Polyamino Acids: Preparation from Reported Proportions of "Prebiotic" and Extraterrestrial Amino Acids

Abstract. *Polyamino acids were thermally prepared from the proportions of amino acids identified (sometimes after hydrolysis) among the products of simulated prebiotic syntheses and (after hydrolysis) in lunar and meteoritic samples. Inferences are made concerning the composition of prebiotic protein and the possible extraterrestrial existence of protein-like polymers.*

Polyamino acids have been prepared by heating suitable proportions of amino acids under simulated prebiotic conditions (*1*). These polymers, which resemble contemporary protein in many ways (*1*), are regarded as models for prebiotic protein. However, the proportions and kinds of amino acids used as reactants have not been those reported among the products of simulated prebiotic syntheses (*2*). These proportions of amino acids [including nonproteinous amino acids (*3*)] may have influenced the composition of primitive protein. We therefore used as reactants the exact proportions of amino acids found in the products (sometimes after hydrolysis) of five "prebiotic" syntheses (*4–8*) and also those reported as constituents (after hydrolysis) of two lunar samples (*9*) and of the Murchison meteorite (*10*).

The amino acid mixtures (5.1 to 8.7 g) were polymerized (*1*) at 175°C under a stream of nitrogen gas for a period of 1.5 to 6 hours. The products, largely or totally soluble in water or in dilute base, were dialyzed for 48 hours against a continual flow of distilled, deionized water, and the nondiffusible portions were then lyophilized after any insoluble materials were removed by filtration. Portions of the polymers were hydrolyzed in 6*N* HCl, under N_2 in sealed tubes, at 109° to 110°C, 48 to 53 hours. The hydrolyzates were analyzed with an amino acid analyzer (Beckman model 120C) (*11*); norleucine was used as an internal standard.

We obtained polymeric material from all reactants tested, in yields of 0.3 to 5.7 percent by weight. Each polymer contained peptide bonds, as indicated (*12*) by absorbance peaks at 1650 (amide I), 1550 (amide II), and 3300 cm^{-1} (amide A), and also by positive biuret reaction. Biuret color intensities ranged from 0.9 to 1.7 absorbance units per gram of polymer per liter; the value obtained for crystalline bovine albumin under these conditions was 1.6. Crude estimates of the range of molecular size [Bio-Gel columns (*13*)] suggested (*14*) that some percentage of each polymer was of molecular weight of at least 10,000; the extreme values for the eight preparations were 24 and 65 percent.

Molar proportions of amino acids in the reactants (boldface) and in hydrolyzates of the products for four of the preparations are shown in Table 1. The close agreement of results from duplicate or triplicate preparations (including three not shown in Table 1) indicates that these polymerizations, as others (*1*), are reproducible. In each preparation, some amount of each amino acid present in the reactants was found in the hydrolyzate of the polymer, except for α-aminoisobutyric acid (*15*) and, possibly, serine and threonine (*15*). To what extent, if any, dicarboxylic amino acids were present as amides was not determined. Minor amounts of unknown compounds (Table 1) were found in hydrolyzates of most preparations; nascent α-amino-*n*-butyric acid was tentatively identified in three cases.

Glycine was the predominant constituent of all but one [that patterned according to (*8*)] of our reactants and of all products. Alanine in most cases was the second most common amino acid present. Large proportions of these amino acids are not common in contemporary protein with the exception of some structural protein [for example, fibroin, elastin, collagen (*16*)]. In addition, nonproteinous amino acids (*3*), when present in the reactants, were found in the products. These results suggest that, if the proportions of amino acids used as reactants are representative of those of abiotic environments (*17*), prebiotic protein may have been rich in glycine and alanine and may have contained nonproteinous amino acids. The stage (or stages) of evolution at which nonproteinous amino acids may have been selected against has yet to be resolved (*18*).

Our "Sagan" preparation (*8*) was of particular interest in that our reactants contained only 9 percent glycine (and 86 percent alanine), whereas the dialyzed product contained 59 percent glycine (and only 30 percent alanine). The enrichment with glycine of the nondiffusible portion of the preparation was probably due to preferential incorporation of glycine, rather than to the decomposition of alanine, because the crude (undialyzed) product contained

Table 1. Amino acid compositions of the reactants (boldface numerals) and of the dialyzed products of four thermal preparations. The reactants were patterned according to the proportions of amino acids reported in the indicated references. Duplicate or triplicate entries result from duplicate or triplicate syntheses. The compositions are expressed to three figures to show the presence of minor constituents (< 1 percent) and to indicate the reproducibility of the syntheses; accuracy to three figures is not implied. Dashes, not present in reactants or products; tr, trace.

