Synthesis of Oligonucleotides by Proteinoid Microspheres Acting on ATP

John R. Jungck and Sidney W. Fox

Departments of Biology and Chemistry and Institute for Molecular and Cellular Evolution, University of Miami, Coral Gables, Florida, U.S.A.

Interaction of a solution of ATP with a suspension of microparticles of basic and acidic thermal proteinoids produces the dinucleotide and trinucleotide of adenine. The basic proteinoid in solution alone promotes the production of dinucleotide. The results with the particles provide a model for the origin of cellular synthesis of polynucleotide. In association with other concepts the results strengthen the concept of cells prior to contemporary nucleic acid and protein.

This report describes experiments that constitute a model for the origin of polynucleotides in a protocell acting upon ATP. The microsystem that serves as a laboratory model for a protocell replicates through budding [8], is semipermeable, ultrastructured, and is composed of partially ordered, catalytically active macromolecules [7]. Both the polymer and the microsystem have been produced under geologically and thermodynamically relevant conditions [7]. In proliferating at the systems level, the proteinoid microsphere grows by accretion [8], i.e. heterotrophically, in accordance with the requirements for a primitive organism [11, 20]. In order to evolve toward a presumably more efficient contemporary cell, such a protocell would have had to acquire autotrophic syntheses of nucleic acid and protein, in a coded relationship.

Many authors [e.g. 2–5, 7, 8, 10, 11, 13–16, 20] have proposed or allowed the concept that the original functional nucleic acid emerged from prior protein and/or cell in primordial evolution through a mechanism of "reverse translation". The alternative idea of a primal nucleic acid rests on "chance" and is testable with difficulty [1] or not at all [6].

To model the origin of nucleic acids in a protocell [2], radioactive ATP has been subjected to basic proteinoid in solution, and to suspensions of proteinoid microspheres of two types. In one type, 0.44 g of acidic proteinoid (his 3.2 mole %, lys 2.3, asp 65, glu 12, gly 3.9, ala 4.4, val 2.1, iso 0.9, leu 2.3, phe 2.0, alloiso 1.0; 125 μmolar equivalents of his) and 0.31 g (500 μmoles) of ATP·2 Na·4 H_2O in 1.0 ml of 20 m*M* $MgCl_2$, 0.05 *M* tris-HCl pH 7.0 buffer and to this was added 1.0 ml of 8-^{3}H-ATP, 0.4 mCi, in the same buffer, the solutions were mixed, and then heated in a boiling water bath for 5 min, and finally incubated at 37 °C for 24 h. The reaction system consisted of a dense layer of microspheres over a gel.

The products were next fractionated by DEAE-cellulose column chromatography (Whatman Column Chromedia, microgranular DE 52), by eluting with an exponentially increasing triethyl-ammonium bicarbonate concentration gradient (pH 7.5) ranging from 0.01 *M* to 0.44 *M*. Three liters of buffer were eluted and subsequently collected in 10 ml fractions. The fractions were analyzed for nucleotide material by measuring the O. D. at 259 nm and by removing a 1.0 ml aliquot, mixing it with 10 ml of Scintisol-Complete (Isolab, Inc., Akron, Ohio), and counting the radioactivity in a Beckman LS-100 liquid scintillation counter. Percentages of di- and tri-nucleotides were obtained by summing all tubes within corresponding peaks and calculating from the total of all eluted fractions.

The two-proteinoid microspheres studied were prepared by suspending 0.44 g of acidic proteinoid, 0.19 g of a basic proteinoid (his 7.1 mole %, lys 45.4, asp 1.2, glu 4.3, gly 8.9, ala 6.7, val 3.1, iso 1.1, leu 2.6, thr 0.5, ser 0.3, and pro 2.9; 125 μmolar equivalents of his), and 0.31 g (500 μmoles) of ATP·2 Na·4 H_2O in 1.0 ml of 20 m*M* $MgCl_2$, 0.05 *M* tris-HCl pH 7.0 buffer and 1.0 ml of 8-^{3}H-ATP, 2 mCi, in the same buffer, mixing, heating in a boiling water bath for 5 min, and then incubating at 37 °C for 24 h. This suspension was also a dense layer of microspheres over a gel. The products were fractionated and analyzed as before.

