally-occurring and atypical, have been synthesized using a wide variety of energy sources; these include alpha, beta, and gamma radiations, ultraviolet light, x-rays, electric discharge, and thermal energy (5).

The thermal method of Harada and Fox (4) was adapted for this research, because it produced larger percentages of the naturally-occurring amino acids than other methods. Thermal energy, in combination with hydrogen sulfide gas, methane, ammonia, and water vapor has not been used in the synthesis of amino acids, although the addition of hydrogen sulfide to the mixture would be consistent with the hypothesized composition of the prebiotic atmosphere since it is included in volcanic gases (10). Further, Friedman and Miller (1) suggested that hydrogen sulfide was necessary for the formation of phenylalanine under primitive earth conditions.

In the present experiment the substances produced by heating hydrogen sulfide, methane, ammonia and water vapor were recovered and analyzed.

Experimental Procedure

The apparatus for this experiment was designed to provide for a flow of the reaction gases through a reaction tube heated to 950°C and to collect the reacted gases in an ammoniacal solution. A vacuum pump was used to facilitate the flow of gases through the system. Controls were established by passing gases through the system at

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room temperature with all conditions and reagents identical to those used during the experimental run. Aseptic conditions were used throughout the experiment. Before each experiment the system was flushed with nitrogen gas for 24 hours to remove any traces of oxygen. Two consecutive experimental runs which produced ninhydrin-positive compounds were performed. In the first run the gas mixture was heated for 7 hours, the second for 1 hour.

TABLE 1. *Natural gas composition.*¹

Component	Percent by Volume
CO ₂	0.89
N ₂	0.42
CH ₄	93.81
C ₂ H ₆	3.63
C ₃ H ₈	0.86
I-C ₄ H ₁₀	
	0.18
N-C ₄ H ₁₀	
C ₅ H ₂	0.08
C ₆ +	0.13
Mercaptal Odorants	0.00006

¹ Gas analysis supplied by Mr. Richard Martzall of the Central Indiana Gas Company of Muncie, Indiana.

Natural gas, which had a composition (Table 1) similar to that thought to have been on the primitive earth (8), was used as a source of methane. Ammonia and water vapor were added to the system by bubbling the gas through 6.5N NH₄OH and H₂S was generated by dripping 8N H₂SO₄ on FeSO₄ chips (Fig. 1). The gas entered the system through a gas outlet, passed through a glass wool filter, passed through a trap (an empty flask), bubbled through a potassium permanganate solution, passed through a trap, and bubbled through 6.5N ammonium hydroxide. The emerging methane, ammonia and water vapor were mixed with hydrogen sulfide emerging from a hydrogen sulfide generator in a mixing flask. The resulting mixture was passed directly to the bottom of the reaction tube (Fig. 2) and flowed upward through the heated Vycor glass chips. The temperature of the chips was constantly monitored with the enclosed thermocouple which was inserted into the middle of the glass chips. After flowing past the heated chips, the reacted gases passed out through an exit tubing of Vycor glass that extended into the reaction tube slightly above the level of the glass chips. The reacted gases passed through another trap and bubbled into a collecting fluid of 3N ammonium hydroxide. The polar constituents of the reacted gases were absorbed in the ammoniacal solution. Some brownish vapors which were not absorbed in the ammoniacal solutions passed through another trap and were collected in hexane. These fluids were saved for analysis.

The NH₄OH collecting fluid was sealed under nitrogen gas, agitated at 75°C for 42 hours, and lyophilized. The resulting solids

were influxed in 4N HCl at 110°C for 9 hours, lyophilized, dissolved in 10% propanol and refrigerated until analysis.

