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FORMATION OF PEPTIDES FROM AMINO ACIDS BY SINGLE OR MULTIPLE ADDITIONS OF ATP TO SUSPENSIONS OF NUCLEOPROTEINOID MICROPARTICLES

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When lysine-rich proteinoid, which catalyzes the formation of peptides from amino acids and ATP, is complexed with acidic proteinoid to form microspheres of mixed constitution, the normal synthesis by basic proteinoid alone is multiplied several-fold. The product consists not only of small peptides but also of a high-molecular-weight fraction of substituted proteinoid.

Suspensions of particles of lysine-rich proteinoid complexed with polyadenylic acid catalyze the synthesis of peptides from each of the amino acids tested with ATP. When equimolar solutions of mixtures of glycine and phenylalanine with ATP are tested in suspensions of complexes of lysine-rich proteinoid and each of various polyribonucleotides, both homopeptides and heteropeptides are produced. Glycylphenylalanine or phenylalanylglycine is the principal product; the preference is related to which polyribonucleotide is in the complex.

The rate of conversion of amino acid to peptide is a function of whether ATP is added in a single batch or in repeated amounts adding to the same amount as in the single batch. Related experiments indicate a relatively rapid initial rate of decay of ATP in this system. These results are discussed relative to the mechanisms for continuous generation in modern organisms, as are the results in peptide formation.

Introduction

A plausible model for the origin of geological synthesis of polypeptides involves the warming or heating of amino acids under hypohydrous conditions (Rohlfing, 1976; Fox, 1978). Both ribosomal and aribosomal synthesis of polypeptides in modern cells, however, occurs with the aid of ATP (Lee and Lipmann, 1974; Lipmann 1974). A proposed evolutionary link between these two is lysine-rich proteinoid, which catalyzes peptide synthesis from amino acids and ATP (Nakashima and Fox, 1980). Pyrophosphate can function as high-energy phosphate instead of ATP (Nakashima and Fox, 1980), in accord with the reasoning (Baltscheffsky, 1971) that the inorganic compound preceded ATP in evolution.

Some earlier experiments had suggested that peptides can be made in catalyses by lysine-rich proteinoid when the proteinoid is complexed with homopolyribonucleotide (Nakashima and Fox, 1972; Fox et al., 1974). In this paper we report new experiments of this sort with individual amino acids and with a pair of amino acids, glycine and phenylalanine.

Experiments employing ATP approach more closely a cellular type of peptide synthesis than do others, such as the thermal syntheses (Fox and Harada, 1960). The thermal syntheses are conducted in the relative absence of water (hypohydrously), and yield very much higher yields than do those described here. The syntheses reported here, however, occur in the presence of water, with energy from ATP instead of heat. As in the cellular syntheses, which are also of relatively low "yield", identification and evaluation of yield of peptides require radioactively labelled amino acids for monitoring the syntheses.

Problems in the evolution of protocellular

metabolism thus require design and interpretation of experiments in a mode of biological chemistry more than in one of traditional organic chemistry with its emphasis on yields in batch synthesis. Conversions have a somewhat different significance for biochemical reactions, especially those reactions involving unstable high-energy phosphates. ATP participates in organisms (Atkinson, 1977) by its continuous generation; models of modern cellular protein synthesis employ *ATP-generating* systems (Nirenberg, 1963). Experimental treatment of the questions raised by these considerations in transitionally evolving cellular systems are reported in this paper.

Materials and methods

Acidic proteinoid

A mixture of amino acids (30 g alanine, 69 g arginine HCl, 1600 g aspartic acid 79 g cystine, 25 g glycine, 57 g histidine HCl, 1600 g glutamic acid, 43 g isoleucine, 43 g leucine, 60 g lysine HCl, 49 g methionine, 54 g phenylalanine, 38 g proline, 35 g serine, 59 g tyrosine, and 39 g valine) was pulverized in a ball mill. One-fourth of this was heated under a stream of nitrogen for 8 h in an oilbath at 195°C.

