EFFECT OF POLYNUCLEOTIDES AND A BASIC PROTEIN ON THE CONDENSATION OF PHENYLALANYL ADENYLATE

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Abstract. The effect of polyuridylate and of histone on the oligomerization of phenylalanyl adenylate was tested. Polyuridylate which pairs with adenine under suitable conditions showed no effect. Histone increased the rate of the polymerization whereas a neutral protein, albumin, had no effect. Simultaneous presence of nucleotides diminished the effect of histone. The implication of these results on the prebiotic polypeptide formation is discussed.

1. Introduction

Amino acyl adenylate spontaneously forms polypeptides in neutral and basic aqueous solutions (Paecht–Horowitz and Katchalsky, 1967). Selective acceleration of amino acyl adenylate condensation by lysine-rich proteinoid microparticles has been observed by Nakashima and Fox (1972). Adenosine (5×10^{-3} *M*) forms a complex with poly(U) (1.5×10^{-2} *M*) in 0.02*M* $MgCl_2$ plus 0.1*M* NaCl aqueous solution at 5 °C (Huang and Tso, 1966). Sulston *et al.* (1968a, b) reported nonenzymatic synthesis of oligoadenylate on poly(U) template. Activation of amino acids by nucleotides and the subsequent formation of polypeptides from the activated amino acids have been proposed as the first step in the evolution of the protein synthesis mechanism (Ishigami and Nagano, 1975; Ishigami *et al.*, 1977). The effect of the addition of polynucleotides and proteins on the peptide formation is reported in the present paper.

2. Materials and Methods

2.1. MATERIALS

[^{3}H]-labeled-L-phenylalanine (Phe) was purchased from the Radiochemical Center Amersham. Sodium salt of polyuridylate (poly(U), average molecular weight of 2×10^6) and of polycytidylate (poly(C), average molecular weight of 7.9×10^5) were obtained from Yamasa Shoyu Co. Ltd. *N,N'*-Dicyclohexylcarbodiimide was purchased from Nakarai Chemicals Co. Ltd and used as a condensing agent for the preparation of phenylalanyl adenylate. Histone (Type II, from calf thymus) and albumin (bovine serum albumin, fraction V) were purchased from Sigma Chemical Co.

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2.2. PREPARATION OF PHENYLALANYL ADENYLATE (Phe–pA)

Phe–pA was prepared by the method of Berg (1958). About 40% of the added adenylate was recovered as the phenylalanyl derivative as calculated from the absorption at 260 nm. After a purification through a Dowex 1 column (1.2 cm x 10 cm, H^+ form), Phe–pA fraction was divided into 100 μl portions and kept at −80 °C. At this temperature Phe–pA in an acid aqueous solution (pH 2.5) did not significantly decompose for several weeks.

2.3. RECORDING OF THE REACTION RATE BY AUTOMATIC TITRATION SYSTEM

Phe–pA in aqueous solution produces a variety of phenylalanine oligomers (containing n residues: Phe_n ($n \geqslant 1$)). In this reaction, one Phe–pA molecule gives two protons; therefore, we monitored the overall reaction by titrating protons with 0.1N sodium hydroxide solution, using an automatic titrator Model TTT2, autoburette Model ABU12 (Radiometer Co. Ltd, Copenhagen). In preliminary experiments, it was found that dissolved carbon dioxide did not interfere with the measurement under our experimental conditions. Reaction vessel is shown in Figure 1. A magnetic stirrer helps the sodium hydroxide solution to diffuse rapidly after it was injected into the reaction vessel: otherwise, Phe_n adhered on the outlet of the alkaline solution. In order to minimize uncertainty in the interpretation of results, each set of measurements was performed with the same pH electrode under the same conditions. A complex electrode, 5 mm ϕ, Model 39030, Beckman Co. Ltd was used for the experiments with histone and with albumin (Figure 3). A similar electrode, 8 mm ϕ, Model 6028–10T, Horiba Co. Ltd was used for other experiments. In the latter case, the electrode was vibrated to stir the solution to avoid the effect of the viscosity difference among different sets of experiments.