In other experiments (Fig. 1), (a) the ATP was incubated in the buffer solution of $MgCl_2$ without proteinoid, and (b) with 0.19 g of the basic proteinoid subsequent to 5 min in a boiling water bath. The material was totally soluble, hence no microspheres were present in either (a) or (b). The products of these two reactions were also fractionated and analyzed as before.

Fig. 1 c presents the profile of products from the action of two-proteinoid microspheres on 8-^{14}C-ATP in 20 m*M* $MgCl_2$ solution. The principal peak, I, in each chromatogram is unchanged ATP. Various other peaks running before (to the left of)I have been identified as adenine, adenosine, cyclic AMPs, AMP, and ADP. II and III have been identified as adenine dinucleotide and adenine trinucleotide, respectively, by comparison

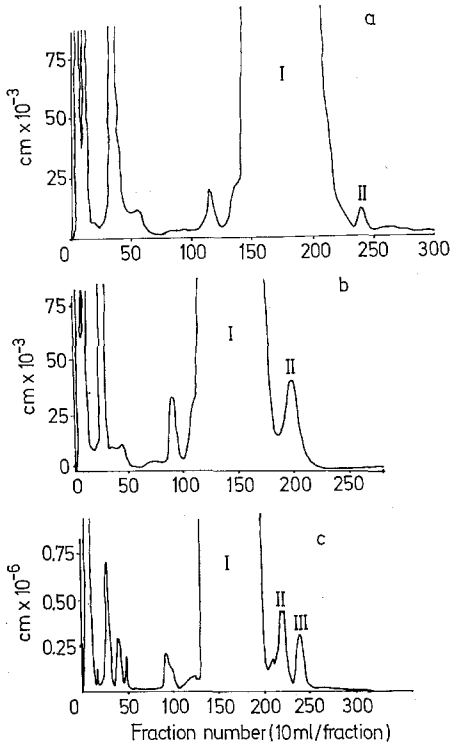

Fig. 1. (a) Products of ATP in 20 mM $MgCl_2$, (b) products of lysine-rich proteinoid and ATP in 20 mM $MgCl_2$ solution, (c) products in suspension of acidic-basic proteinoid microspheres in 20 mM $MgCl_2$

chromatography of commercially available di- and trinucleotides (Miles Laboratories, Kankakee, Ill.) and of di- and tri-nucleotides prepared thermally by refluxing AMP in DMF in a fashion similar to that reported by Pongs and Ts'O [22].

In addition, the oligonucleotides were characterized by comparison thin layer chromatography on PEI-cellulose F precoated 0.1 mm thick on aluminum sheets (Brinkman Instruments, Inc., Westbury, N.Y.) with the solvents: (a) n-PrOH/NH_3/H_2O, 55:10:35, and (b) iso-PrOH/NH_3/H_2O, 70:10:20. The products were alkali-labile. Chain length was characterized by incubating a 0.1 ml sample of a concentrated column chromatography fraction with 10 lambda of *E. coli* alkaline phosphatase (Calbiochem) solution (0.1 mg/ml in 0.05 M tris-HCl buffer) at 37 °C for 4 h, removal of alkaline phosphatase by chromatography, hydrolyzing in 0.3 N KOH at 37° for 24 h, neutralizing, and rechromatographing in the same solvent. The ratio of AMP to adenosine of the material with RT of dinucleotide was 0.84 and of that with RT of trinucleotide was 1.63. An aliquot of peak II in Fig. 1a was also hydrolyzed and analyzed on an amino acid analyzer. No other amino acids than glycine were present in significant amount; glycine can be ascribed to hydrolytic breakdown of adenine [12]. This seems to exclude the possibility that proteinoid served to bind mononucleotide so that the complex was eluted at an RT corresponding to a dinucleotide. How much synthesis occurs at 37° and at 100° is not known.

