NUCLEIC ACID-LIKE STRUCTURES
II. POLYNUCLEOTIDE ANALOGUES AS POSSIBLE PRIMITIVE PRECURSORS OF NUCLEIC ACIDS*

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Abstract. Activated derivatives of purine-containing deoxynucleoside- diphosphates spontaneously oligomerize to produce pyrophosphate- linked oligodeoxynucleotide analogues. These analogues are of potential interest as models of primitive, polynucleotide precursors. The efficiency of oligomerization (ImpdGpIm and ImpdApIm much greater than ImpdIpIm) appears to reflect a combination of stacking forces and the specific geometric orientations of the stacked units. Under favorable conditions, chain lengths greater than 20 have been obtained for oligomers containing pdGp in the absence of a template. In the presence of a complementary template, the activated derivatives of pdGp and pdAp oligomerize much more extensively. An acyclo-analogue of G has also been shown to undergo template-directed oligomerization on poly(C). These observations suggest the possibility that primitive information transfer might have evolved in much simpler systems and that this function was taken over by polynucleotides at a later stage in evolution.

1. Introduction

We have recently shown (Schwartz and Orgel, 1985a) that a new family of polynucleotide analogues can be synthesized on polynucleotide templates by oligomerization of activated 2′-deoxynucleoside-3′, 5′-diphosphates. These products are linked by pyrophosphate groups rather than by phosphodiester linkages. As a first step in investigating the possibility that such molecules or related ones might have been primitive, prebiotic precursors of polynucleotides, we have studied the non-template-directed oligomerization of the activated derivatives ImpdApIm, ImpdGpIm and ImpdIpIm (I in Figure 1). In the present paper we report

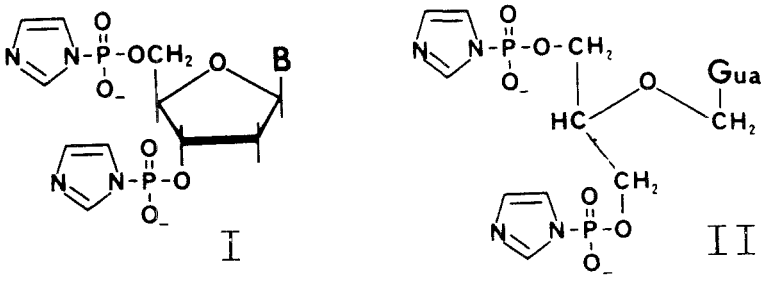

Fig. 1. Structures of activated monomers. B is adenine, guanine or hypoxanthine.

* For the previous paper in this series see Schwartz and Orgel, 1985a.

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that monomers based on adenosine and guanosine will, under appropriate conditions, oligomerize to produce substantial yields of long oligomers even in the absence of a template. The effects of a number of minerals and amino acid polymers on the oligomerization have also been studied.

2. Materials and Methods

Deoxynucleosides and polynucleotides, Alkaline Phosphatase (Type III from *E. coli*) and Bovine Pancreatic Ribonuclease (Type I-A) were purchased from Sigma Chemical Company. Phosphodiesterase I from *Crotalus adamanteus* venom was purchased from P-L Biochemicals. The diphosphates of dG and dI were prepared according to the method of Bennett *et al.* (1976), except that purification of the products was carried out by chromatography on DEAE-Sephadex (A25, Pharmacia) in the chloride form with a linear gradient of 0.1 M to 0.4 M NaCl at pH 4.0. Desalting was carried out by absorbing the diluted fractions on a column in the bicarbonate form and eluting with 1.0 M TEAB until no chloride was detectable with $AgNO_3$ and then bringing off the product in 1.0 M TEAB. TEAB was removed by repeated evaporation under vacuum in the presence of ethanol. Phosphorylation of dA was performed according to the method of Beld *et al.* (1984), subsequent to protection of the adenine moiety (Ti *et al.*, 1982). Purification was as described above.