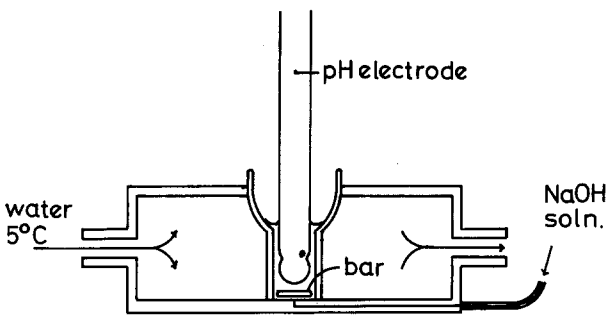

Fig. 1. Schematic representation of the reaction vessel with a pH electrode and a hole at the bottom to supply NaOH solution from automatic titrating device. Reaction mixture (0.6 ml ~ 1.2 ml) was stirred by a small magnetic bar at the orifice of the hole and kept at 5 °C by circulation of cold water.

2.4. ANALYSIS OF PRODUCTS BY PAPER CHROMATOGRAPHY

[^{3}H]–Phe_n produced were analyzed quantitatively by paper chromatography. The chromatography was carried out for 6 hr at room temperature (23 ~ 26 °C) on Toyo 51A

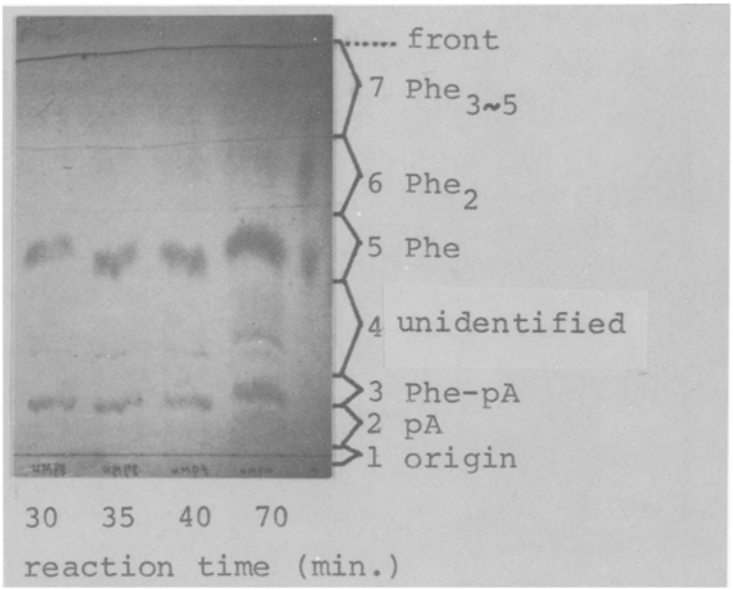

Fig. 2. A typical paper chromatogram of the reaction products developed with ninhydrin spray. The paper strip was cut into the seven segments as shown and the radioactivity was measured by a scintillation counter.

paper (0.16 mm thick) by an ascending technique with a solvent system of butanol-acetic acid-water (12:3:5). After drying the paper, nucleotides and polynucleotides were identified under a UV lamp. Ninhydrin, dissolved in ethanol, was sprayed on the paper to detect Phe and its derivatives. Their chromatographic mobilities are shown in Figure 2. Reaction products were identified by comparing their chromatographic mobilities with those of authentic preparations (Phe–pA, pA, $Phe_{1\sim5}$. The amount of Phe represents the sum of Phe–pA and Phe, since most Phe–pA hydrolyzes to Phe + pA before the analysis. A broad band ($Rf = 0.27$) on the section 4 (Figure 2) was not identified; its position and the time course of its appearance in the reaction mixture (Figure 4) suggests that it is longer polyphenylalanines. Each section (1 to 7) on the paper was cut out as shown in Figure 2 and oxidized by a sample oxidizer, Packard Model 306 tri-carb, and radioactivity of $[^3H]$–Phe_n was measured by a liquid scintillation system, Beckman LS–233. Occasionally, the separation between Phe_2 and Phe_3 was not sufficient and the sum was measured.

