Thermal Copolymerization of Amino Acids to a Product Resembling Protein

Attempts to produce a true proteinoid from all of the common amino acids (*1*) by concerted application of information now accumulated (*2, 3*) have yielded such materials. Chromatograms of acid hydrolyzates of the dialyzed products have displayed spots with R_f's of all of the amino acids except tryptophan, which was found in the unhydrolyzed polymer by Hopkins-Cole test. A critical feature of producing such proteinoids is employment of considerable molar excess of dicarboxylic amino acid (*4*).

To prepare the proteinoid, 2.0 g of L-glutamic acid was heated for 1 hr in an oil bath at 170°C, and into this melt was stirred a finely ground mixture of 2.0 g of DL-aspartic acid with 1.0 g of an amino acid mixture used for microbial assay (*5*). The mixture was heated for 3 hr under a blanket of CO_2 in the oil bath at 170°C. After being allowed to cool, the resultant glass was vigorously rubbed with 20 ml of water which converted the product to a granular precipitate. This was allowed to stand overnight and was then filtered and washed with 10 ml of water and 10 ml of ethanol. The solid was next washed by dialysis in a cellophane bag in an agitated water bath for 4 days. Yields, by weight, were usually much in excess of 15 percent. A chromatogram of a hydrolyzed sample of the clear soluble fraction

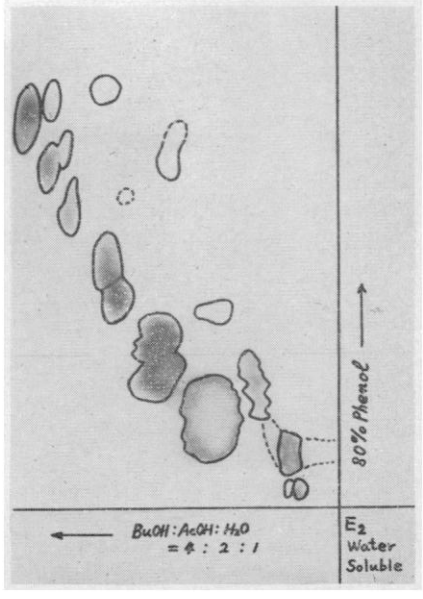

Fig. 1. Chromatogram of hydrolyzed sample of polymer of the common amino acids.

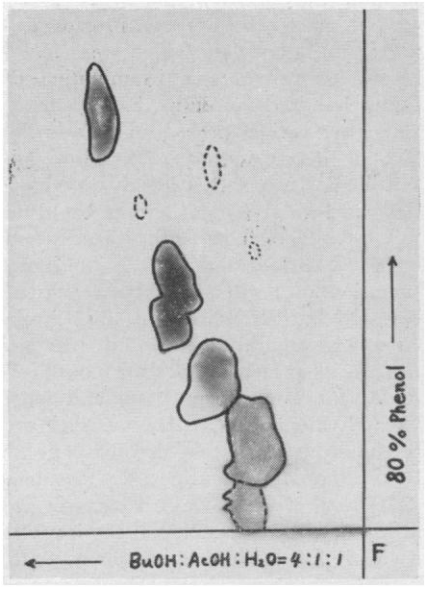

Fig. 2. Chromatogram of hydrolyzate of polymer of aspartic and glutamic acids, glycine, alanine, and leucine.

of nondiffusible proteinoid is shown in Fig. 1. Hydrolyzates of the solid had the same pattern on chromatography. The polypeptide nature of the polymer was substantiated by biuret tests and infrared analysis.

Variations on the synthesis have included replacement of glutamic acid by L-glutamine without preheating, and the added use of 85 percent phosphoric acid (*6*). In each of these, chromatograms similar to that shown in Fig. 1 were obtained.

The recovery of five amino acids, as shown in Fig. 2, from the same five reacted, precludes explanation of the results as bacterial contamination (*7*). The possibility of trapping of amino acids in the solid fraction was eliminated by a negative ninhydrin test and by chromatographic results of the hydrolyzed dialysis residue of the soluble sodium salt of the proteinoid; these chromatograms resembled that shown in Fig. 1. The possibility of difficultly soluble diketopiperazines was similarly eliminated (*8*). An unanswered question, however, is that of the extent to which all of the amino acids are present in each of the peptides of the presumed mixture obtained.

One of the first proteinoids showed a mean chain weight of 4900 and contained 15 percent of glutamic acid, 71 percent of aspartic acid, and 14 percent of the other amino acids by DNP assay (*2*). The N-terminal amino acids were 48 percent of glutamic acid, 13 percent of aspartic acid, and 39 percent of the others; these analyses indicate a nonrandom arrangement of residues. Microbial assay by the Shankman Laboratories of Los Angeles reveals 0.2 to 2.0 percent of each of 13 amino acids, smaller proportions of serine, threonine, and cystine, and large proportions of the dicarboxylic amino acids. Studies of the effects of time, temperature, ratios of reactants, and phosphoric acid have revealed ways of increasing the percentage of basic and neutral amino acids (*9*).

Although these results do not prove that primordial protein was produced thermally, nor prove interpretations of biochemical origins suggested by unexpected results (*10*) from such experiments, the entire outlook appears worthy of more serious consideration because of these findings. It becomes possible to visualize in fuller detail an overlapping origin of anabolic reactions, enzymic protein, and genic nucleic acid (*11*). In the purely preparative realm, the scope for synthesis of peptides, including proteinoids, is enlarged by recognition of the possibilities from copolymerization of amino acids (*12*).

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References and Notes

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2. K. Harada and S. W. Fox, *J. Am. Chem. Soc.* 80, 2694 (1958).
3. G. D. Maier, M.S. thesis, Iowa State College (1956).
4. Probable need for molar excess of either aspartic acid or glutamic acid appeared partly in experiments by one of us (S.W.F.) at the Scripps Institution of Oceanography, University of California, during the summer of 1957. The hospitality of Dr. Denis L. Fox is gratefully acknowledged.
5. K. A. Kuiken, W. H. Norman, C. M. Lyman, F. Hale, L. Blotter, *J. Biol. Chem.* 151, 615 (1943).
6. The technical assistance of Mrs. Donna Keith is acknowledged.
7. Mr. Allen Vegotsky has also carried a methanol-sterilized sample of polymer through to a result like that shown in Fig. 1.
8. We thank Mr. George W. Knight in the Laboratory of Dr. Karl Dittmer for repeating the essential synthesis and analysis from written directions.
9. A detailed description of such studies is in preparation.
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12. This project is supported by grant RG-4666 of the National Institutes of Health, U.S. Public Health Service, grant G-4566 of the National Science Foundation, and by the General Foods Corporation. This report is contribution No. 97 of the Oceanographic Institute, Florida State University.

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