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Synthesis of Peptides from Amino Acids and ATP with Lysine-Rich Proteinoid

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Summary. Lysine-rich proteinoids in aqueous solution catalyze the formation of peptides from free amino acids and ATP. This catalytic activity is not found in acidic proteinoids, even though the latter contain some basic amino acid. The pH optimum for the synthesis is about 11, but is appreciable below 8 and above 13. Temperature data indicate an optimum at 20°C or above, with little increase in rate to 60°C. Pyrophosphate can be used instead of ATP, with lesser yields resulting. The ATP-aided syntheses of peptides in aqueous solution occur with several types of proteinous amino acid.

Key words: Prebiotic synthesis – Proteinoid – Lysine-Rich – ATP – Pyrophosphate – Peptides – Cellular synthesis

Introduction

Various methods for the synthesis of peptides under geological conditions have been reported (Fox and Dose, 1977). The thermal polymerization of amino acids at temperature of 65°C or above (Rohlfing, 1976; Snyder and Fox, 1975) under hypohydrous conditions has been reported at length; many of the products have been extensively characterized. Some of the polymerizations have been conducted in the presence of minerals (Rohlfing and McAlhaney, 1976; Fox and Dose, 1977).

Since cells contain much water and mainly make peptides by using energy from ATP, instead of heat, a model for the synthesis of peptide bonds by a mechanism using ATP in the presence of water has been sought. In the model now identified and reported here, the necessary catalytic agent is lysine-rich proteinoid. This possibility was first indicated in aqueous suspensions of phase-separated particles composed of lysine-rich proteinoid and polyadenylic acid (Fox et al., 1974).

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In other methods in the literature, synthesis of peptides from amino acids in aqueous solution has been recorded with the aid of ATP and free imidazole (Weber et al., 1977) or by the action of ethyl metaphosphate (Mizutani and Ponnamperuma, 1978), which is ungeological. Paecht-Horowitz and A. Katchalsky (1973) claimed formation of peptides of alanine in a mixture of ATP, montmorillonite and a zeolite; that claim remains unconfirmed in the context of negative results reported from another laboratory (Warden et al., 1974). Such synthesis would anyhow not be occurring in the presence of the kind of catalytic polymer that has been characterized as a precellular component (Fox, 1978).

In this paper, we report experiments yielding peptides from free amino acids in aqueous solution with the energetic aid of ATP, as catalyzed by lysine-rich proteinoid.

Experimental

Proteinoids were made by the method usually employed (Fox and Waehneldt, 1968). A typical preparation for lysine-rich proteinoid, such as A, was from 92.4 g of lysine hydrochloride, 7.8 g of glutamic acid, 7.8 g of aspartic acid, and 78.0 g of an equimolar mixture of basic-neutral amino acids common to protein. These were ground in a ball mill, and the mixture was then heated in an oilbath at 190-210°C for 6 h. The product was ground, taken up in water, continuously dialyzed for two or three days and lyophilized. Lysine-rich proteinoid B was made in the same way by a second chemist at another time.

The acidic proteinoids were typically made by heating two parts each by weight of aspartic acid and glutamic acid and one part of an equimolar mixture of the basic-neutral proteinous amino acids. To one mixture of these was added 5% of its weight of histidine, to the other 20%.

Amino Acid Analysis. Polymers were hydrolyzed 24 h with 700 parts of 6N HCl at 105°C. Acidic proteinoids yield amino acids quantitatively upon hydrolysis, but basic proteinoids give recoveries in the range of 20–70% (Fox and Dose, 1977). All compositions (Table 1) are calculated without regard for ammonia; the figures are mole percent of a total amino acid content equal to 100.

The acidic proteinoids contain over 75% of total dicarboxylic amino acid. The greater histidine content of the 2:2:1 proteinoid made from a mixture containing 20% histidine over that from the one having 5% histidine is consistent with earlier observations (Fox et al., 1962).

Synthesis of Peptides by Proteinoid and ATP. The syntheses reported in Table 2 were carried out with 200 mg of ATP trisodium salt, 20 mg of radioactive glycine (100 μC_i), 200 mg of the proteinoid, and 80 mg of $MgCl_2$. These were made up to 1.0 ml in water. Of this 1.0 ml, 50 μl was used in each small tube and incubated as specified, usually at 25°C where temperature is not specified. In some cases, toluene or chloroform was added to prevent wild growth, which was not seen in any case. In later repetitions, all solutions were filtered through presterilized Nalgene filter units (0.2 micron pore size) into glassware sterilized at 130° C for 30 min.

