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Oligomerization of Cytosine-Containing Nucleotide Analogs in Aqueous Solution

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Summary. Bisphosphoimidazolides of 2′-deoxycytidine and of its acyclic analog $\tilde{C}$ can be oligomerized in aqueous solution in the presence of Mn(II). Under certain conditions, a range of products extending to at least the 20mer can be obtained. These products are of interest as possible templates for oligomerization of the complementary monomers.

Key words: Chemical evolution — Nucleic acid analogs — Oligonucleotides — Metal ion catalysis

Introduction

Polyribonucleotides have been prepared in aqueous solution by using enzymatically synthesized polynucleotides as templates for the oligomerization of nucleoside phosphoimidazolides such as 2-MeImpG (Joyce et al. 1984). Pyrophosphate-linked polynucleotide analogs have similarly been synthesized by the oligomerization of bisphosphoimidazolides of 2′-deoxyribonucleosides or of acyclic nucleoside analogs on polynucleotide templates (Schwartz and Orgel 1985; Visscher and Schwartz 1988). Recently, a pyrophosphate-linked analog, oligo(pdCp), has been chemically synthesized and shown to catalyze the oligomerization of the complementary monomer (Visscher et al. 1989a). These studies have been undertaken as part of a program to develop models for primitive self-replicating systems. An obvious question to be answered is how the first templates for any such system might have arisen spontaneously. Pyrophosphate-linked polynucleotide analogs are also synthesized, albeit less efficiently, by oligomerization of bisphosphoimidazolides in the absence of a template (Schwartz et al. 1987). A limitation in these studies, however, has been the observation that only the purine-containing analogs produce long oligomers in aqueous solution. Because oligomers containing largely purines are not capable of serving as templates in either of the systems that have been studied to date, the question arises as to how the first pyrimidine-rich templates might have formed. Sawai (1988) has reported that the oligomerization of ImpC in aqueous solution is catalyzed by Pb(II). Under these conditions, 2′-5′-phosphodiester-linked oligomers are produced up to about the hexamer. It is not known whether 2′-5′-linked oligonucleotides have template activity. In the pyrophosphate-linked system, we have found that manganese ions are more effective than magnesium in catalyzing the oligomerization of purine-containing analogs in aqueous solution (Visscher and Schwartz 1989). We now report that the oligomerization of pyrimidine-containing analogs is also much improved in the presence of manganese, and that under certain conditions, oligomers as long as the 20mer are formed.

Materials and Methods

The preparation of $\tilde{C}$ was accomplished by condensation of silylated cytosine (Ogilvie et al. 1984) with 1,3-dibenzyloxy-2-chlo-

Abbreviations: $\tilde{C}$, 1-[(1,3-dihydroxy-2-propoxy)methyl]cytosine; $p\tilde{C}p$, the bisphosphate of $\tilde{C}$; dN (N = C, T, G, or A), the 2′-deoxynucleoside of cytosine, thymine, guanine, or adenine; pdNp, the 3′,5′-bisphosphate of dN; ImpdNpIm, the 3′,5′-bisphosphoimidazolide of dN; oligo(pdCp), 3′-5′-linked oligomers of pdCp; 2-MeImpG, the 5′-phospho-2-methylimidazolide of G; EDTA, ethylenediamine tetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; Bis-Tris, bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane

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romethoxypropane and a catalytic amount of tetra-*n*-butylammonium iodide in dry acetonitrile as described for the preparation of 1-[(1,3-dibenzyloxy-2-propoxy)methyl]guanine (Ogilvie et al. 1982). The protecting benzyl groups were removed by treating 1-[(1,3-dibenzyloxy-2-propoxy)methyl]cytosine with BCl_3 as described in Ogilvie et al. (1984).

$\tilde{C}$ was bisphosphorylated as described in Visscher and Schwartz (1988). The 3',5'-bisphosphate of 2'-deoxycytidine was prepared as described for the preparation of 2'-deoxyadenosine-3',5'-bisphosphate (Schwartz et al. 1987). pdTp was a gift of C.G. Bakker.

The bisphosphoimidazolide of dT was prepared as described in Schwartz et al. (1987). The bisphosphoimidazolides of dC or $\tilde{C}$ were prepared using a modification of the procedure described for the preparation of 5'-phosphoimidazolides of nucleotides by Joyce et al. (1984). The bisphosphates (70 μmol) of $\tilde{C}$ or dC were suspended in 1 ml of tetrahydrofuran plus 0.5 ml of triethylamine. To this suspension was added a solution containing 250 μmol of imidazole, 250 μmol of triphenylphosphine, 250 μmol of 2',2'-dithiodipyridine, and 500 μl of triethylamine in 2 ml of tetrahydrofuran. After 24 h at room temperature, the product was precipitated by treating with 100 μl of $NaClO_4$-saturated acetone and 100 ml acetone–diethylether (1:4). After washing the suspension five times with acetone–diethylether (1:4), the product was stored under vacuum over P_2O_5 and KOH.