2.5. REACTION CONDITIONS AND OTHER PROCEDURES

The following concentrations of the reaction mixture used in our experiment was similar to the one described by Sulston *et al.* (1968a, b): Phe–pA, 23 mM; $MgCl_2$, 75 mM; $NaCl_2$, 200 mM; poly(U) or poly(C), 45 mM (equivalent of residues); uridine 5′-phosphate (pU) 45 mM; histone or albumin, 3.46 mg/600 μl. Total volume of the reaction system was 600 μl and the reaction was carried out at 5 °C. The concentration of poly(U) and poly(C)

adopted here was twice as large as that of Phe–pA, since poly(U) and polyadenylate (poly(A)) form a triple-strand complex (1 poly(A); 2 poly(U)) at this high ionic concentration (Massoulie *et al.*, 1964; Stevens, 1964). The pH of the reaction mixture (initially 2 ~ 3) was first adjusted to about 6 by an addition of 1*N* NaOH (10 ~ 20 μl) and then to 7.0 by a quick addition of 0.1*N* NaOH from the titrator. The observation of the reaction rate started at this point by the measurement of subsequent delivery of NaOH solution to keep it at pH 7.0. A small portion (10 μl) of the sample solution was taken every 2 min and acidified by an addition of 2 μl of acetic acid and kept at −20 °C until the analysis for the amount of Phe and its oligomers. Phe–pA was hydrolyzed to Phe + pA by the addition of acetic acid.

3. Results and Discussion

3.1. EFFECT OF poly(U)

The effect of poly(U) on the polypeptide formation from Phe-pA was tested. Four sets of experiments were carried out: (1) reaction without nucleotides or polynucleotides; (2) with poly(U); (3) with poly(C); (4) with uridine 5′-phosphate (pU).

The addition of Phe–pA immediately turned the mixture containing poly(U) from transparent to milky white. This suggested the formation of a complex between Phe–pA

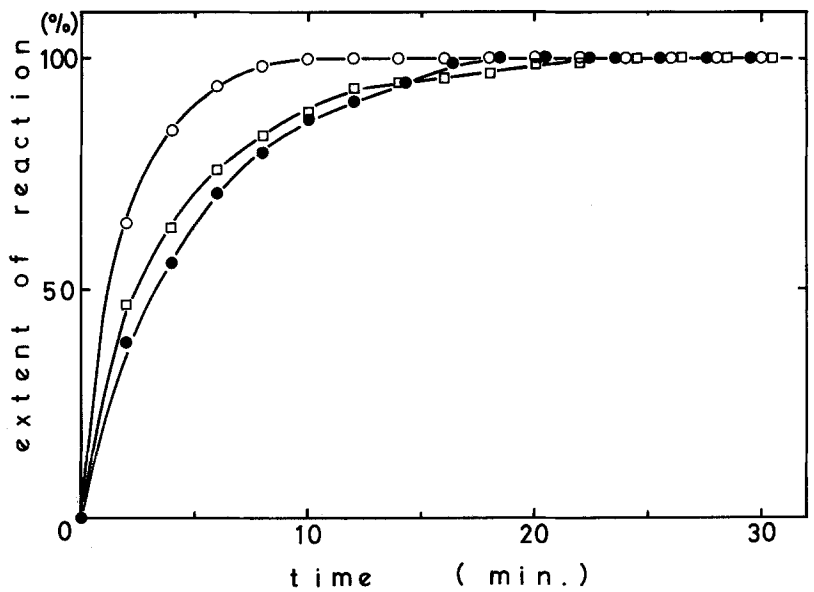

Fig. 3. Effect of histone and albumin on the polymerization reaction of Phe–pA. Time course of the reaction is represented by the amount of the proton release. Released proton was measured by automatic titration of 0.1*N* NaOH. Total volume of the reaction mixture was 600 μl and the system was kept at 5 °C and pH 7.0. □: without added proteins; ○: with histone (3.46 mg/600 μl); ●: with albumin (3.46 mg/600 μl).