The product was taken up in water, and dialyzed in a stirred pipette washer with constant water flow for 3 days. The material in the dialysis sacs was brought to pH 6.25 with NaOH, and filtered. The soluble fraction was lyophilized, and labelled 2 : 2 : 1 proteinoid SF.

Lysine-rich proteinoid

A mixture of 50 g of lysine HCl and 50 g of an equimolar mixture of all other common proteinous amino acids was pulverized in a ball mill and heated in an oilbath at 200°C for 6 h under nitrogen.

After cooling, the product was taken up in 500 ml of water and dialyzed over 48 h with four changes of 10 l water each.

Formation of oligoglycine from acidic proteinoid and lysine-rich proteinoid

Acidic proteinoid, 90 mg, was dissolved in 2.0 ml of Tris-HCl buffer (pH 7.2). Lysine-rich proteinoid (60 mg) was dissolved in 4.0 ml of buffer. Each solution was returned to pH 7.2 by addition of 1 N NaOH. An 18-mg amount of acidic proteinoid and 6.0 mg of lysine-rich proteinoid, each in solution, were mixed. Complex microspheres separated. To this suspension was added 0.50 mg ATP tripotassium salt from a solution of 5.0 mg ATP potassium salt in 1.0 ml of 0.05 M Tris buffer (pH 7.2), 0.30 mg of glycine from a 2 M solution, and all was made to 1.0 ml. Of this solution, 5.0 μl was added promptly to dissolve 2.5 μCi [^{14}C]glycine and 1 μl of $CHCl_3$. From control tubes were omitted: (a) lysine-rich proteinoid; (b) acidic proteinoid; and (c) both lysine-rich and acidic proteinoid (LRP-AP). The four tubes were incubated at 25°C for 5 days.

To each tube was then added 30 μl of 28% NH_4OH. From each, 10 μl was applied to a strip of Whatman No. 1 paper. Chromatograms were developed with propanol-1/H_2O (3 : 1 v/v) for 3 days and then dried in air overnight. The radioactivity was counted on a Packard Radiochromatogram scanner connected to a Spectra-Physics Autolab Minigrator. The results are in Table 2.

Digestibility of phenylalanine-enriched proteinoid formed from proteinoid, ATP, and phenylalanine

In another synthesis using phenylalanine (selected for the digestibility of many of its derivatives by proteases), the incubation mixture contained 18 μg acidic proteinoid, 6 μg lysine-rich proteinoid, 0.5 μg ATP potassium salt, and 0.3 μg [^{14}C]phenylalanine (1.0 μCi), 1.0 μl of 0.05 M Tris buffer (pH 7.2) and the solution was saturated with

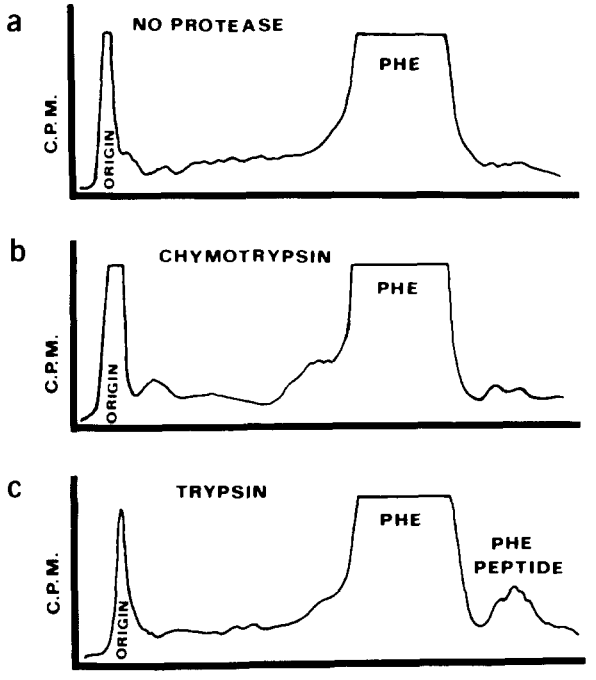

Fig. 1. Effects of proteases on chromatographic composition resulting from polymerization of phe with ATP in suspension of acidic: basic proteinoid microspheres. (a) Shows control; (b) shows small amount of oligopeptide released by chymotrypsin from origin fraction of (a); and (c) shows substantial release by trypsin.