Oligomerizations were carried out as described in Schwartz and Orgel (1985). The conditions are given in the tables.

After a reaction period of 2 or 4 weeks at 0°C, the reaction mixtures were quenched by adding 9 μl of EDTA (1.0 M, pH 9) and water to a total volume of 50 μl. Storage was at −25°C. Prior to analysis any surviving imidazolides were hydrolyzed overnight at room temperature by adding 2–4 μl of the quenched reaction mixture to 100 μl of sodium acetate (0.1 M, pH 4.0).

Analyses were performed by HPLC on RPC-5 (Joyce et al. 1984) in 0.02 M NaOH with a linear gradient of $NaClO_4$ (0.0–0.04 M over 60 min) at a flow rate of 1.0 ml/min. Peak detection was by absorbance monitoring at 254 nm. The cyclic-monomers, cyclic-dimers, and the pentamer of pdCp were verified by isolating these products from the RPC-5 column and treating with alkaline phosphatase and phosphodiesterase from venom, as described in Visscher and Schwartz (1988) and Schwartz et al. (1987).

Results and Discussion

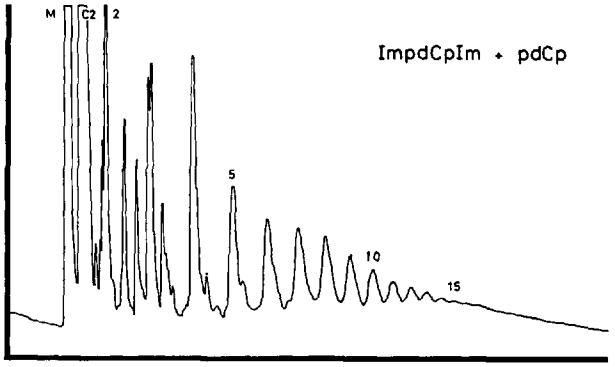
The mechanism of oligomerization of bisphosphoimidazolides at pH 6.5 probably involves a gradual hydrolysis to produce free phosphate groups, which then attack a neighboring phosphoimidazolide, producing either an internucleotide or an internal pyrophosphate bond. We have previously reported that Mn(II) catalyzes the oligomerization of ImpdGpIm and ImpdApIm at pH 6.5 more effectively than Mg(II), probably due to an inhibition of internal cyclization of the monomer (Visscher and Schwartz 1989). Table 1 compares the results for ImpdTpIm and ImpdCpIm at pH 6.5 with those previously obtained for ImpdApIm and ImpdGpIm. Although the total yield of oligomers produced from both pyrimidines is nearly doubled in the presence of Mn(II), there is no reduction in the extent of cyclization. This behavior can be correlated to some extent with reported differences in the structures of the Mn(II) complexes of purine and pyrimidine nucleotides. In the case of purine nucleotides, Mn(II) can interact with a phosphate group as well as with the purine ring system of the same molecule (Pezzano and Podo 1980). This interaction probably has the effect of restricting the freedom of motion of one of the two phosphates, reducing the rate of the cyclization reaction, and thereby favoring intermolecular condensation. A different situation exists with regard to the pyrimidines, for which Mn(II) can interact with the pyrimidine ring, but not simultaneously with a phosphate group of the same molecule (Pezzano and Podo 1980). It is not clear, however, why the oligomerization yields are increased in the presence of Mn(II). Of possible relevance is the description of a 1:1 complex of Mn(II) with cytidine 5'-phosphate in the solid state (Aoki 1976). In this structure, phosphate groups of neighboring molecules are closely coordinated with each other to form a three-dimensional network. Conceivably, an interaction of this kind may provide a more favorable environment for internucleotide condensation than the Mg(II) complex.

