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Template-Catalyzed Oligomerization with an Atactic Glycerol-Based Polynucleotide Analog

J. Visscher and Alan W. Schwartz

Laboratory for Exobiology, Faculty of Science, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands

Summary. We have tested the effect of a nonstereoregular (atactic) template, based on the acyclic nucleotide analog pC̃p, on the oligomerization of the complementary bis-phosphoimidazolide monomer, ImpG̃pIm. Although there is little catalysis when the template is added together with magnesium ions, the substitution of manganese for magnesium produces a substantial increase in the extent of oligomerization as well as increasing the effect of the template. These results are discussed in relation to the hypothesis that prochiral nucleotide analogs may have been precursors to the first RNA molecules.

Key words: Chemical evolution — Nucleic acid analogs — Oligonucleotides — Template-directed synthesis

Introduction

The problems inherent in the synthesis of the enantiomerically pure nucleosides, which would appear to be necessary for the first self-replicating RNA molecules to arise, have led to suggestions that RNA may have been preceded by a more primitive set of autocatalytic molecules (Joyce et al. 1987). These precursors, although retaining the base-pairing properties of nucleic acids, might have had simpler backbone structures. Prochiral nucleotide analogs of the kind shown in Fig. 1 have received considerable attention in this context. A central point is that although the monomers are not themselves chiral, a possibility for the generation of chirality exists as a result of polymerization, as each residue can assume a D-like or an L-like configuration (Spach 1984; Joyce et al. 1987). In earlier work, it has been demonstrated that the oligomerizations of bisphosphoimidazolides of G̃ and Ã are catalyzed by the complementary polyribonucleotide templates, producing pyrophosphate-linked polynucleotide analogs (Visscher and Schwartz 1988). We have now synthesized a nonstereoregular (i.e., atactic) set of pyrophosphate-linked oligomers based on pC̃p and examined the effect of these products on the oligomerization of ImpG̃pIm.

Materials and Methods

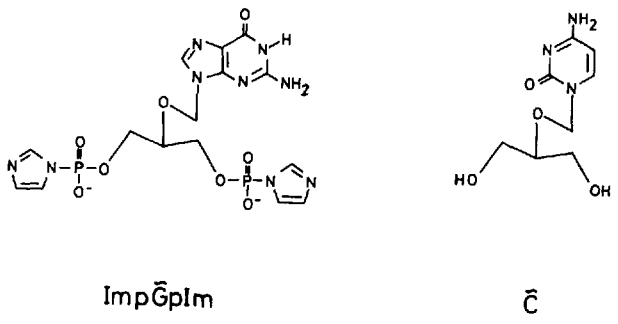
Alkaline phosphatase (type III from *Escherichia coli*) was purchased from Sigma Chemical Company. Phosphodiesterase I from *Crotalus adamanteus* venom was purchased from P-L Biochemicals. EDAC was purchased from Janssen Chimica.

The preparation of 1-[(1,3-dihydroxy-2-propoxy)methyl]-cytosine (C̃) and the bis-phosphorylation of C̃ were accomplished as described in Visscher and Schwartz (1990). The synthesis of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (G̃) and the bis-phosphorylation of G̃ were described in Visscher and Schwartz (1988).

The cyclic pyrophosphate of pC̃p was prepared by treating 0.1 mmol pC̃p with 1.0 mmol EDAC and 0.4 mmol $MgCl_2$ in 4.0 ml 0.4 M imidazole (pH 6.5 with HCl). After 1 h at 40°C the reaction was 95% complete. Purification was accomplished by chromatography on DEAE-Sephadex (A25, Pharmacia) with a

Offprint requests to: A.W. Schwartz

Abbreviations: Ã, 9-[(1,3-dihydroxy-2-propoxy)methyl]adenine; G̃, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine; ImpG̃pIm, the bisphosphoimidazolide of G̃; C̃, 1-[(1,3-dihydroxy-2-propoxy)methyl]cytosine; pC̃p, the bisphosphate of C̃; cpC̃p, the cyclic pyrophosphate formed from pC̃p; CTP, cytidine 5′-triphosphate; EDAC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; EDTA, ethylenediamine tetraacetic acid; TEAB, triethylammonium bicarbonate; Tris, tris(hydroxymethyl)aminomethane