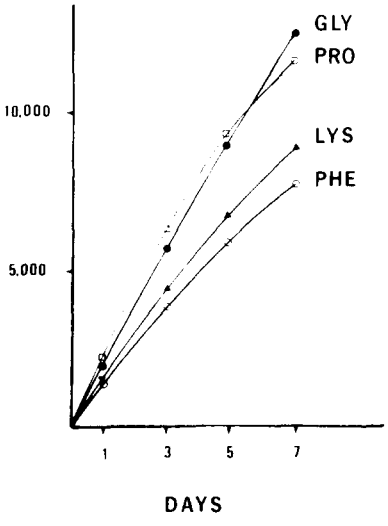

Fig. 2. Time-course of formation of oligopeptides from ATP and gly, lys, phe, or pro under standard conditions in suspension of lysine-rich proteinoid/poly(A) particles.

chloroform (single drop). After incubation for 72 h at 25°C, 1.0 μl was mixed with 5.0 μl of 0.05 M Tris—HCl buffer (pH 7.2) containing 10 μg of chymotrypsin or trypsin. The solution containing chymotrypsin was 0.2 M in $CaCl_2$, that containing trypsin was 0.001 M in $CaCl_2$. After incubation at 25°C for 24 h, samples were chromatographed. The results are in Fig. 1. Readings in Figs. 1a, 1b, and 1c were each made on the Packard Chromatogram Scanner with linear range set at 1×10^3.

Formation of oligopeptides of each of four amino acids in suspensions of microparticles composed of lysine-rich proteinoid and homopolyribonucleotide

To prepare nucleoproteinoid microparticles in suspension, 0.4 ml of Tris buffer (pH 7.2) containing 3 mg of poly(A) (Sigma) was mixed with 0.6 ml of buffer containing 6 mg of lysine-rich proteinoid. To this was added 0.5 mg of ATP dissolved in 0.1 ml.

Also added was 10 μCi of individual ^{14}C-labelled amino acid in 10 μl of buffer saturated with chloroform. In comparative experiments of this sort at 25°C (Fig. 2) 2.0-μl samples were removed at 1, 3, 5, and 7 days. These were chromatographed on strips of Whatman No. 1 paper with propanol-1/H_2O (3 : 1), except in the case of lysine peptides, which were chromatographed on Whatman No. P81 with 0.4 M potassium acetate (pH 9.7). Counting was done as described. In all studies, radioactive amino acids were purified just before the experiments, by paper chromatography and elution.

Experiment in decay of ATP

Microspheres formed by mixing 0.4 ml of Tris—HCl buffer (0.05 M, pH 7.2) containing 18 mg acidic 2 : 2 : 1-proteinoid (SF) with 0.6 ml of the same buffer containing basic