Amino acid	Amino acid composition (moles per 1000 moles of amino acids)											
	Miller* (*4*)		Fox* (*5*)		Lunar A (*9*)				Meteorite* (*10*)			
Glycine	**506**	430	**571**	691	**555**	693	691	687	**360**	535	532	
Alanine	**273**	194	**91**	119	**142**	119	120	129	**174**	155	154	
Aspartic acid	**3**	7	**103**	128	**129**	129	128	119	**57**	68	54	
Glutamic acid	**5**	53	—	—	**46**	30	28	28	**94**	40	46	
Leucine	—	—	**39**	17	—	—	—	—	—	—	—	
Threonine†	**0**	2	**0**	8	**31**	9	9	10	**0**	1	3	
Serine†	**0**	3	**79**	4	**98**	10	8	11	**0**	2	3	
Isoleucine	—	—	**0**	1	—	—	—	—	—	—	—	
Proline	—	—	—	—	—	—	—	—	**50**	24	27	
Valine	—	—	**117**	28	—	—	—	—	**61**	48	61	
β-Alanine	**120**	202	—	—	—	—	—	—	**20**	34	29	
α-Amino-*n*-butyric acid	**40**	51	**0**	5†	**0**	11†	10†	11†	**47**	46	46	
α-Aminoisobutyric acid	**4**	0	—	—	—	—	—	—	**107**	0	0	
β-Aminoisobutyric acid	—	—	—	—	—	—	—	—	**30**	44	43	
Sarcosine	**40**	22	—	—	—	—	—	—	—	—	—	
N-Methylalanine	**8**	33	—	—	—	—	—	—	—	—	—	
Unknowns‡	**0**	2	—	—	**0**	tr	5	5	**0**	3	3	

* Only those amino acids quantified in literature reports were included in our reactants. † Identification tentative. ‡ Calculated assuming a norleucine color equivalent of 1.000.

about 92 percent alanine, comparable to its proportion in the reactants. These results indicate a nonrandom incorporation of amino acids (*1*) during thermal polymerization (less strikingly evident from compositions listed in Table 1) and further suggest the presence of a high proportion of glycine in primitive protein (*19*).

Our study has shown that polyamino acids are formed thermally from the proportions of amino acids obtained (*4–8*) by the action of spark discharge, heat, or ultraviolet light on five different geologically plausible atmospheres or hydrospheres. These findings are interpreted to support a proposed molecular evolutionary continuum (*5*), from primitive gases to amino acids to polyamino acids. Our "Miller" and "Sagan" preparations are perhaps the more pertinent in this context, because their syntheses (*4, 8*) apparently yielded free amino acids. However, our "Fox" preparation is also of interest in that the amino acids were obtained [after hydrolysis (*5*)] not from primitive atmospheres of conjectural composition (*1*), but from identified constituents of interstellar space (*20*). Fox and Windsor (*5*) indicate that free amino acids could result naturally from their product [which possibly was hexamethylenetetramine (*20*) and not a polyamino acid (*5*)], and they implicate such amino acids in the evolutionary sequence: ". . . simple compounds → amino acids → proteinoids [polyamino acids] → microspheres. . . ." Our results show that polyamino acids are formed from their reported proportion of amino acids.

The uncertainties concerning the compositions of primitive atmospheres also do not apply to our "meteorite" and "lunar" preparations. The amino acids we used, however, were those quantified after the hydrolysis of samples (*9, 10*). Although some free amino acids have been reported (*10, 21*), the indigenous lunar compounds probably (*22*) existed largely as (unidentified) precursors of amino acids. This study does indicate, however, that if the amino acids ever existed in free form, they could have polymerized thermally. The results, supported by evidence of a thermal lunar history (*23*), suggest that protein-like polymers could exist extraterrestrially.