ImpG̃pIm

C̃

Fig. 1. Structures of the glycerol-based analogs C̃ and ImpG̃pIm (the bisphosphoimidazolide of G̃)

linear gradient of 0.1–0.4 M TEAB at pH 8. The cyclic product was identified by enzymatic analysis, as described by Schwartz et al. (1987), and by ^{31}P-NMR (^{31}P-NMR shifts relative to phosphoric acid in D_2O were: pC̃p, 2.8 ppm, singlet; cpC̃p, −9.8 ppm, singlet). The cyclic pyrophosphate was resistant to the action of bacterial alkaline phosphatase. Treatment with venom phosphodiesterase resulted in the production of pC̃p.

The polymerization of cpC̃p was carried out following Tohidi and Orgel (1990). Samples of 1 μmol cpC̃p (as triethylamine salt), each dissolved in 10 μl 0.1 M imidazole (pH 6.5 with HCl), were spotted on a glass plate and incubated over P_2O_5 at 83°C. After 3 days more then 70% of the cyclic-pyrophosphate had reacted to form oligomers and polymers. Analysis by HPLC (see below) showed that the length of the polymers extended to about 60 monomer-units. A collection of 100 reactions was treated with 10 units of alkaline phosphatase in 3 ml 0.04 M tris-HCl (pH 8) containing 0.02 M $MgCl_2$, to remove terminal phosphate groups. After incubation for 4 h at 37°C, oligomers with chain lengths ≥20 monomer-units were isolated by fractionating on Q-Sepharose (Fast Flow, Pharmacia) in a linear gradient of 0.1 M to 1.5 M TEAB (pH 8). The identity of the products as oligomers of pC̃p was confirmed by enzymic digestion with phosphodiesterase combined with HPLC, and by ^{31}P-NMR (^{31}P-NMR shift relative to phosphoric acid in D_2O was −9.0 ppm, singlet).

The in situ bis-imidazolation of pG̃p was carried out by treating 0.1 M pG̃p with 10 equivalents each of imidazole and EDAC at pH 6.5 and 0°C for 2 h as described in Visscher and Schwartz (1988). Reaction mixtures were prepared by adding 2.5 μl of the imidazolating mixture to 10 μmol NaCl, 0.5 or 2 μmol $MgCl_2$ or $MnCl_2$ and, when required, 0.25 μmol of the template (monomer equivalents) in a total volume of 10 μl. The final reaction conditions are given in Table 1. After a reaction period of 7 days at 0°C the reaction mixtures were quenched by adding 4 μl of EDTA (1.0 M, pH 9) and water to a total volume of 30 μl. Storage was at −25°C. Prior to analysis any surviving imidazolides were hydrolyzed by adding 1 μl of the quenched reaction mixture to 50 μl 1.0 M NaOH and incubating at 37°C for 4 h. The sample was then diluted with water to 1 ml. Analyses were performed by HPLC on RPC-5 in 0.02 M NaOH with a linear gradient of $NaClO_4$ (0–0.04 M over 60 min) at a flow rate of 1 ml/min. Peak detection was by absorbance monitoring at 254 nm. The cyclic-dimer, dimer, and tetramer of pG̃p were identified by isolating these products from the RPC-5 column and carrying out sequential degradation with phosphodiesterase as described in Visscher and Schwartz (1988).

Table 1. Product distributions in the oligomerization of ImpG̃pIm

C̃p(pC̃p)$_n$pC̃ (with $n \geq 18$) template (M)	Magnesium (M)	Manganese (M)	Incorporation of monomer into oligomers of length n (%)		
			$n \geq 2$	$n \geq 3$	$n \geq 4$
0.025	0.2	—	6	1	Trace
—	0.2	—	3	—	—
0.025	0.05	—	3	—	—
—	0.05	—	1	—	—
0.025	—	0.2	27	13	6
—	—	0.2	18	6	2
0.025	—	0.05	16	8	4
—	—	0.05	9	3	1

Conditions: 0.025 M monomer, 1.0 M NaCl, metal chloride as shown and, when required, 0.025 M template [monomer equivalents of C̃p(pC̃p)$_n$pC̃]. Reactions were at pH 6.5 and 0°C for 7 days. For further details see text