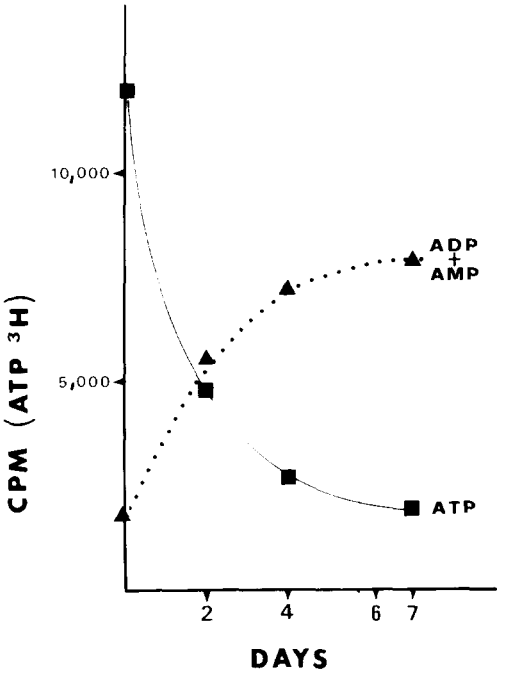

Fig. 3. Decay of ATP during reaction of glycine in presence of suspension of LRP-AP microparticles.

proteinoid. To this was added 0.5 mg of ATP in 0.1 ml of buffer and 0.3 mg glycine in 0.1 ml. The ATP contained 10 μCi of [^{3}H]ATP and 2 μl of chloroform. Samples were taken at 0, 2, 4, and 7 days.

The products were chromatographed, 1 μl each on Eastman 6064 cellulose chromatogram sheets with isobutyric acid/2 N NH_4OH (2 : 1 v/v). The strips were counted as described (Fig. 3).

TABLE 1

Single and multiple treatment with ATP

Control	Single	Multiple
Tube as described in text	Tube as described in text	Tube as described in text
Nothing added	2.0 mg ATP K_3 salt added at once	0.5 mg ATP K_3 salt added at: 3 days, 5 days, 7 days.
		Total 2.0 mg

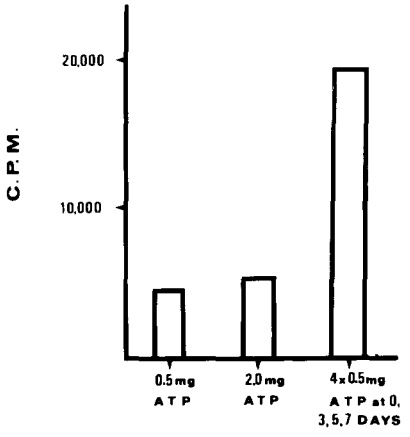

Fig. 4. Comparative effects of single charge of ATP in peptide syntheses with that of divided addition of same amount.

The samples were made up to include in each tube 1.0 ml composed of 18 mg of (acidic) 2 : 2 : 1-proteinoid in 0.4 ml of Tris—HCl buffer (0.05 M, pH 7.2), 6.0 mg of (basic) lysine-rich proteinoid in 0.4 ml of the same buffer, and 0.3 mg of glycine containing 250 μCi of [^{14}C]glycine. To each was added 50 μl chloroform. Various tubes were treated as in Table 1. Results are graphed in Fig. 4.

Identification of peptides formed

For the formation of dipeptides from mixed amino acids, nucleoproteinoid microparticles, prepared as described, were treated with mixtures of glycine and phenylalanine. Each tube contained 3.0 mg of polynucleotide, 6.0 mg of lysine-rich proteinoid, 0.5 mg of ATP potassium salt, 0.50 mg of phenylalanine having 5 μCi, and 0.30 mg glycine having 5 μCi in 1.0 ml of Tris buffer (pH 7.2) (0.05 M), and chloroform. The tubes were incubated 6 days at 25°C and chromatographed on Whatman No. 1 paper with propanol-1/H_2O (6 : 1). Spots were com-

pared by R_F with authentic gly-gly, gly-phe, phe-gly, and phe-phe.

Identity of phe-gly and gly-phe formed was confirmed by elution with 5% acetic acid, drying in a tube, and dansylation. To each tube was added 25 μl of triethylamine carbonate solution (pH 8.0) and 25 μl of dansyl chloride in a 1% acetone solution. The tube was incubated overnight, dried in a desiccator, and its contents hydrolyzed with 100 μl of 6 N HCl at 90°C for 12 h. After drying, the contents were extracted with 50 μl of ethyl acetate twice and chromatographed on thin-layer Eastman silicagel chromagrams (13179 without fluorescent indicator No. 6061) using chloroform/benzyl alcohol/acetic acid (50 : 50 : 3). The dansyl-phe and dansyl-gly were compared with standards.

