SELECTIVE FORMATION OF PARTICLES BY BINDING OF PYRIMIDINE POLYRIBONUCLEOTIDES OR PURINE POLYRIBONUCLEOTIDES WITH LYSINE-RICH OR ARGININE-RICH PROTEINOIDS

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SUMMARY

Thermally prepared arginine-rich (lysine-free) or lysine-rich (arginine-free) proteinoids show specific reactions with enzymically synthesized homopolynucleotides, as judged by formation of spherules under standard conditions. When arginine is omitted, the proteinoid reacts with pyrimidine polynucleotides; when lysine is omitted, the polymer reacts more readily with purine polynucleotides. With polyadenylic acid, but not with polycytidylic acid, the reactions of various lysine-rich proteinoids show different concentration dependencies.

Thermal polymers of amino acids have served as models to explain the terrestrial origins of self-ordered macromolecules, enzymes, cells, ultrastructures including membranes, and self-replication[1]. Experiments explaining how such microsystems might have in part evolved to microparticles having a more contemporary type of biosynthesis have been reported[2].

One experimental step in the direction of contemporary cells has been the production of very small spherules by reactions of basic lysine-rich proteinoids with nucleic acids[3]. Investigations employing homopolynucleotides with varied lysine-rich proteinoids have revealed selective interactions[4]. (Homopolynucleotides have also been found to have selective effects on the condensation products of adenylates of twenty mixed amino acids[5].)

In this paper, differences in behavior between lysine-rich proteinoids and arginine-rich proteinoids are reported. Such experiments may throw light on the evolution of lysine-rich histones and arginine-rich histones[6].

The total experimental program is designed to provide insight into the molecular basis of genetic systems, their origins, and how and when such phenomena entered the evolutionary stream.

MATERIALS AND METHODS

Basic proteinoids were prepared by the procedure previously described[7]. The lyophilized polymers were stored at -20°. Amino acid composition of each of the proteinoids is presented in Table I. Two additional

Table I

Amino Acid Composition of Thermal Proteinoids (Mole %) and Related Data

Amino Acid	Proteinoid No. 60Y[a]	Lysine-rich (histidine-free)[a]	Lysine-rich (arginine-free)[a]	Arginine-rich (lysine-free)[b]
Lysine	50.9	53.7	74.2	0.0[c]
Histidine	3.0	0.0[c]	1.7	2.2
Ammonia	7.0	4.7	4.3	21.0
Arginine	4.4	3.7	0.0[c]	12.0
Aspartic acid	6.5	2.2	1.0	2.9
Glutamic acid	8.0	7.3	3.1	4.8
Proline	2.1	5.1	2.3	3.7
Glycine	5.8	8.2	4.1	12.0
Alanine	3.9	3.1	1.9	5.4
Valine	2.4	3.0	2.1	5.5
Alloisoleucine[c]	0.8	1.4	1.1	2.0
Isoleucine	1.1	1.6	1.1	3.1
Leucine	3.5	5.4	2.6	5.2
Ornithine[c]	0.0	0.0	0.0	19.4
Unidentified[d]	0.4	0.6	0.4	1.3
% Yield	0.81	0.32	0.39	1.9
Recovery on hydrolysis	69	22	54	30

[a]From 60 mole % lysine, other amino acids 4.0 or 4.4% each.

[b]From 60 mole % arginine, no lysine, 4.4% each of others.

[c]Not included in reaction mixture.

[d]Quantitation on basis of leucine equivalent.

proteinoids were prepared. One was a second arginine-rich proteinoid formed by heating for 7 hr. at 165°, instead of at 196°; histidine was omitted. The other was made from 60 mole % of L-ornithine hydrochloride replacing the arginine, also by heating at 165°.

Each of the polyribonucleotides (Miles) was dialyzed against 0.5 N NaCl for 8 hours and then against tris-HCl buffer (0.05 M, pH 7.0) for 18 hr. before use. The concentration was adjusted by checking O.D. $_{260\ m\mu}$ at pH 7.0, with use of εp values furnished by the vendor.

In the experiment recorded in Table II, 1.0 ml. of polynucleotide solution (1.0 mg. per ml.) in tris-HCl buffer (0.05 M, pH 7.0) was added to 1.0 ml. of proteinoid solution (1.0 mg. of polymer per ml. of buffer). The mixture was shaken immediately and stood for 30 minutes at room temperature. The turbidity which resulted is expressed by optical density at 600 mμ. The particles ranged in diameter from 0.5 to 1.5 μ.