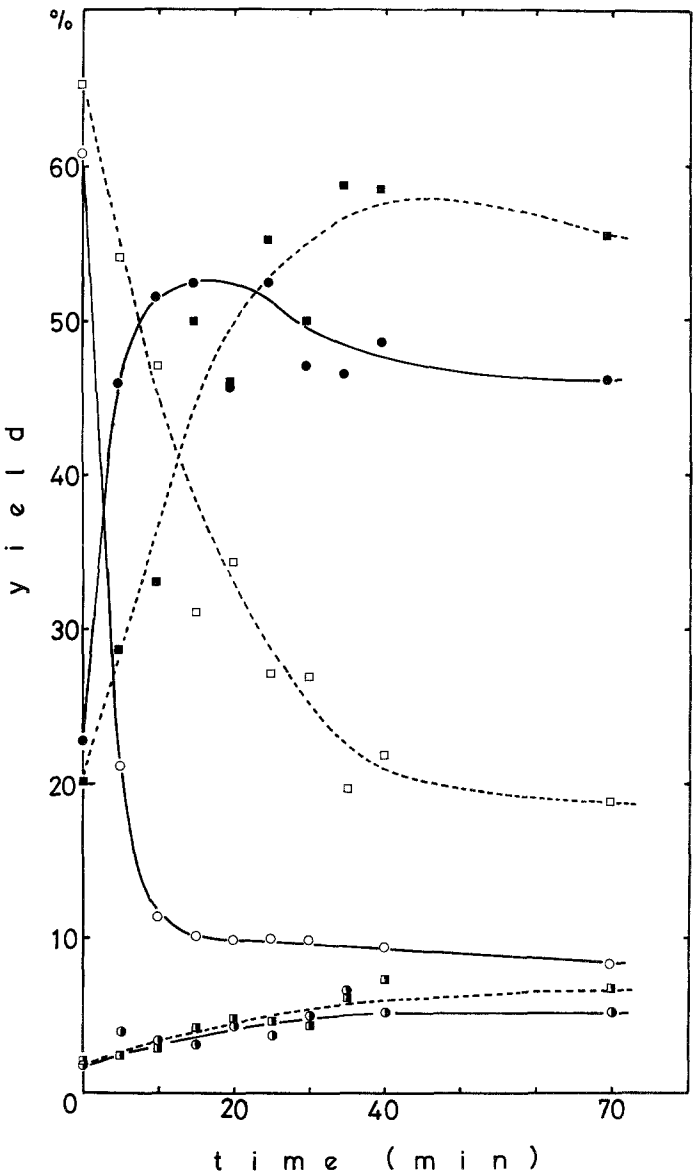

Fig. 4. Time course of the relative amounts of reaction products in the presence and the absence of histone.

	(With histone)	(Without histone)
Phe (Phe–pA + Phe)*	——○——	---------□---------
Phe_2 + Phe_3	——●——	---------■---------
The section 4 in Figure 2	——◑——	---------◨---------

* Phe–pA was hydrolyzed to Phe + pA in acid solution before analysis.

and poly(U); however, no increase in the formation of polypeptides was observed. About 20% of Phe–pA used as the starting material was hydrolyzed to Phe and pA. Neither the addition to poly(C) nor pU had any effect on the polymerization.

3.2. EFFECT OF HISTONE

In order to study the effect of basic proteins, histone was added to the reaction mixture. The proton release (Figure 3) and the change in the amount of phenylalanine derivatives (Figure 4) show that the total reaction rate and the formation of $Phe_2 + Phe_3$ were accelerated in the presence of histone. The acceleration was about three-fold at the initial phase of the reaction. On the other hand, no effect was observed when a neutral protein, bovine serum albumin, was added (Figure 3). Lewinsohn *et al.* (1967) reported that the polymerization and the hydrolysis of Phe–pA proceeded rapidly under basic conditions. Histone might offer local basic surroundings to the reacting Phe–pA molecules. The addition of poly(U), poly(C), or pU reduced the effect of histone (Figure 5). It is known that poly(A) and histone form coacervate droplets (Oparin *et al.*, 1964; Evreinova, 1966). Basic proteinoids also have been reported to form microparticles with synthesized homopolynucleotides (Waehneld and Fox, 1968; Yuki and Fox, 1969). In the present experiment, amorphous droplets about 0.03 mm in diameter appeared in the reaction mixture only when histone was added. From these previous works and from the results reported here,