The identity of gly-gly formed was confirmed also through high voltage electrophoresis, through hydrolysis to glycine only, by study of the kinetics which showed the synthesis to be second-order, through chromatography of the dansylated gly-gly, and through hydrolysis of the dansylglygly to dansylgly + gly.

Freedom from contamination was shown also by the absence of product when any one component was omitted from the reaction and by check runs carried out through sterilized Nalgene filters.

Results

Table 2 shows the effects of the acidic and lysine-rich proteinoids on the formation of oligoglycines. A measurable amount of small peptide is not indicated for acidic proteinoid alone but the material at the origin is definitely above background. In each region of the chromatogram the amount is greater with basic proteinoid than with acidic proteinoid. The largest yield is observed when equal amounts of both acidic and basic proteinoids are together in the aqueous medium, in which they are then

TABLE 2

Effects of acidic, lysinerich and mixtures of these proteinoids on oligoglycine formation (5 days at pH 7.2).

Proteinoid	CPM at origin	CPM dipeptide and tripeptide
None	5500	<1000
Acidic proteinoid	10500	<1000
Basic proteinoid	14000	3000
Acidic/basic proteinoid (microspheres)	35000	11000

however present as phase-separated microparticles (Fox, 1976).

Figure 1 presents evidence that a large part of the high-molecular-weight product remaining at the origin is peptide-bound material.

The sensitivity of the machine for this comparison was set at a minimum, in contrast to the earlier report of formation of phe peptides (Fox et al., 1974), in which maximal sensitivity was employed. Figure 1a displays the control without any enzyme, the small peptide peak being minimal as explained. Figure 1b illustrates only a very small release of peptide when chymotrypsin was allowed to react. In Fig. 1c, in which trypsin had acted on the high-molecular-weight fraction, the oligophe peak is substantial. This should be compared with the size of the origin peak, not the phe peak. Together, Figs 1a—1c and the earlier result (Fox et al., 1974) indicate that the synthesis resulting in a component of the "origin" fraction is major.

Figure 2 shows that amino acids are each converted to peptides by poly(A)/LRP particles in suspension, at rates that differ in minor degree for the various amino acids.

In Table 3, the catalytic effect of imidazole is compared with those of LRP-AP suspension and of nucleoproteinoid microparticles. This comparison is judged in the dipeptide-tripeptide fraction only. As already indicated, the fraction of substituted proteinoid found

TABLE 3

Analysis of small peptides synthesized

Catalytic agent	Homopeptide in total oligopeptide	Heteropeptide in total oligopeptide	Dansyl gly/ heteropeptide fraction	Dansyl phe/ heteropeptide fraction
Imidazole	75%	25%		
Acidic-basic ptd microspheres	32	68		
Poly(A)-basic ptd particles	30	68	82[a]	18
Poly(G)-basic ptd particles	42		—?	—?
Poly(C)-basic ptd particles	37	58	24	76[a]
Poly(U)-basic ptd particles		64	32	68[a]

[a]Major product.

at the origin of the original chromatogram is substantial but has not been fully analyzed.

The effect of imidazole on glycine and phenylalanine is the production predominantly of the homopeptides glycylglycine and diphenylalanine with only 25% of a mixed heteropeptide fraction. This is in contrast to the other syntheses catalyzed by LRP in association either with AP or with polynucleotide. In the latter cases the heteropeptides are the main product and the homopeptides represent a minor fraction.

None of the results are all-or-none but the preferences appear to be significant. With the LRP-AP microspheres, the favoring of one sequence over the other in the heteropeptide fraction is not so pronounced as in the nucleoproteinoid suspensions. The results with the LRP-AP system suggest a moderately larger production of phe-gly than of gly-phe (about 60 : 40). The results with nucleoproteinoid particles are unequally divided.