Table II

Effect on $A_{600\ m\mu}$ of Omission of Basic Amino Acids from Lysine-rich Proteinoid No. 60Y in Binding with Polyribonucleotides

Proteinoid	Poly A	Poly U	Poly C
No. 60Y	0.273	0.745	1.307
Lysine-rich (arginine-free)	0.132	0.744	1.421
Lysine-rich (histidine-free)	0.277	0.755	1.295

The experiments of Fig. 1 and Table III were set up similarly except that the polynucleotides were made up in solution so that the final concentration was 0.10 mmoles per ml. of final solution calculated as equivalent to the mononucleotide concentration. These were thus in the concentration range of 32-36 μg/ml., in contrast to the 500 μg/ml. of final solution for Table II.

The differences in result between the table and the figure are further explained by the studies of concentration dependence of Fig. 2. In each

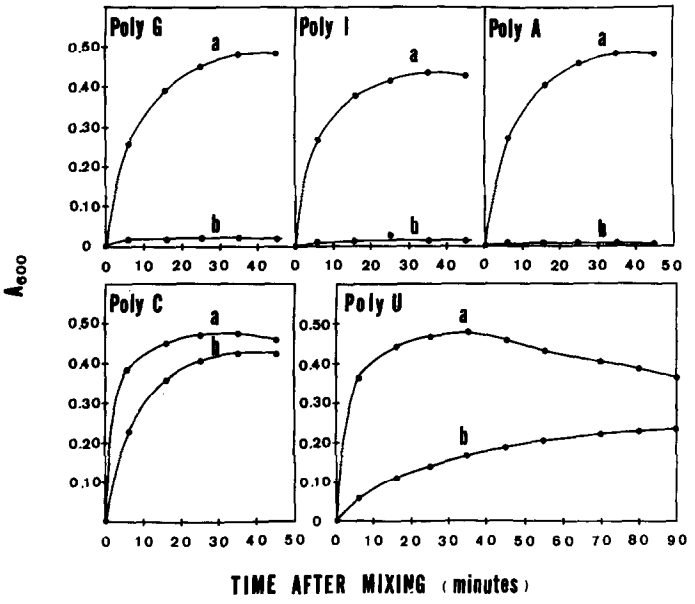

Fig. 1. Formation of microparticles from lysine-rich proteinoid no. 60Y (a) or lysine-rich (arginine-free) proteinoid (b). Polymer (a) binds with all polynucleotides to form particles, whereas (b) reacts thus with the pyrimidine polynucleotides only. Formation of microparticles measured at A_{600}. The polynucleotides and the proteinoids do not absorb at 600 mμ.

Table III

Comparison of Lysine-rich Proteinoid with Arginine-rich Proteinoid in Binding with Polynucleotides

Polyribonucleotide	Lysine-rich (arginine-free) Proteinoid		Arginine-rich (lysine-free) Proteinoid	
	Turbidity	Number of Spherules	Turbidity	Number of Spherules
Poly C	0.253	1.0×10^5/ml	0.002	-
Poly U	0.050	7.5×10^2/ml	0.058	1.3×10^3/ml
Poly A	0.001	-[a]	0.060	1.4×10^3/ml
Poly G	0.003	-	0.218	4.2×10^4/ml
Poly I	0.003	-	0.248	1.0×10^5/ml

[a] - signifies < 1 spherule/ml.

of these, 1.0 ml. of solution of polynucleotide in tris-HCl buffer (0.05 M, pH 7.0) was added to 1.0 ml. of proteinoid solution (2.0 mg./ml.) in the buffer at room temperature. The turbidity resulting 10 minutes after mixing was determined by optical density measurements at 600 mμ.

RESULTS

The particles did not form unless both polymers were brought together in solution. The particles formed were centrifuged. Characteristic absorption patterns at 235 mμ and 260 mμ confirmed that the particles were composed both of proteinoid and polynucleotide.

Table II shows that poly C forms particles most abundantly, poly U less so, and poly A least of the three polynucleotides tested. Omission of histidine has virtually no effect. Omission of arginine, however, yields approximately half as much reaction with poly A only.

In Fig. 1 the formation of turbidity between lysine-rich (arginine-free) proteinoid and polynucleotides equivalent to 0.10 mmoles mononucleotide per ml. was measured as a function of time.