Table 1. Amino acid composition[a] of proteinoids

Amino Acid	Proteinoids				
	2:2:1 (2.6% his)	2:2:1 (6.6% his)	Equimolar	Lysine-richA	Lysine-richB
Ala	3.4	3.4	12.1	7.5	8.8
Arg	0.8	0.6	3.9	2.3	2.3
Asp	62.0	62.4	4.6	5.0	4.9
Cys/2	1.8	0.9	5.2	–	–
Glu	15.2	15.5	10.3	9.6	11.3
Gly	1.6	1.5	8.4	5.8	8.6
His	2.6	6.6	3.3	1.6	2.5
Ile	1.3	1.1	8.4	9.3	5.2
Leu	1.0	1.3	6.0	4.0	3.7
Lys	1.5	1.5	8.4	34.5	34.4
Met	– [b]	1.5	6.8	5.4	4.0
Phe	1.5	1.7	4.6	4.1	6.5
Pro	– [b]	– [b]	2.7	2.4	2.0
Ser	– [b]	– [b]	0.2	0.4	0.1
Thr	4.8	– [b]	0.3	0.2	0.7
Tyr	1.6	1.8	5.2	4.1	2.0
Val	1.4	1.5	9.7	3.8	3.2

[a] Molar ratios, calculated without ammonia
[b] Not included in reaction mixture

Table 2. Relative yields of oligopeptides from glycine, ATP, and proteinoids in solution (7 days, pH 8.0)

Proteinoid	Yield (cpm)
None	< 300
Acidic 2:2:1 (high his)[a]	< 300
Acidic 2:2:1 (high his)[b]	< 300
Equimolar	< 300
Lysine-rich A	7200
Lysine-rich B	9500

[a] 2.6% histidine
[b] 6.6% histidine

The syntheses of Figs. 1–4 were carried out with in situ histidine-rich proteinoid. This was prepared in tubes by heating at 190°C, for 6 h, 4.0 parts by weight of free histidine, 2.0 parts of cystine, 1.0 parts of lysine HCl, and 1.0 part of an equimolar mixture of 18 amino acids (Fox and Waehneldt, 1968). To the tube was then added the amounts of ATP and radioglycine of the previous paragraph. Incubation was performed as indicated for each experiment. The products of Figs. 1–4 were chromatographed on Whatman No. 1 paper with three parts of propanol-1 dissolved in one part of H_2O v/v for 3 days, and the chromatogram examined in a Packard 7201 Radiochromatogram Scanner. The radioactivity was measured for the diglycine and triglycine peaks identified by comparison with standards. The proportional area under each peak was determined by a Spectra-Physics Autolab Minigrator. The diglycine, the major peptide component, was also converted to the dansyl derivative (Gray and Hartley, 1963) and identified by TLC on Polygrams with benzene: acetic acid (4:1 v/v); both authentic dansyl derivative and the experimental product had R_f of 0.19.