These particles indicate a decided preference for synthesis of the gly-phe type when the polynucleotide is poly(A). With poly(C) or poly(U), the main product in the heteropeptide fraction is the obverse one of phe-gly. The result with poly(G) is not clear. Success was not attained in separating dansyl derivatives from poly(G), although the presence of dansylheteropeptides was clearly indicated.

As Fig. 3 indicates, ATP decays rapidly in the peptide synthesis reaction. This rapid decay is consonant with the rapid initial synthesis of phenylalanine peptides under comparable conditions (Fox et al., 1974).

Figure 4 shows that, if the ATP is supplied in repeated small fractions, the conversion is more complete than if the same total amount is supplied at once. Since the glycine is present in considerable molar excess over the ATP, the most likely explanation for the higher conversion for repeated reaction of ATP is that it is more efficiently used, due to rapid decay of any lot of ATP (Fig. 3).

Except for poly(G), in which the characterization of peptides is incomplete, the "yield" of small peptides is in each case (Table 3) 0.1—0.2%. Again, however, the emphasis in these experiments has been kinetic, i.e. on relative rates of conversion. In this respect, the molar proportions of glycine have been several times those of the ATP used, whereas the inverse would have been chosen had size of yield been a meaningful objective. Experiments designed for that objective should take into account peptide recoverable from the "origin" fraction, and also the fact that substantial proportions of diketopiperazine are in some cases found in independent fractions (Syren and Fox, 1981, unpublished).

When the experiment of Fig. 4 was repeated under aseptic conditions by use of sterilized Nalgene filters, the results were essentially the same.

Discussion

The first evidence that proteinoid rich in basic amino acid catalyzes the synthesis of peptides from amino acids and ATP was obtained in suspensions of complexes of lysine-rich proteinoid with poly(A) (Fox et al., 1974). Earlier suggestions of such reactions emerged from selective incorporation of preformed aminoacyl adenylates into suspensions of microparticles comprising lysine-rich proteinoid and polyhomoribonucleotide (Nakashima and Fox, 1972), and from formation of glycylhydroxamate at the surface of acidic proteinoid/lysine-rich proteinoid particles in suspension when glycine, hydroxylamine, ATP, and $MgCl_2$ were allowed to react (Ryan and Fox, 1973). In a related study, proteinoid catalyzed the condensation of preformed aminoacyl adenylates (Krampitz and Fox, 1969). The reaction to form peptides from amino acids has been recently reported in a detailed exploration of lysine-rich proteinoid in aqueous solution (Nakashima and Fox, 1980). The proteinoid is thus active in either: (a) solution; (b) suspension of mixed proteinoid particles; or (c) in a suspension of nucleoproteinoid microparticles (Fox and Nakashima, 1980).

Although lysine-rich proteinoid has received the most attention in some of these experiments, the use of proteinoids sufficiently rich in any basic amino acid is probably the common denominator of compositional requirement for catalytic activity. In addition, while acidic proteinoids moderately rich in basic amino acids are inactive in producing small peptides (as in Table 2), more basic polymers rich in either histidine or lysine (Nakashima and Fox, 1980) have been found to be active. These are being compared with polymers rich in arginine (Syren and Fox, 1980, unpublished).

Similar studies of basic amino acids in esterolytically and phosphatatically active proteinoids were carried out earlier (Rohlfing and Fox, 1967; Oshima, 1968); histidine content was significant in those.

Of related interest is the fact that lysine-rich proteinoid has been shown to catalyze also the formation of internucleotide bonds (Jungck and Fox, 1973; Fox et al., 1974). The ability to catalyze the formation of *both* peptide and internucleotide bonds has led to an explanation for the origin of coordinated templated synthesis of proteins with nucleic acids in protocells (Fox, 1981a). According to this concept, the protocells arose from self-ordered proteinoid. The self-ordered proteinoid constituted an answer to the classical chicken-egg question (Fox, 1978), and also explained adequate individual catalytic activities by providing multiple copies of each active macromolecule.