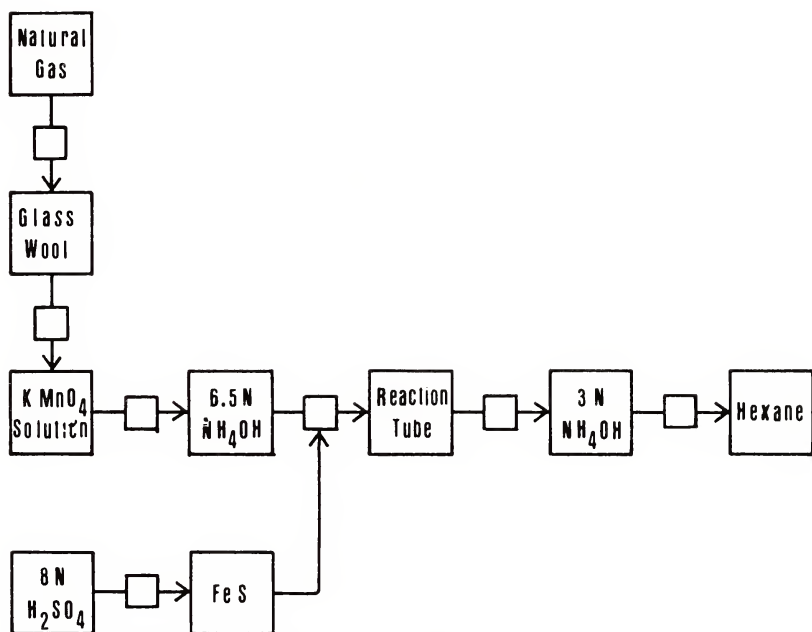


FIGURE 1. Diagrammatic representation of the flow of reaction gases. Traps are indicated by $\square$.

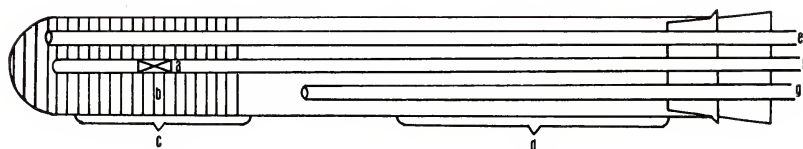


FIGURE 2. Diagram of reaction tube. a) Location of thermocouple; b) Coarsely crushed Vycor glass; c) Portion heated by heating element; d) Portion filled with Fiberglass insulation; e) Inlet tubing; f) Thermocouple tubing; g) Outlet tubing. All glass components were made of Vycor brand glassware.

The ammonium hydroxide soluble products were separated on Silica Gel G thin layer plates with phenol: water (75:25; w/v) as the developing solvent and on cellulose plates with n-butanol:acetic acid: water (80:20:20; v/v/v). The developed plates were air-dried and sprayed with a solution of ninhydrin:n-butanol:acetic acid (0.3:100:3; w/v/v) (12). Color was produced by heating the plates in an oven at 60°C for 30 minutes. Unknown compounds were identified by comparing their R_f values with those of known amino acids. The size and intensity of spots produced by the ninhydrin-positive compounds were used to estimate the amino acid composition of the products.

The hexane soluble fraction was analyzed spectrophotometrically for groups absorbing the U.V. region. (Coleman double beam spectrometer, Model 124.)

Results and Discussion

Thin layer chromatographic analysis of the products revealed ninhydrin-positive compounds whose R_f values were comparable to alanine and phenylalanine (Table 2).

TABLE 2. R_f values of amino acids separated by thin layer chromatography.

Amino Acid	Sorbent	Unknown ¹	Standard ¹
First Run			
Alanine	Cellulose	.326	.329
	Silica Gel	.266	.265
Phenylalanine	Cellulose	.684	.686
	Silica Gel	.543	.550
Second Run			
Alanine	Cellulose	.283	.286
	Silica Gel	.271	.260
Phenylalanine	Cellulose	.673	.670
	Silica Gel	.520	.521
X1	Silica Gel	0.33	
X2		.066	
X3		.128	
X4		.292	
Glycine			.218
Serine			.184

¹ Each value represents an average of 3 to 5 experiments.