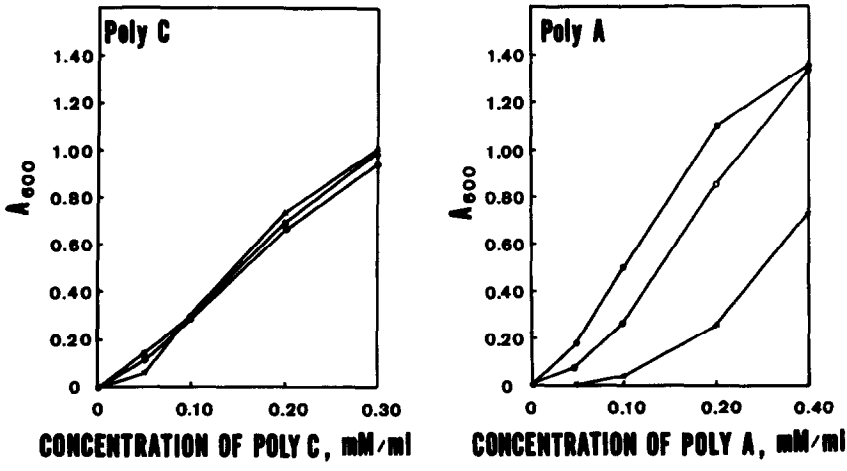

Fig. 2. Concentration dependence of poly C and poly A in formation of microparticles with lysine-rich proteinoid no. 60Y. For poly A, the uppermost curve depicts reaction with proteinoid no. 60Y; the middle curve, lysine-rich (histidine-free) proteinoid; the lowest curve, lysine-rich (arginine-free) proteinoid.

Proteinoid no. 60Y, containing both lysine and arginine (Table I), reacts to form complexes with each of the five polyribonucleotides (Fig. 1). The lysine-rich (arginine-free) proteinoid reacts at the concentration ratios of Fig. 1 only with poly C and poly U.

The results of Table III confirm and extend the results in Table II. Table III shows, moreover, that the lysine-rich (arginine-free) proteinoid reacts with the pyrimidine polynucleotides only. The arginine-rich (lysine-free) proteinoid however reacts maximally with two of the purine polynucleotides, poly G or poly I. Reactions with poly U or poly A are relatively minor, whereas that with poly C is zero. The results obtained with arginine-rich (lysine, histidine-free) proteinoid or ornithine-rich proteinoid prepared at 165° were similar to those obtained with arginine-rich (lysine-free) proteinoid prepared at 196°. Comparisons of turbidities with number of particles showed a close correspondence between the two.

Fig. 2 shows the effects of concentration of polynucleotides on formation of particles, and further confirms the results of Table II.

DISCUSSION

The results reported confirm the tendency of sufficiently basic polyamino acids to react to form nucleoproteinoid microparticles [3]. By the use of homopolynucleotides and proteinoids lacking individual amino acids, however, this assembly is seen not to be simply the consequence of neutralization of oppositely charged macromolecules. Omission of individual basic amino acids alters the specificity of formation of microparticles. In other studies [4], we have shown that the omission of a "neutral" amino acid, leucine, alters the specificity of the type of interaction reported here.

The results reported indicate a high degree of selectivity in the interaction of basic polyamino acids with individual homopolynucleotides, partly as a function of the content of lysine or arginine. When lysine and arginine are both present, the polyamino acid reacts with each of the five homopolyribonucleotides to form microparticles. When arginine is omitted, the proteinoid fails to react in this way with any of the purine polynucleotides, but does so with the pyrimidine polynucleotides. When lysine is omitted from the arginine-rich or the ornithine-rich proteinoid, the basic

polymer reacts most readily with the purine polynucleotides. The fact that ornithine is equivalent to arginine, rather than to lysine, in these reactions is noteworthy.

Whether the results and interpretations presented here provide exact insight into contributions of polyamino acids and polynucleotides to the molecular bases of the contemporary code, they illustrate one kind of experiment that can be performed to make possible the collection and testing of data for general relevance to these problems. In the formation of nucleoproteinoid spherules from polynucleotides and basic proteinoids, specificities are observed. Correspondence between primitive protein and polynucleotides could broadly thus have been established in archeobiochemical situations. Related experiments and their interpretation are described elsewhere [1, 2, 5, 8].

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