The diimidazolides were prepared by a modification of a standard method used for the preparation of 5′-phosphoimidazolides of nucleotides (Joyce *et al.*, 1984). For pdGp or pdIp (0.6 mmol as triethylamine salts), the diphosphate was dissolved in 1.3 ml of DMSO plus 0.2 ml of triethylamine. For pdAp, 1.3 ml of DMF plus 0.2 ml of triethylamine was substituted. This solution was added dropwise to 2.5 mmol each of triphenylphosphine, 2′, 2′-dithiodipyridine (aldrithiol-2) and imidazole, dissolved in 3.3 ml of DMF plus 0.45 ml of triethylamine. Reaction was for 2 hr at room temperature under dry conditions, with magnetic mixing. Precipitation of the product was carried out in a dry mixture of 50 ml of acetone, 50 ml of ethyl ether, 4 ml of triethylamine and 0.5 ml of $NaClO_4$-saturated acetone. The products were washed and dried as described in Joyce *et al.* (1984).

Reaction mixtures were prepared as previously described (Schwartz and Orgel, 1985a) and contained 0.1 M diimidazolide, 0.4 M $MgCl_2$, 0.1 M NaCl, 0.4 M imidazole (pH 6.5 with HCl) and, when required, 0.1 M poly(U), poly(C), or poly(A). Reaction tubes were allowed to react in a water bath at 4 °C for three weeks. At the conclusion of the reaction period, reactions were quenched by addition of 10 μL of 1 M KEDTA (pH 9) and 80 μL of H_2O. In some cases poly(U) and poly(C) templates were destroyed prior to HPLC analysis by incubation with ribonuclease.

Enzyme digestions: For Ribonuclease digestion 10 μL of the quenched reaction mixture were added to 100 μL of Tris-HCl (0.05 M, pH 7.6) containing 10 units of enzyme. Incubation was at 37 °C for 4 hr. For Alkaline Phosphatase digestion, 10 μL were added to 100 μL of Tris-HCl (0.04 M, pH 8.0) containing 0.02 M

$MgCl_2$ and 0.1 units of enzyme. Incubation was as above. For Phosphodiesterase-I digestion, 10 μL was added to 100 μL of Tris-HCl (0.2 M, pH 9.0) containing 0.04 M $MgCl_2$ and 0.2 units of enzyme. Incubation was as above. Before HPLC analysis, 100 μL of sodium acetate (0.1 M, pH 4.0) was added to a sample containing 0.1 μL total nucleotide and the sample was allowed to stand at room temperature overnight to hydrolyze surviving imidazolides.

A number of reactions were also run in the presence of mineral samples or amino acid polymers, to test for the possibility of catalytic effects. Clay Mineral Standards (Ward's) used were kaolinite No. 7; montmorillonites Nos. 23, 24, and 31; and attapulgite No. 43. Other minerals tested included synthetic manganate (gift of G. Arrhenius), cacoxenite (gift of P. B. Moore), ferric hydroxide (Schwartz and Orgel, 1985b) and powdered Pueblito de Allende meteorite. The ground minerals (20–30 mg) were washed in imidazole buffer containing magnesium and sodium chlorides at the concentrations to be used in the reactions, which were then carried out as above. After one week, the supernatants were removed by centrifugation in an Eppendorf model 5414 centrifuge at 12 000 rpm and the minerals were extracted with 50 μL of 0.75 M $K_4P_2O_7$. To the combined supernatants and extracts was added 10 μL of 1 M KEDTA and hydrolysis and analysis were carried out as usual. Polyamino acids were purchased from Sigma. Oligomerizations were conducted in the presence of 0.3 M polyaspartic acid, polyglutamic acid and polyarginine. Proteinoids were a gift of S. W. Fox. A series of sixteen proteinoids including 'equimolar', '2:2:1', and others including basic, acid and neutral preparations were tested as saturated solutions (for details on the preparation of proteinoids, see Chapter 5 of Fox and Dose, 1977).