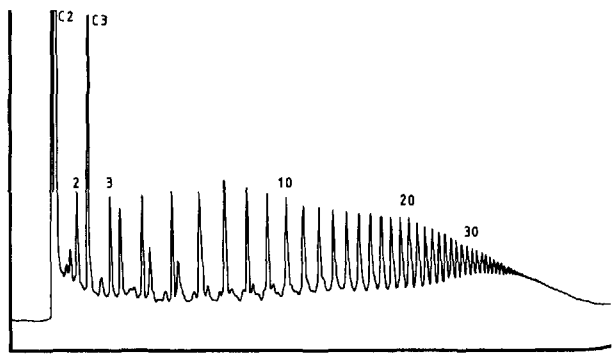


Fig. 2. HPLC analysis on RPC-5 of the products of the polymerization of cpC̃p. C2 and C3 show the positions of cyclic dimer and trimer, respectively.

Results and Discussion

The range of oligomers obtained from the thermal polymerization of cpC̃p is illustrated in Fig. 2. The products extended to at least the 60-mer. After removal of terminal phosphates and isolation of those oligomers with chain lengths of 20 and more, we carried out the experiments summarized in Table 1. In previous work on the catalysis of the oligomerization of nucleotide analogs based on deoxyribose bisphosphates, we demonstrated that the substitution of pyrophosphate for phosphodiester linkages between deoxyribose units does not destroy the ability of the polynucleotide analog to catalyze the synthesis of its complement (Visscher et al., 1989). In contrast to the earlier results, we observed only a marginal effect of the glycerol-based template on the oligomerization in the presence of magnesium (Table 1). Attempts to reduce the effects of charge repulsion between the pyrophosphate chains by the addition of spermine or spermidine to the system produced no improvement in the yields.

We also studied the effect of substituting manganese for magnesium in the reaction. We had pre-