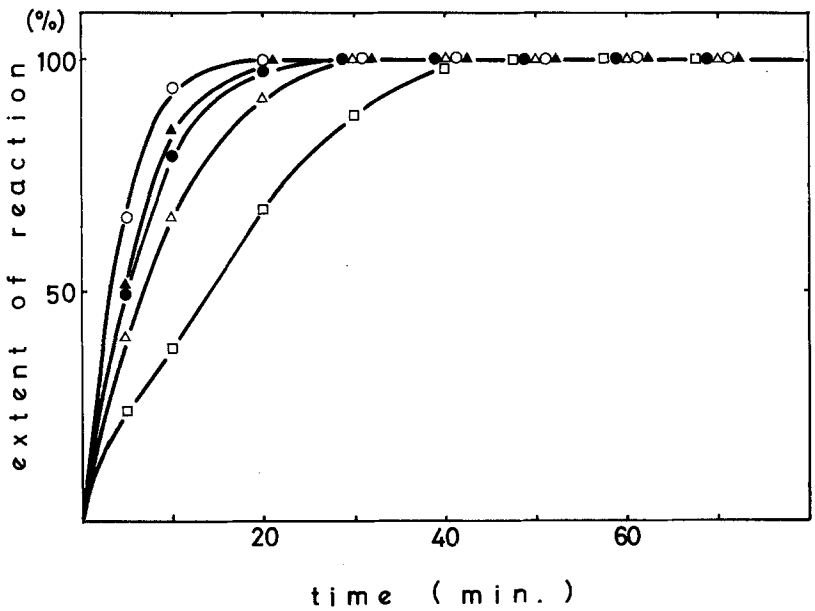

Fig. 5. Effect of histone and (poly)nucleotides on the polymerization reaction of Phe–pA. Experimental conditions and the representation of the reaction are the same as those in Figure 3. □: without addition; ○: with histone (3.46 mg/600 μl). ●: with histone and poly(U) (45 mM equivalent of residues) △: with histone and poly(C) (45 mM); ▲: with histone and pU (45 mM).

it might be said that protocells which contained basic polypeptides could have grown faster than the others.

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References

Berg, P.: 1958, *J. Biol. Chem.* **233**, 608.
Evreinova, T. N.: 1966, *Condensation of Substances and Action of Enzymes in Coacervates*, Izd, Nauka, Moscow.
Huang, W. M. and Tso, P. O. P.: 1966, *J. Mol. Biol.* **16**, 523.
Ishigami, M.: 1974, *Viva Origino* **2**, 35.
Ishigami, M. and Nagano, K.: 1975, *Origins of Life* **6**, 551.
Ishigami, M., Nagano, K., and Tonotsuka, N.: 1977, *Biosystems* **9**, 299.
Massoulie, J., Blake, R., Klotz, L. C., and Fresco, J. R.: 1964, *Compt. Rend. Acad. Sci.* **259**, 3105.
Lewinsohn, R., Paecht-Horowitz, M., and Katchalsky, A.: 1967, *Biochim. Biophys. Acta* **140**, 24.
Nakashima, T. and Fox, S. W.: 1972, *Proc. Nat. Acad. Sci., U.S.A.* **69**, 106.
Oparin, A. I., Serebrovskaya, K. B., Vasileva, N. V., and Balaevskaya, T. O.: 1964, *Dokl. Akad. Nauk. SSSR* **154**, 471.
Paecht-Horowitz, M. and Katchalsky, A.: 1967, *Biochim. Biophys. Acta* **140**, 14.
Stevens, C. L. and Felsenfeld, G.: 1964, *Biopolymers* **2**, 293.
Sulston, J., Lohrmann, R., Orgel, L. E., and Miles, H. T.: 1968a, *Proc. Nat. Acad. Sci., U.S.A.* **59**, 726.
Sulston, J., Lohrmann, R. Orgel, L. E., and Miles, H. T.: 1968b, *Proc. Nat. Acad. Sci., U.S.A.* **60**, 409.
Waehneldt, T. V. and Fox, S. W.: 1968, *Biochim. Biophys. Acta* **160**, 239.
Yuki, A. and Fox, S. W.: 1969, *Biochem. Biophys. Res. Commun.* **36**, 657.