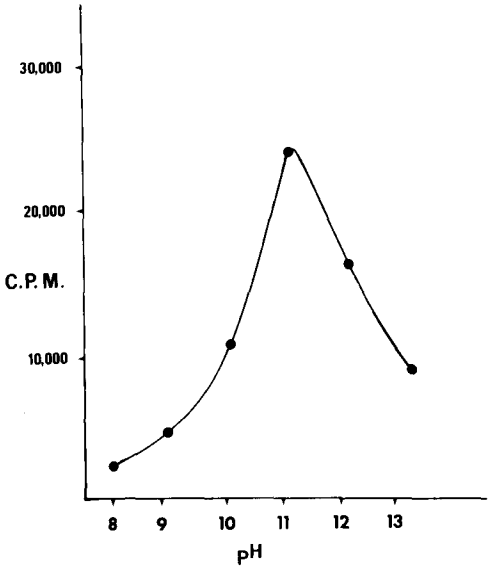

Fig. 1. Synthesis of glycine oligopeptides as a function of pH

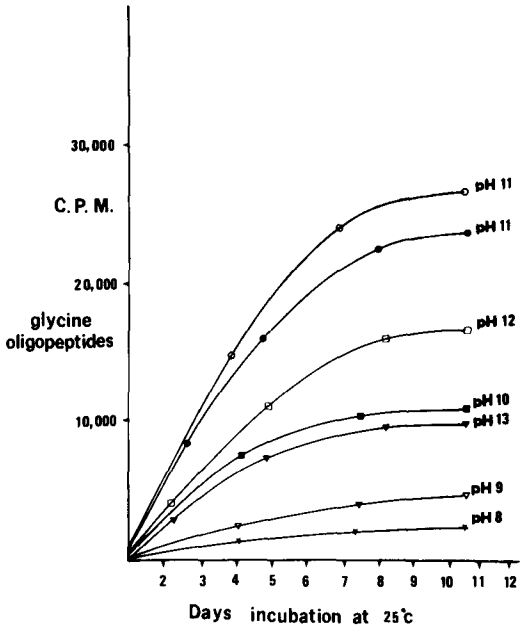

Fig. 2. Time-course of synthesis of oligoglycines at 6 pH values in the range of 8–13

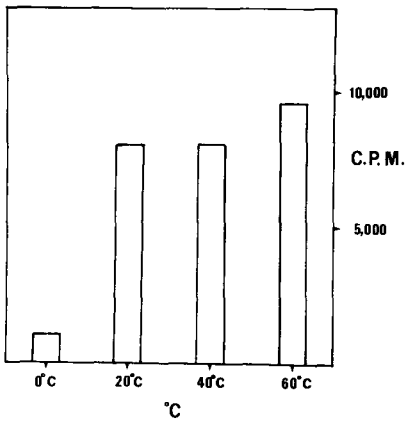

Fig. 3. Effect of temperature of synthesis of oligoglycines by proteinoid and ATP

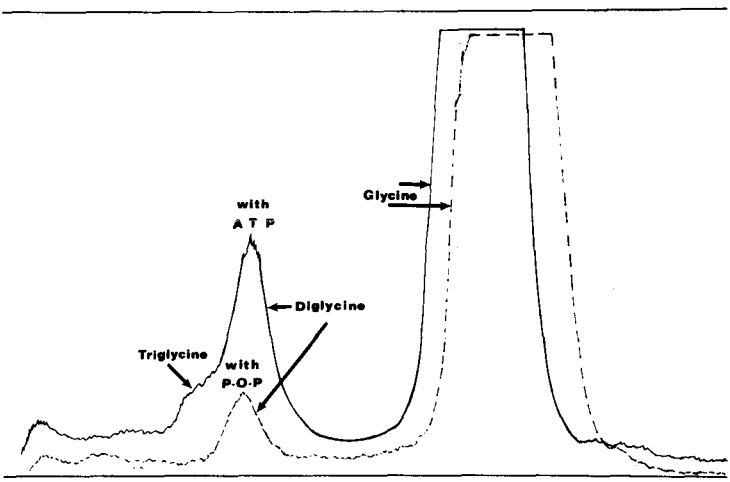

Fig. 4. Effects of pyrophosphate and ATP on the synthesis of oligoglycine by proteinoid and ATP

Results

Analyses of those proteinoids subsequently used in the experiment of Table 2 are presented in Table 1. The preponderance of basic amino acids in the lysine-rich proteinoid, and of dicarboxylic amino acids in the acidic proteinoids, is evident.

In Table 2 is indicated the difference in activity between the more basic proteinoids and the acidic proteinoids for the catalysis of synthesis of peptides from amino acids and ATP at pH 8.0. The equimolar proteinoid, which is essentially neutral, is without activity. The acidic and equimolar proteinoids contain some lysine. When ATP or lysine-rich proteinoid was omitted from the test-tube synthesis, no peptides resulted. Both ATP and a proteinoid dominated by basic amino acid content must, accordingly, be present.

Figure 1 presents a plot of oligoglycine synthesis as a function of pH. The optimum is at 11. The range is broad, extending from below 8 to 13.