It is the hydrolysis of one of the two phosphoimidazolide groups of each molecule that provides an opportunity for cyclization. Thus, we have also studied the oligomerization of equimolar mixtures of the bisphosphoimidazolides and bisphosphates of dC and $\tilde{C}$ at pH 8.0, a pH at which the rate of hydrolysis is much reduced (Kanavarioti 1986). These results are presented in Table 2 and Figs. 1 and 2. By increasing the concentrations to 0.2 M, we were able to increase the total yield of oligomers for dC to 57%. The results with $\tilde{C}$ are less spectacular, although oligomers longer than the decamer can be detected in the chromatogram. The 13% yield of oligomers with chain lengths of 10 or more (23% of all oligomers) produced with dC is particularly interesting as these are potential templates for the oligomerization of ImpdGpIm. This fraction ap-

Table 1. Oligomerizations at pH 6.5 (0.5 M Bis-Tris HCl, 0.1 M NaCl, 2 weeks at 0°C)

Monomer (0.1 M)	MCl_2 (0.1 M)	CM (%)	M (%)	Incorporation of monomer into oligomers of length *n* (%)		
				$n \geq 2$	$n \geq 4$	$n \geq 10$
ImpdTpIm	Mg	45	30	18	2	—
ImpdTpIm	Mn	50	12	34	3	—
ImpdCpIm	Mg	44	30	27	4	—
ImpdCpIm	Mn	45	7	49	12	Trace
ImpdApIm	Mg	44	17	35	9	—
ImpdApIm	Mn	26	11	59	32	6
ImpdGpIm	Mg	53	14	34	13	4
ImpdGpIm	Mn	35	6	56	30	7

MCl_2 is the metal chloride, CM is the cyclic pyrophosphate of the monomer, and M is unreacted monomer



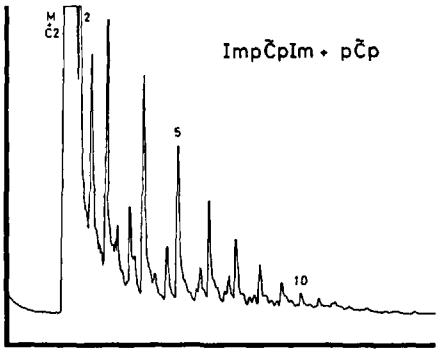


Fig. 1. Products of the oligomerization of ImpdCpIm + pdCp at pH 8.0 (C2 is the cyclic dimer; cyclic monomer is not shown)
Fig. 2. Products of the oligomerization of ImpC̃pIm + pC̃p at pH 8.0 (C2 is the cyclic dimer; cyclic monomer is not shown)

pears to extend beyond the 20mer (Fig. 1). The products undoubtedly contain a mixture of 3′-3′, 3′-5′, and 5′-5′ linkages (Visscher et al. 1989b), and thus, our expectation is that their activity as templates will be less than that of the 3′-5′-linked oligomers that we have recently reported on (Visscher et al. 1989a). A different situation may exist in the case of oligomers of the prochiral analog pC̃p (Fig. 2). These atactic oligomers possess a much more regular backbone geometry (Joyce et al. 1987) than the structurally heterogeneous oligomers of pdCp and may therefore display template activity even when randomly oligomerized. We expect to be able to test this supposition shortly.

We do not suggest that structures of this type necessarily represent molecules that were abundant on the prebiotic Earth. No reasonable prebiotic synthesis has yet been demonstrated for either C̃ or dC. Although mechanisms that could account for bisphosphorylation are known (Lohrmann and Orgel 1971; Schwartz et al. 1975), activation of the analog pC̃p is problematical because of the strong tendency to cyclize. Although this may be a formidable difficulty, it is in many ways less serious than the objections to β-D-ribonucleosides (Joyce et al. 1984, 1987). Furthermore, recent work on the oligomerization of cyclic pyrophosphates suggests that cyclization can be reversed (L.E. Orgel, personal communication). The studies described here may only be a first step toward developing a truly plausible model.

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Table 2. Oligomerizations at pH 8.0 (0.5 M Tris HCl, 0.1 M NaCl, 4 weeks at 0°C)

						Incorporation of monomer into oligomers of length n (%)			
ImpdCpIm	pdCp	MCl_2		CM (%)	M (%)	$n \geq 2$	$n \geq 4$	$n \geq 10$	$n \geq 14$
0.05 M	0.05 M	Mg	(0.2 M)	16	52	29	3	–	–
0.05 M	0.05 M	Mn	(0.2 M)	14	47	38	21	1	–
0.10 M	0.10 M	Mn	(0.6 M)	10	30	57	32	13	8
ImpC̃pIm	pC̃p								
0.10 M	0.10 M	Mn	(0.6 M)	21	54	23	7	1	–

MCl_2 is the metal chloride, CM is the cyclic pyrophosphate of the monomer, and M is unreacted monomer

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