4. Results and Discussion

It has previously been shown that a number of minerals are without effect on the poly(C)-directed oligomerization of 2-MeImpG (Schwartz and Orgel, 1985b). A large number of mineral samples have now also been tested for their possible effect on the oligomerization of ImpdGpIm in the presence and absence of poly(C), by comparison of the HPLC results with those of control reactions. Neither stimulation of the reaction nor inhibition were observed for either reaction in the presence of any of the clay minerals, or of hydroxyapatite. Partial inhibition was observed, however, in the presence of manganate and ferric hydroxide. None of the amino acid polymers had a stimulatory effect on the template-free oligomerization, although in some cases a slight inhibition was observed.

HPLC chromatograms of the products of oligomerization reactions are shown in Figure 2. After three weeks of reaction, both ImpdApIm and ImpdGpIm produce substantial yields of oligomers. In both template-directed and template-free reactions, the most significant limiting factor is the cyclization of monomer to produce the 3′:5′ cyclic pyrophosphate. In template-free reactions, 50–60% of the

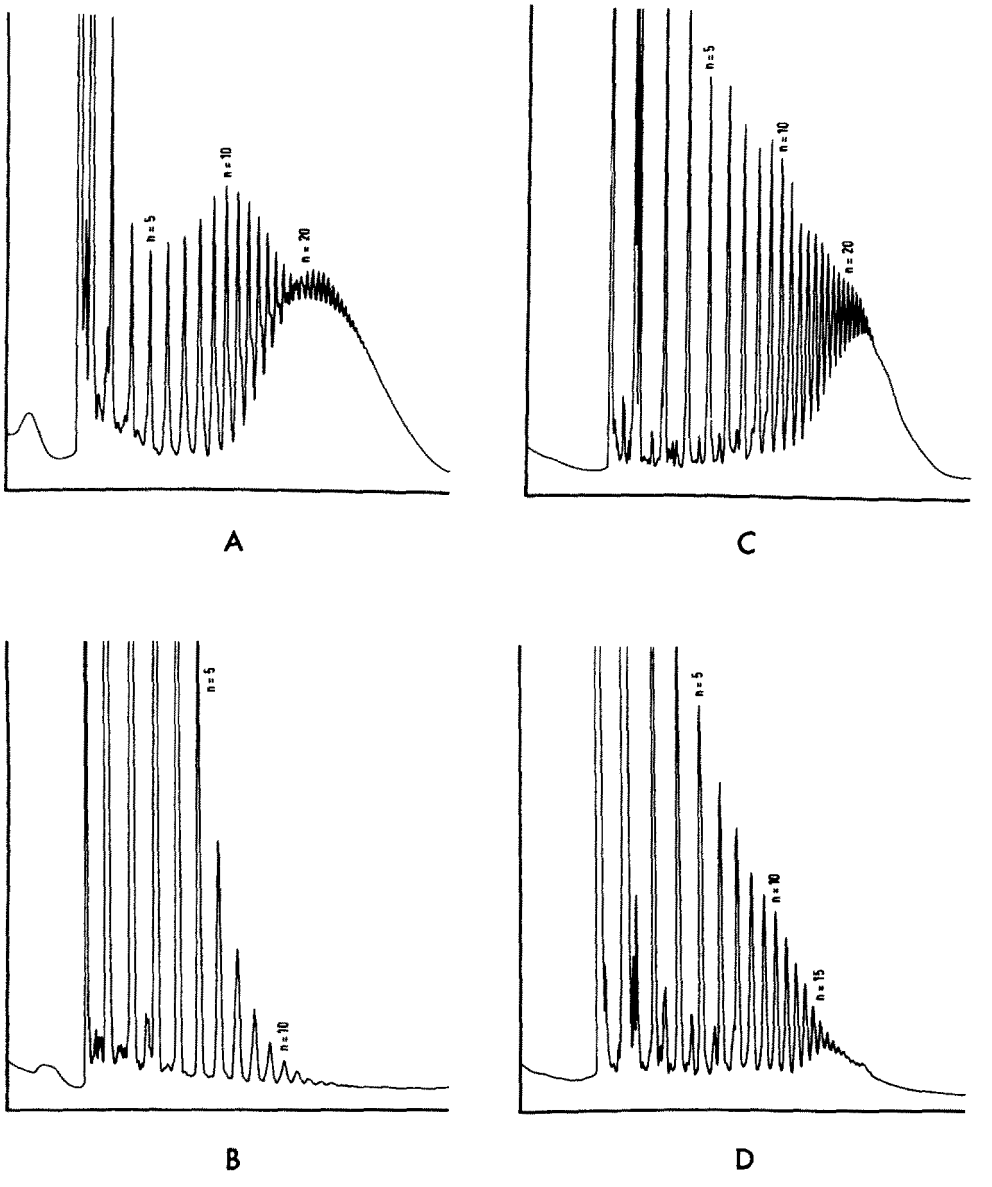

Fig. 2. Chromatographic analyses of reaction products. [A] ImpdApIm + poly(U); [B] ImpdApIm alone; [C] ImpdGpIm + poly(C); [D] ImpdGpIm alone; All mixtures contained 0.1 M diimidazolide, 0.4 M $MgCl_2$, 0.1 M NaCl, 0.4 M imidazole-HCl (pH 6.5) and, where indicated, 0.1 M template. The reaction was carried out at 4 °C for three weeks. Analysis on RPC5 in 0.02 M NaOH with a linear gradient of $NaClO_4$ (0 to 0.04 M over 60 min) at a flow rate of 1.0 ml min^{-1}. Peak detection was by absorbance monitoring at 254 nm.

starting material can be consumed in this manner. The cyclization of dimer and trimer appear to be much slower reactions. After three weeks reaction, only about as much cyclic oligomer has accumulated as the remaining linear product (these products can be resolved under slightly different chromatographic conditions). The identities of the cyclic products were established by demonstrating resistance to bacterial alkaline phosphatase and by incubation with venom phosphodiesterase and observing the sequential production (for example for trimer) of linear trimer, dimer and finally the monomer.