The substance identified as phenylalanine accounted for about 15% of the products and alanine, 30%. Six other ninhydrin-positive compounds were produced, but their R_f values did not correspond to any of the 20 natural amino acid standards. Quantitative tests for the free sulfhydryl, disulfide, and thiocyanate groups were negative. No ninhydrin-positive compounds were found in either of the control experiments.

The hexane soluble fraction absorbed light in the 250 to 270 m μ range, suggesting that some of the products were aromatic.

The presence of hydrogen sulfide gas on the primitive earth may have had a significant effect on the prebiotic production of amino acids. Harada and Fox (4) found that when water, methane and ammonia were heated to 950°C that glycine represented 60.3% of the amino acids produced, alanine, 18% and phenylalanine, 0.8%. In our experiments glycine was not identified, alanine accounted for 30% of the amino acids produced, and phenylalanine, 15%.

Harada and Fox (5) suggested that the addition of sulfur-containing compounds to this type of reaction mixture would enhance the production of hydrogen cyanide. Hydrogen cyanide is involved

in the Strecker synthesis which has been suggested as the synthetic mechanism for amino acids in experiments that duplicate prebiotic conditions (3, 8). Hydrogen cyanide and aldehydes are thought to be produced in the reaction tube. The subsequent heating of these compounds results in the formation of hydroxynitriles, which in turn react to form aminonitriles in the presence of ammonia. Upon hydrolysis, the aminonitriles form the corresponding amino acids.

The results of this work also support the view of Friedman and Miller (1) that the presence of hydrogen sulfide in the primitive oceans was necessary for the production of phenylalanine.

Literature Cited

1. FRIEDMAN, N., and S. L. MILLER. 1969. Phenylalanine and tyrosine synthesis under primitive earth conditions. *Science* **166**:766-767.
2. GABEL, N. W., and C. PONNAMPERUMA. 1967. Model for origin of monosaccharides. *Nature* **216**:453-455.
3. HARADA, K. 1963. Asymmetric synthesis of α -amino-acids by the Strecker synthesis. *Nature* **200**:1201.
4. ———, and S. W. FOX. 1964. Thermal synthesis of natural amino-acids from a postulated primitive terrestrial atmosphere. *Nature* **201**:335-336.
5. ———, and ———. 1965. The thermal synthesis of amino acids from a hypothetically primitive terrestrial atmosphere, p. 187 to 194. *In* S. W. Fox [ed.] *The origins of prebiological systems and of their molecular matrices*. Academic Press, New York, N. Y. 482 p.
6. HODGSON, G. W., and C. PONNAMPERUMA. 1968. Prebiotic porphyrin genesis: porphyrins from electric discharge in methane, ammonia and water vapor. *Proc. Nat. Acad. Sci. U. S.* **59**:22-28.
7. MILLER, S. L. 1955. Production of some organic compounds under possible primitive earth conditions. *J. Amer. Chem. Soc.* **77**:2351-2360.
8. ———, and UREY, H. C. 1959. Organic compound synthesis on the primitive earth. *Science* **130**:245-246.
9. PONNAMPERUMA, C., C. SAGAN and R. MARINER. 1963. Synthesis of adenosine triphosphate under possible primitive earth conditions. *Nature* **199**:222-226.
10. RITTMAN, A. 1962. *Volcanoes and their activity*. Interscience Publishers, New York, N. Y. (Translated from the second German edition by E. A. Vincent.) 305 p.
11. SANCHEZ, R. A., J. P. FERRIS and L. E. ORGEL. 1968. Studies in prebiotic synthesis. *J. Mol. Biol.* **38**:121-128.
12. STAHL, E., ed. 1965. *Thin layer chromatography; a laboratory handbook*. Springer-Verlag, New York, N. Y. 553 p.
13. YOUNG, R. S. 1965. Morphology and chemistry of microspheres from proteinoid, p. 347 to 356. *In* S. W. Fox [ed.] *The origins of prebiological systems and of their molecular matrices*. Academic Press, New York, N. Y. 482 p.