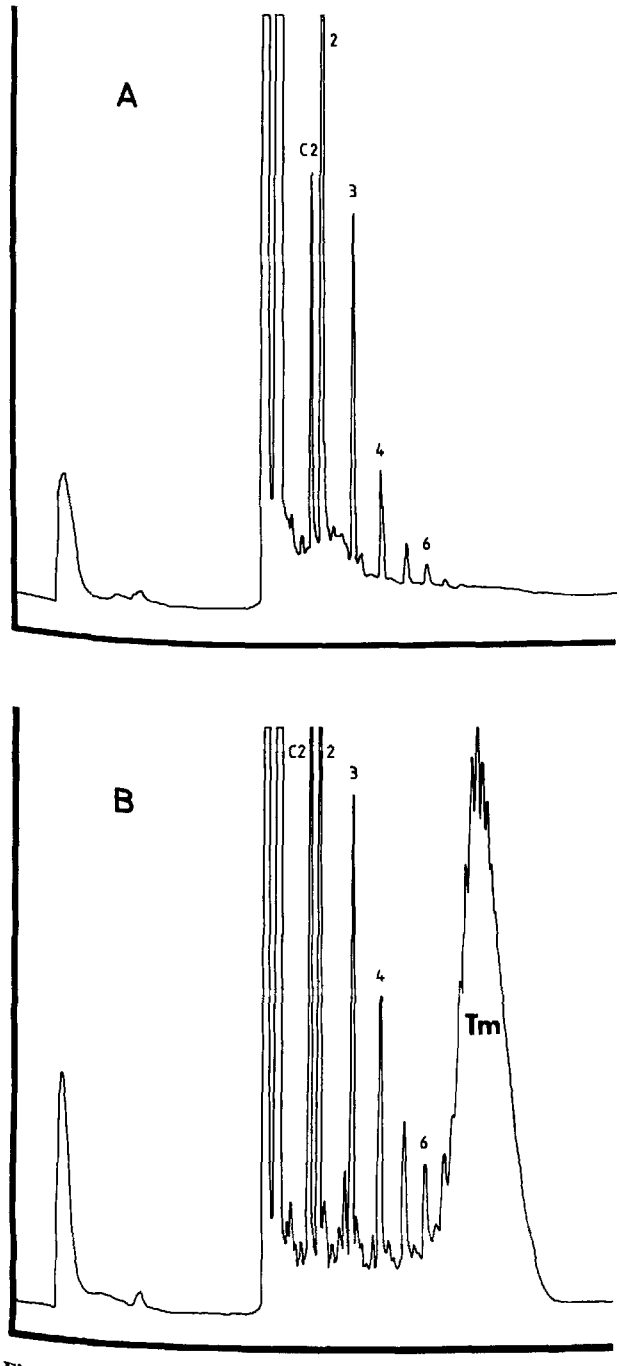


Fig. 3. Oligomerization of ImpG̃pIm. **A** 0.025 M ImpG̃pIm, 0.05 M $MnCl_2$, 1.0 M NaCl, pH 6.5. (Reaction was at 0°C for 7 days. For exact conditions see Table 1 and text.) **B** As A, but with the addition of 0.025 M template [monomer equivalents of C̃p(pC̃p)$_n$pC̃, $n \geq 18$]. The position of the template mixture is indicated by Tm. Control experiments established that no spurious peaks were produced by degradation of the template under the conditions of the reaction and analysis.

viously reported that although manganese has a favorable effect on the oligomerization of ImpG̃pIm in the absence of a template, it appeared to reduce the catalytic effect of a polyribonucleotide template (Visscher and Schwartz 1989). Somewhat to our surprise, therefore, we found that there was a substantial increase in oligomerization with manganese, both in the presence and absence of the template. It is possible that this difference may be due to an interaction of Mn(II) with the pyrophosphate groups of the analog template. Recent studies of coordination complexes between Mn(II) and CTP in water suggest that the metal coordinates with the phosphates of CTP rather than with the cytosine ring-system (Sigel et al. 1987). This type of interaction, which is stronger for Mn(II) than for Mg(II), may help to stabilize the catalytic complex between the pyrophosphate-linked template and monomer plus products, without interfering with base-pairing by cytosine.

The presence of an atactic template increased the total yield of oligomers from 18 to 27% in 0.2 M $MnCl_2$ and from 9 to 16% in 0.05 M $MnCl_2$ (Table 1). Under the more dilute conditions, the effect of the template was more obvious. As can be seen in Fig. 3, the longer oligomers (4–6-mers) are most noticeably affected. Although modest in terms of yields, these results essentially confirm the prediction by Joyce et al. (1987) that the flexibility of a glycerol-based pyrophosphate-linked polynucleotide analog might be sufficient to permit chain elongation even when the individual units of the polymer are partially atactic. The increase in the height of the cyclic dimer peak in the presence of the template, however, may indicate that chain elongation becomes more difficult with increasing lengths of products. This could reflect the statistics of distribution of isotactic segments of the template along the chain. It is therefore not yet clear whether chain extension is actually occurring on an atactic template or is dependent on the existence of short isotactic regions and chain sliding. It will be of interest to compare these results with those obtained with a fully isotactic template.

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References

Joyce GF, Schwartz AW, Miller SL, Orgel LE (1987) The case for an ancestral genetic system involving simple analogues of the nucleotides. Proc Natl Acad Sci USA 84:4398–4402

Schwartz AW, Visscher J, Bakker CG, Niessen J (1987) Nucleic acid-like structures II. Polynucleotide analogues as possible primitive precursors of nucleic acids. Origins Life 17:351–357

Sigel H, Tribolet R, Malini-Balakrishnan R, Martin RB (1987) Comparison of the stabilities of monomeric metal ion com-

plexes formed with adenosine 5′-triphosphate (ATP) and pyrimidine nucleoside 5′-triphosphates (CTP, UTP, TTP) and evaluation of the isomeric equilibria in the complexes of ATP and CTP. Inorg Chem 26:2149–2157

Spach G (1984) Chiral versus chemical evolutions and the appearance of life. Origins Life 14:433–437

Tohidi M, Orgel LE (1990) Polymerization of the cyclic pyrophosphates of nucleosides and their analogues. J Mol Evol 30:97–103

Visscher J, Schwartz AW (1988) Template-directed synthesis of acyclic oligonucleotide analogues. J Mol Evol 28:3–6

Visscher J, Schwartz AW (1989) Manganese-catalyzed oligomerizations of nucleotide analogues. J Mol Evol 29:284–287

Visscher J, Schwartz AW (1990) Oligomerization of cytosine-containing nucleotide analogs in aqueous solution. J Mol Evol 30:3–6

Visscher J, Bakker CG, van der Woerd R, Schwartz AW (1989) Template-directed oligomerization catalyzed by a polynucleotide analog. Science 244:329–331

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