The last experiment, and those to be described, were performed with in situ proteinoid, i.e., proteinoid prepared by heating amino acids in the tube, which was then used directly, as a closer modelling of events initially based in geological conditions. In this and other cases, controls were run with the unheated mixture of amino acids. No peptide was formed in control tubes containing only unheated amino acids. The proteinoid polymer is thus essential.

The effect of varying pH, as a function of time, was studied over a period of 12 days (Fig. 2). As in Fig. 1, the optimum is again at pH 11. The two topmost curves give some indication of degree of reproducibility of these experiments, two sets of the pH 11 experiment having been carried out simultaneously. In one of the repetitions of this experiment, the pH was determined both at the beginning and at the end of the experiments. Virtually no change in pH was observed. In Fig. 3, the effect of variation in temperature is seen.

Because of the proposal that pyrophosphate preceded ATP in the evolution of anabolism (Baltscheffsky, 1971) the experiment of Fig. 4 compared equimolar amounts of these two energy-rich phosphates. The ratio of yield from ATP to that from pyrophosphate is 3.7. In other experiments, the yields of peptide from ATP were all about four times as large as from pyrophosphate. The yield from pyrophosphate was however significant.

The yield from this experiment, based on glycine, is 0.40% for diglycine and 0.12% for triglycine. This results at an essentially contemporary physiological temperature of 25° C and in a continuing dilute aqueous medium throughout the experiment. Yields, however, are not relevant to mechanisms of the cellular type (ATP, hydrous conditions) in contrast to those of the geological type (heat, hypohydrous conditions). Cellular processes involve ATP formed and utilized under kinetically controlled conditions at extremely low concentrations (Atkinson, 1977). The larger picture requires a two-step evolution in peptide synthesis from geological matrix to physiological-type synthesis.

Experiments using lysine, phenylalanine, or proline instead of glycine gave peptides of each of those amino acids. The proteinoid-ATP mechanism is thus not restricted to glycine, the first amino acid tested. The results thus suggest that the proteinoid-ATP mechanism is applicable to other amino acids.

Discussion

The tendency of proteinoids rich in lysine to catalyze peptide bond synthesis from amino acids and ATP contrasts with the effects of acidic proteinoid. This preference is consistent with the generally high pH optima of polymerases and with the observation that suspensions of lysine-rich proteinoid-Poly A complexes catalyze the synthesis of peptides from phenylalanine and ATP (Fox et al., 1974).

The kind of result reported provides the essence of an explanation for how an initial thermal copolycondensation of amino acids evolved to a contemporary type of cellular synthesis of polyamino acids, in which energy-rich phosphate is used. The concept of the origin of the first precellular catalytic copolyamino acid has been shown to comport with other aspects of early evolution (Fox, 1978). Those studies also explain how a kind of protein arose when there was no protein to make proteins, a problem

defined by Blum (1955). Although the production of *large* copolyamino acids by a proteinoid-ATP mechanism is yet to be reported, the sequence of a prebiotic geological-type thermal peptide bond sequence followed by a more contemporary physiological-type peptide bond synthesis using ATP is now modelled. It is lysine-rich proteinoid that, either alone, or in complexes with acidic proteinoid, catalyzes the synthesis of internucleotide bonds (Jungck and Fox, 1973). The same complexes subjected to artificial silicification have been found also to resemble microfossils from ancient sediments (Francis et al., 1978).

The observation that the high-energy phosphate is available from pyrophosphate is consistent with the view (Baltscheffsky, 1971) that pyrophosphate played early roles in metabolic evolution, to be largely replaced later by ATP. The origin of pyrophosphate has been explained directly from phosphate (Baltscheffsky, 1971) and through cyanate and apatite (Miller and Parris, 1964).

The possibility for the origin of ATP prebiotically has been discussed (Ryan and Fox, 1973), based on explanation in principle for the origins of adenine (Oró, 1960), ribose (Oró and Cox, 1962), and adenosine (Fuller et al., 1972). More recently, models for the origins of cellular ATP have been provided by photoillumination of ADP and P_i in nonaqueous solution (Fox et al., 1978) which may mimic hydrophobic zones in aqueous suspensions of modern cells or proteinoid microspheres. Of the various ribonucleoside triphosphates, ATP was found to be most effective in synthesis of peptide bonds (Ryan and Fox, 1973).