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References and Notes

1. S. W. Fox, *Nature* **205**, 328 (1965); *Naturwissenschaften* **56**, 1 (1969).
2. Generally, factors such as yield of polymer, specific function, or the compositions of contemporary proteins have influenced the proportions of amino acids used as reactants (*1*). However, we are informed by S. W. Fox that A. Wood has thermally polymerized the amino acids reported in (*7*).
3. The term "nonproteinous" denotes those amino acids not commonly found in proteins; the term "proteinous" is used in (*9*).
4. S. L. Miller, *Biochim. Biophys. Acta* **23**, 480 (1957), table II, run 1.
5. S. W. Fox and C. R. Windsor, *Science* **170**, 984 (1970), experiment 4.
6. C. U. Lowe, M. W. Rees, R. Markham, *Nature* **199**, 219 (1963), table 1, last column.
7. K. Harada and S. W. Fox, in *The Origins of Prebiological Systems*, S. W. Fox, Ed. (Academic Press, New York, 1965), p. 187, table 1, column 1.
8. C. Sagan and B. N. Khare, *Science* **173**, 417 (1971), table 2.
9. K. Harada, P. E. Hare, C. R. Windsor, S. W. Fox, *ibid.*, p. 433, figure 2 ("Lunar A") and table 1, sample 12033 ("Lunar B").
10. J. R. Cronin and C. B. Moore, *ibid.* **172**, 1327 (1971), table 1 (Murchison).
11. "Model 120C Amino Acid Analyzer," instruction manual (Beckman Instrument Co., Palo Alto, Calif., 1965).
12. R. D. B. Fraser and E. Suzuki, in *Physical Principles and Techniques of Protein Chemistry*, S. I. Leach, Ed. (Academic Press, New York, 1970), part B, p. 213.
13. M. R. Heinrich, D. L. Rohlfing, E. Bugna, *Arch. Biochem. Biophys.* **130**, 441 (1969).
14. The exclusion value (10,000) of Bio-Gel P-10 applies rigorously only to spherical, noncharged, nonassociated molecules.
15. α-Aminoisobutyric acid was found in a "modified meteorite" poylmer (using one-fourth the glycine and four times the aspartic acid in the reactants). Materials eluting in the serine-threonine regions (*11*) were common. These amino acids are thermolabile [J. R. Vallentyne, *Geochim. Cosmochim. Acta* **28**, 157 (1964)], and the observed peaks may have been due to other compounds.
16. A. L. Lehninger, *Biochemistry* (Worth, New York, 1970), p. 94.
17. Our reactants included only those amino acids quantified by other investigators and could not include unidentified amino acids, materials removed by fractionation (for example, the inorganic material in extraterrestrial samples), or unidentified precursors of amino acids.
18. M. Saunders and D. L. Rohlfing, Abstract, Annual Meeting of the Southeast Regional Division of the American Chemical Society, Nashville, Tenn., 1971.
19. Our "Sagan" preparation was also of interest in that cystine, first reported by Sagan and Khare (*8*) to be a product of simulated prebiotic syntheses, was incorporated (perhaps as cysteine) into our polymer.
20. The synthesis by Fox and Windsor (*5*) has been discussed by H. R. Hulett, *Science* **174**, 1038 (1971); Y. Wolman, S. Miller, J. Ibanez, J. Oro, *ibid.*, p. 1039; S. W. Fox and C. R. Windsor, *ibid.*, p. 1040.
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24. We thank R. Boan for conducting many of the amino acid analyses; the School of Pharmacy for use of their Infracord; and S. W. Fox, D. L. Claybrook, and J. M. Dean for pertinent discussions. Supported by NASA grant NGR 41-002-030.

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Leukemia, Lymphoma, and Osteosarcoma Induced in the Syrian Golden Hamster by Simian Virus 40

Abstract. *Leukemia, lymphoma, and osteogenic and anaplastic sarcomas develop in Syrian golden hamsters inoculated intravenously at 3 weeks of age with simian virus 40, which is a popova virus. Previously, only RNA and herpes DNA viruses have been recognized as capable of inducing leukemia and lymphoma in mammals. The significance of these findings is emphasized in relation to the nature of viral agents that may be involved in analogous diseases of man.*

Avian and murine leukemia and lymphoma are induced by a number of closely related RNA viruses (*1*). However, oncogenic DNA viruses, except those that belong to the herpes group (*2, 3*), have not been shown to possess leukemogenic or lymphomagenic properties. Various workers, therefore, have proposed that RNA viruses will most likely prove to be etiologically associated with the analogous diseases in man. Since this view is based only on negative evidence (*4*), the question of whether a DNA virus of known oncogenic potential other than a member of the herpes group, could induce, under appropriate conditions, leukemia or lymphoma in the experimental animal was investigated. The weanling rather than the newborn Syrian golden hamster and the known oncogenic agent simian virus 40 (SV40), a DNA virus that belongs to the papova (*5*) group, were used.

In this experiment, 300 weanling male Syrian hamsters, 21 to 22 days old, with an average weight of 44 g (range 35 to 55 g; 83 percent weighed 40 to 50 g) were used. Of these, 250 were inoculated via the femoral vein and 50 were held as uninoculated controls. The animals that were inoculated intravenously were divided into four groups (Table 1). Each of 100 received $10^{8.5}$ median tissue culture infective doses ($TCID_{50}$) of SV40 stock virus, strain VA 45-54 (*6*), suspended in 1 ml of culture medium. Each of 50 animals