The contribution to peptide bond synthesis of lysine-containing proteinoids is consistent with the knowledge that transpeptidation by ribosomes depends upon groups having a pK_a of 7.5—8.0 (imidazole or N-terminal α-amino) and perhaps one of pK_a 9.4 due to ϵ-amino of lysine (Harris and Pestka, 1977).

The inactivity of acidic proteinoid in solution of pH 7.2 (Table 2) is in contrast to the activity of the lysine-rich proteinoid. When both of these polymers are present in the same concentration in which they were tested individually, they form phase-separated microparticles, as has long been known (Fox, 1976). Such mixed microspheres are a closer model for modern cells (Snyder and Fox, 1976), or for ribosomes, which contain both acidic and basic proteins, the latter mainly (Spirin and Gavrilova, 1969). Notably, the activity of the complex is several times as large as that of lysine-rich proteinoid alone at its same concentration (Table 2). The question of whether the acidic proteinoid potentiates the active structure of lysine-rich proteinoid has been examined without obtaining an answer. Physical surface effects may be responsible.

The total mechanism is somewhat more complex than that involving simply synthesis

of small peptides, as is inferred from the incorporation into the fraction at the origin of the chromatograms (Fig. 1). For this latter fraction, which represents most of the high-molecular-weight radioactivity, lysine-rich proteinoid is more catalytic than acidic proteinoid, and the combination of the two is several times as active. This combination is also more effective for formation of small peptides, as earlier stated.

Preliminary examination of the "origin fraction" (Fig. 1) suggests that it has several components. Logically, this fraction consists partly of radioactive oligoglycine combined with proteinoid. Some evidence for such a component of this fraction is found in Fig. 1. In related work in which proteinoid catalyzed the homocondensation of aminoacyl adenylates (Krampitz and Fox, 1969), enlarged proteinoid molecules resulted.

This kind of result is germane to macromolecular replication, which is often, but not always, assumed to have been template-directed by nucleic acids (cf. Eigen and Schuster, 1979). The results reported in this paper permit visualizing more clearly the "feasibility of protein-directed synthesis of proteins without nucleic acids" (Dillon, 1978) ab initio, and in early evolutionary stages of protein synthesis (Fox, 1981b).

Integrated examination of the results of Fig. 1 and Table 3 brings out that synthesis of peptides from single amino acids is substantial for each of the amino acids, but a dominant amount of heterodipeptide is found when two amino acids are allowed to react under some conditions. Further studies may examine the relationship to self-ordering of amino acids in simpler systems, in which mixtures of only amino acids were heated (Fox, 1978). In the present studies, the sequence is a function of the polynucleotide within the system. The directing agent is thus inferred to be the polynucleotide-polyamino acid complex *and* the reactant amino acids (cf. Fox et al., 1953; Lipmann, 1974).

Such observations are related to the views of others that self-ordering of amino acids has been significant at several stages of evolution (Calvin, 1969; Dillon, 1978). The authors cited proposed the relicts of primordial self-ordering in modern systems. This suggestion has been attacked by Jukes and Margulis (1980), and defended by Fox (1980a).

The ease with which dipeptides accumulate in these experiments is reminiscent of the fact that ribosomal activity is often monitored by assaying dipeptides (Moldave and Grossman, 1974). The K^+ that Pestka (1968) finds necessary is present in our experiments as the salt of ATP.

The finding that lysine-rich proteinoid can catalyze the synthesis of peptides from amino acids in the presence of ATP and much water provides an understanding of how geological synthesis of peptides in the relative absence of water evolved easily to a protocellular synthesis of peptide bonds (Fox and Nakashima, 1980). The formation of sufficient lysine-rich proteinoid and a protocell incorporating that catalyst would have been necessary. Another difference in requirement is that some thermal syntheses need trifunctional amino acid; this does not apply to proteinoid-catalyzed syntheses (Fig. 2 and Table 3).