The origin of acidic and neutral amino acids from cosmic sources has been documented (Fox and Dose, 1977), while the origin of lysine and histidine has been suggested by simulation experiments (Hayatsu et al, 1971; Harada and Fox, 1964; Taube et al, 1967).

The evolution of copolyamino acid synthesis on the primitive Earth can be conceptualized most simply as beginning with a thermal polycondensation followed later by particles containing both basic and acidic proteinoid (Snyder and Fox, 1975; cf. also Rohlfing, 1975). The basic proteinoid, either in mixed proteinoid particles, salted-out basic proteinoid, or in proteinoid-polynucleotide particles, could then catalyze the synthesis of cellular peptides from free amino acids and ATP. This last kind of proteinoid-polynucleotide particle has provided data interpretable in the context of the origin of the genetic code (Nakashima and Fox, 1972; Fox, 1978).

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References

Atkinson, D.E. (1977). Cellular energy metabolism and its regulation. New York: Academic Press

Baltscheffsky, H. (1971). In: Chemical evolution and the origin of life. R. Buvet, C. Ponnamperuma eds., pp. 466–474. Amsterdam: North-Holland

Blum, H.F. (1955). Time's arrow and evolution, p. 170. Princeton: Princeton University Press

Fox, S.W. (1978). In: The nature of life. W.H. Heidcamp ed. Baltimore: University Park Press

Fox, S.W., Dose, K. (1977). Molecular evolution and the origin of life. New York: Dekker

Fox, S.W., Waehneldt, T.V. (1968). Biochim. Biophys. Acta **160**, 246–249

Fox, S.W., Jungck, J.R., Nakashima, T. (1974). Origins Life **5**, 227–237

Fox, S.W., Harada, K., Rohlfing, D.L. (1962). In: Polyamino acids, polypeptides, and proteins. M.A. Stahmann, ed. Madison: University of Wisconsin Press

Fox, S.W., Adachi, T., Stillwell, W., Ishima, Y., Baumann, G. (1978). In: Light transducing membranes. D.W. Deamer ed., pp. 61–75. New York: Academic Press

Francis, S., Margulis, L., Barghoorn, E.S. (1978). Precambr. Res. **6**, 65–100

Fuller, W.D., Sanchez, R.A., Orgel, L.E. (1972). J. Mol. Biol. **67**, 25–33

Gray, W.R., Hartley, B.S. (1963). Biochem. J. **89**, 59P

Harada, K., Fox, S.W. (1964). Nature **201**, 335–336

Hayatsu, R., Studier, M.H., Anders, E. (1971). Geochim. Cosmochim. Acta **39**, 935–951

Jungck, J.R., Fox, S.W. (1973). Naturwissenschaften **60**, 425–427

Miller, S.L., Parris, M. (1964). Nature **204**, 1248–1250

Mizutani, H., Ponnamperuma, C. (1978). In: Origin of life. H. Noda ed., pp. 273–277. Tokyo: Japan Sc. Soc. Press

Nakashima, T., Fox, S.W. (1972). Proc. Nat. Acad. Sci. U.S. **69**, 106–108

Oró, J. (1960). Biochem. Biophys. Res. Commun. **2**, 407–412

Oró, J., Cox, A.C. (1962). Federation Proc. **21**, 80

Paecht-Horowitz, M., Katchalsky, A. (1973). J. Mol. Evol. **2**, 91–98

Rohlfing, D.L. (1975). Origins Life **6**, 203–209

Rohlfing, D.L. (1976). Science **193**, 68–70

Rohlfing, D.L., McAlhaney, W.W. (1976). BioSystems **8**, 139–145

Ryan, J.W., Fox, S.W. (1973). BioSystems **5**, 115–118

Snyder, W.D., Fox, S.W. (1975). BioSystems **7**, 222–229

Taube, M., Zdrojewski, Z., Samochocka, K., Jezierska, K. (1967). Angew. Chem. **79**, 239

Warden, J.T., McCullough, J.J., Lemmon, R.M., Calvin, M. (1974). J. Mol. Evol. **4**, 189–194

Weber, A.L., Caroon, J.M., Warden, J.T., Lemmon, R.M., Calvin, M. (1977). BioSystems **8**, 277–286

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