The most efficient oligomerizations in the absence of a template were obtained with ImpdGpIm, which gave conversions of monomer to oligomer of 40%. Of these products 18% had chain lengths of 10 or more. In the presence of a complementary template, oligomerization competes favorably with cyclization and oligomer yields in excess of 60% have been obtained. Although template-directed reactions are susbtantially faster during the first few days of reaction, the most obvious effect of the presence of a complementary template on the oligomerizations of ImpdApIm and ImpdGpIm is the production of higher yields of the longest oligomers after extended periods of reaction (Figure 2). In contrast to ImpdGpIm and ImpdApIm, ImpdIpIm reacted inefficiently. A 33% total yield of oligomers was obtained after three weeks, but no products with chain lengths higher than 8 could be detected (Figure 3). Attempts to improve the reaction by addition of a 'template' consisting of poly(A) were unsuccessful (The wobble-pair A:I has been suggested as the basis for a hypothetical primitive genetic code (Orgel, cited in Crick, 1968)).

The inefficient oligomerization of ImpdIpIm is unexpected. We believe that stacking plays an important role in permitting the formation of long oligomers in the absence of a complementary template. pI is known to stack at least as strongly in aqueous solution as pG, although much less strongly than pA (Neurohr and Mantsch, 1979). A possible explanation for our results may be the known aggregation of guanosine nucleotides to form stacked, H-bonded tetramers (Bouhoutsos-Brown *et al.*, 1982). Monomers participating in such aggregates might be constrained to orientations with O–6 directed inward (relative to the tetramer), thereby discriminating against head to head stacking of monomers. Although tetramers might well be stacked head to head, individual deoxyribose units would still be in position to permit pyrophosphate formation. In support of this hypothesis, we have observed that the addition of potassium ions to reaction mixtures enhances the production of higher oligomers from impdGpim (Bouhoutsos-Brown *et al.*, 1982) but not from impdApim or impdIpim.