Although the peptides that have been most studied are small, the suggestion has been made that small peptides and small oligonucleotides were all that was necessary early in evolution (Carlin, 1980; White, 1980). Whether the results of Table 3 are relatable to the modern genetic code is not clear. Reason to believe that there were other genetic codes than the "universal" one are at hand (Fox, 1974, 1978; Jungck, 1978; Barrell et al., 1979). The results do display a parallelism in reaction for the two pyrimidine polynucleotides. This parallelism is to that observed earlier in static interactions of arginine-rich and lysine-rich proteinoids with polynucleotides (Yuki and Fox, 1969). This parallelism suggests for

the dipeptides a synthetic mechanism in which one kind of amino acid is bound by the polynucleotide while the other amino acid is reacted with the bound one.

The origin, characterization, and significance of thermal proteinoids has been described in detail (Fox and Dose, 1977); the plausibility of their occurrence on the primitive Earth has been explained (Rohlfing, 1976). The origin of modern large polynucleotides has not been explained; however, the formation of the internucleotide bond has been shown, both in solution of lysine-rich proteinoid and in suspension of acidic/lysine-rich proteinoid microparticles (Jungck and Fox, 1973). Polynucleotides of significant activity may thus have first appeared in an early evolving cell (Ehrensvärd, 1962) rather than in the geological realm. Explanations for the origin of ATP have been given (Ryan and Fox, 1973).

In exhibiting the ability to synthesize peptide bonds and to influence amino acid sequence, the particles, which have earlier been referred to as model protoribosomes (Waehneldt and Fox, 1968; Lehninger, 1975) are showing what appears to be the primordia of ribosomal function, i.e. peptidyl transferase and translocase activities (Spirin and Gavrilova, 1969). Indeed, the constructionistic approach (Fox, 1977) represented by these studies and the partial reconstitution of ribosomes (Spirin, 1976; Nomura, 1973) are supplementary types of study.

In addition to the ability of proteinoid microspheres to synthesize peptides, some of them have been reported earlier to possess the ability to synthesize ATP photochemically (Fox et al., 1978, 1980), and to have other kinds of metabolic activity (Fox, 1980b). More recently, they have been found to have photoelectrical activity (Przybylski and Fox, 1981, oral communication at meeting of Society for Neuroscience, Los Angeles, October, 1981).

The visualization of evolution of protein synthesis as a two-stage process (Fox, 1981b) leads to a definition of that aspect of evolution as one of simultaneous coded nucleic acid-and-protein synthesis having arisen from a non-templated self-ordered proteinoid genetic mechanism. Such a picture is an enlargement of the answer to a chicken-egg problem (Fox, 1978). Strictly speaking, a genetic mechanism evolved to a coded genetic mechanism or, in other words, a non-templated mechanism evolved to a templated mechanism (Fox, 1981b).

The experiments showing the rapid decay of ATP in such systems (Fig. 3) and the higher conversion to peptides when repeated smaller charges were used (Fig. 4) brings the model closer to the mechanism operative in modern cells. Continuous generation of the unstable highly reactive ATP characterizes modern cells, as stated in the Introduction. The synthesis reported here is thus energized by ATP, it is operative in the presence of water, and the ATP is most effective when supplied continuously. The system thus represents an organizational unit that is ready for the evolution of associated biochemical pathways.

The glycine is in several-fold molar excess over ATP. The benefit of repeated doses of ATP would theoretically not be expected if all reaction components were in aqueous solution. The peptide bond synthesis is however occurring at the surface of catalytic solids; conventional solution kinetics do not apply. The use of fresh charges is similar to continuous generation and use of ATP in modern organisms.

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