In Table 1, the proportions of radioactive material chromatographing before ATP, the ATP, and the trinucleotide/dinucleotide ratio have been presented for each of four experiments. Only the experiments in suspensions of proteinoid microspheres yielded both peaks for oligonucleotides. ATP is however converted in magnesium-containing buffer to dinucleotide to a small extent. In the presence of basic proteinoid, which has other known catalytic activities [23], the conversion is several times as large. The basic proteinoid thus becomes a model for proto-RNA polymerase activity; other basic proteinoids are being screened to assess the variation of such activity with composition. In the primitive cell, such proteinoids might have been the evolutionary forerunners of more active polymerases. The basic proteinoid of course provides opportunities for binding of oligonucleotides. The formation of at least a trimer of mononucleotide may be crucial, according to Eigen [3], in order to provide codons with adequate stability. Trimers of mononucleotides are the smallest coding units [19], perhaps because they are the smallest oligonucleotides capable of having the stability requirements.

The finding that an oligonucleotide can result from ATP in a minimal cell suggests a close relationship to activation of amino acids by ATP in the protocell [24], and to the simultaneous formation of protein and nucleic acid from intermediate aminoacyl adenylates [18]. (AppA was reported as arising from mixed aminoacyl adenylates [18]. The low yields of AppA reported are however difficult to distinguish from small proportions that might have been produced by DCCD.)

Control experiments have been run also with AMP instead of ATP. No oligonucleotides resulted. This observation reaffirms that ATP is the source of energy for the synthesis of phosphodiester linkages in these experiments. The use of nucleoside triphosphates in *in vitro* enzymic synthesis of RNA is well-known [9]. The mechanism is being further investigated.

The geological plausibility for the archaic occurrence of ATP rests upon a number of demonstrations employing hypohydrous or anhydrous conditions [24].

Theoretically, the advantages of a cell, or particulate, for biosyntheses of polymers are many. Some of these advantages include (a) the possibility of faster reactions, (b) the possibility of evolution of the proteinoids

Table 1. Products and yields of adenine dinucleotide and trinucleotide in various systems reacting with ATP (at 37 °C, 2 days)

Products	System			
	20 mM $MgCl_2$ solution	basic proteinoid solution	with acidic proteinoid microsphere suspension	with acidic-basic proteinoid microsphere suspension
Adenine, adenosine, cAMP, AMP, ADP [%]	12.6	14.7	14.8	13.0
ATP, recovered [%]	86.8	82.9	82.9	84.7
Oligo A, eluting beyond ATP [%]	0.7	2.2	2.2	2.3
Trinucleotide / Dinucleotide	0.0	0.0	0.2	0.5

to powerful enzymes, since enzymelike activities have been demonstrated for these polymers [23]; (c) the general utility for evolution of macro-molecular synthesis maintained in proximity to supporting reactions by the structure within a cell [7], and (d) *especially the maintenance of a thermodynamically favorable hypohydrous environment,* since reactions at surfaces are not those of dilute aqueous solution.

Consonant with the emphasis on particulates, Oparin [21] has described the work of Serebrovskaya, who reports the enhanced formation of polyadenine from ADP in the presence of polynucleotide phosphorylase in histone-RNA droplets. While this experiment does not illuminate the evolutionary origin of a first polynucleotide, it illustrates the biochemical value of a cellular entity.

Selective interactions of polynucleotides with *poly*-amino acids have been demonstrated; these specificities include cognition in either direction [25], which supports the possibility of the origin of translation from an inverse (protein→nucleic acid) evolutionary precursor. Some of the polyamino acid-polynucleotide particles studied in this context reveal a codonic bias [17], and suggest the need for enhancement of the interactions by enzymes at a stage in evolution later than that modelled. This and other intellectual interstices remain to be filled. All of the results, including those presented here, plus the ease of formation of a minimal replicating cell, are consistent with a flowsheet of

amino acids → protoprotein (proteinoid) → protocell → (nucleic acid-governed) contemporary cell.

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