We have earlier reported that the dimonophosphate of the acyclic nucleoside analogue 9-(1, 3-dihydroxy-2-propoxymethy)-methyl guanine (II in Figure 1) can also be oligomerized on a poly(C) template (Schwartz and Orgel, 1985a). Compounds of this type are interesting since – being initially achiral – they can be regarded as possible precursors of chiral polynucleotides (Joyce *et al.*, in preparation). In order to test the hypothesis that nucleic acid analogues with novel backbones might have played a role in chemical evolution, it will be necessary to

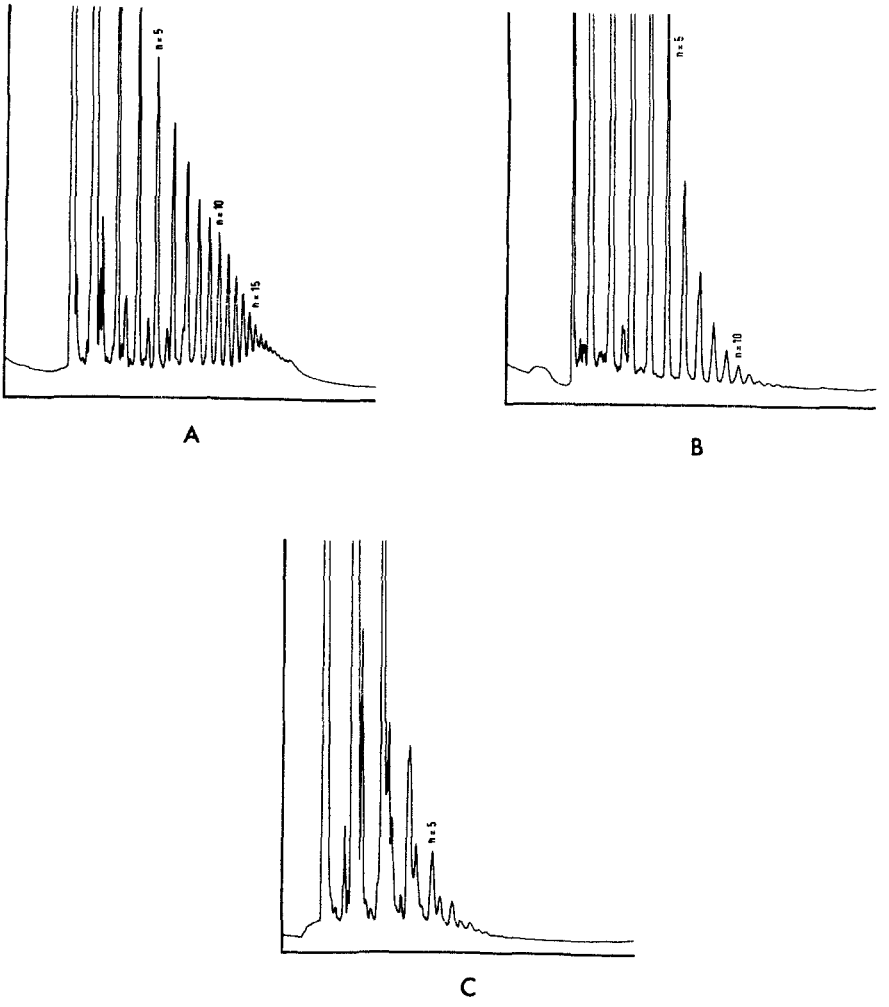

Fig. 3. Chromatographic analyses of reaction products. Conditions as in Figure 1. [A] ImpdGpIm; [B] ImpdApIm; [C] ImpdIpIm.

synthesize and test such oligomers as templates. We are currently planning to carry out such a test.

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Abbreviations

(A) adenosine; (C) cytidine; (U) uridine; (G) guanosine; (I) inosine; dA, dG, dI, the 2′-deoxynucleosides of A, G, and I; pdN (N is A, G, or I), the 5′-phosphate of dN;

dNp, the 3′-phosphate of dN; ImpdNpIm, the 3′, 5′-diphosphoimidazolide of N; pN, the 5′-phosphate of N; 2-MeImpG, the 2′-methylimidazolide of pG; poly(A), polyadenylic acid; poly(U), polyuridylic acid; poly(C), polycytidylic acid; TEAB, triethyl ammonium bicarbonate; KEDTA, the potassium salt of ethylene diamine tetraacetic acid; DMF, dimethyl formamide; DMSO, dimethyl sulfoxide.

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