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Hydrogen Bonding in the Template-Directed Oligomerization of a Pyrimidine Nucleotide Analogue

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Abstract. We have studied the oligomerization of an activated, achiral nucleotide analogue related to the pyrimidine barbituric acid in the absence and in the presence of a complementary, pyrophosphate-linked oligomer. Although no template-directed catalysis of the oligomerization was observed with water as solvent, catalysis of the oligomerization was demonstrated in a mixture of dimethylformamide with water. Poly(U) also stimulated the oligomerization, but was less effective than the analogue. Environments in which similar effects may be observed, and some potential implications for prebiotic chemistry, are discussed.

Key words: Nucleotide analogues — Oligomerization — Prebiotic chemistry — Molecular recognition — Solvent polarity — Hydrogen bonding

Introduction

The template-directed oligomerization of mononucleotides (Inoue and Orgel 1982) depends upon the formation of a hydrogen-bonded, helical complex between a polynucleotide template and complementary monomers and oligomers. Under certain conditions, such as in the polycytidylic-acid-directed oligomerization of guanosine 5′-phosphoro(2-methyl)imidazolide, a double-stranded, Watson-Crick–paired duplex is formed (Miles and Frazier 1982). Other examples of catalysis which probably depend upon a combination of Watson-Crick and Hoogsteen base pairs have also been described (Inoue and Orgel 1982; Huang and Ts'o 1966). The stabilities of nucleic acid duplexes in water depend to a major extent upon stacking interactions between neighboring bases. The weakness of such interactions between pyrimidines explains the failure to achieve polypurine-directed oligomerization of pyrimidine nucleotides (Stribling and Miller 1991). One of the major problems in constructing scenarios for the nonenzymatic replication of RNA sequences has been the recognition that, because stretches of purines could not be transcribed efficiently, a serious block to replication existed (Joyce and Orgel 1993). Recent experiments using hairpin-loop initiators suggest that strings of purines may be transcribable, but only for *uninterrupted* sequences (Hill et al. 1993).

We have reported the synthesis and oligomerization in aqueous solution, as well as in organic solvents, of several new nucleotide analogues which can be regarded as derivatives of barbituric acid (Van Vliet et al. 1994a,b). Oligomers I and II (Fig. 1), which are 5,5-di(phosphoethyl)-2,4,6-triamino- and 2,4,6-trioxopyrimidines, respectively, can theoretically form a hydrogen-bonded complex (as shown in Fig. 2A). However, a spectroscopic study of oligomers I and II detected no decrease in extinction coefficient on mixing in aqueous solution, nor was hyperchromicity observed upon hydrolysis of oligomer I (Van Vliet et al. 1994a). The weakness of the stacking interactions between the pyrimidine ring systems suggested by these results made it unlikely that template-directed oligomerization of monomer I by oligomer II would be successful in aqueous solution—a

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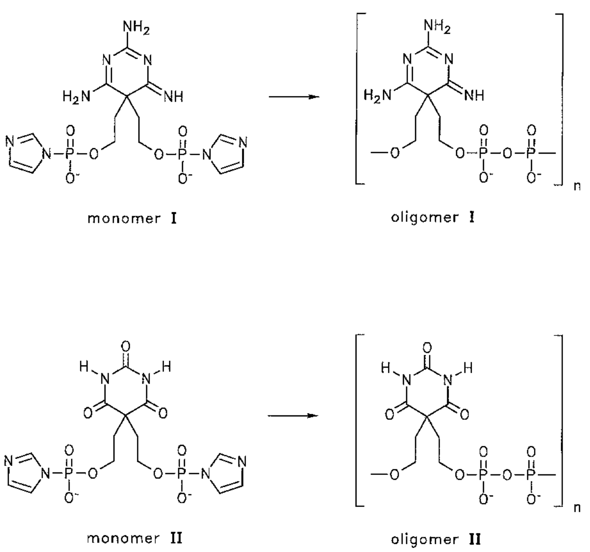

Fig. 1. The structures of monomers and oligomers studied.

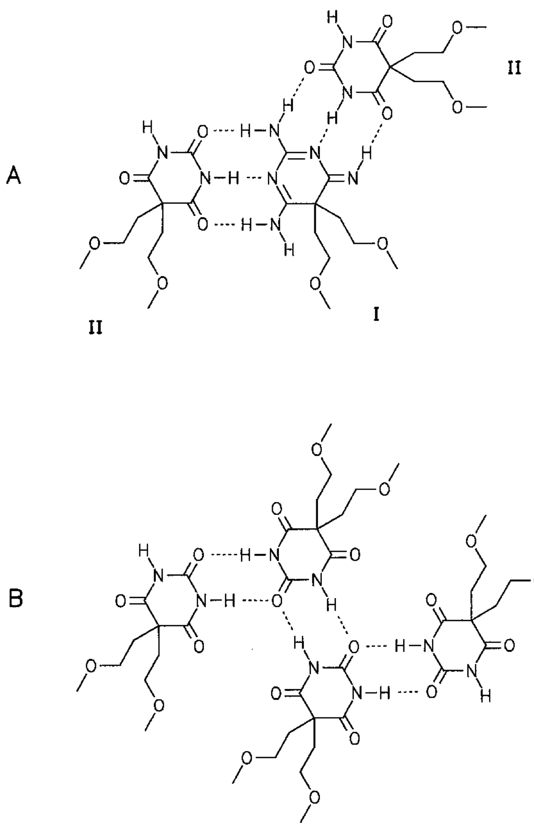

Fig. 2. Possible structures of hydrogen-bonded complexes of oligomers I and II. **A** 2:1 complex of oligomer II with monomer I. **B** Self-complementarity of oligomer II.

conclusion which was confirmed in preliminary experiments. Because decreasing the polarity of the solvent might sufficiently increase the role of hydrogen bonding (Petersen and Led 1981) so as to favor a template-directed oligomerization, we conducted experiments in DMF/H_2O (1:1, v/v).

Materials and Methods

Ribonuclease (type I-A from bovine pancreas), polyuridylic acid (poly(U)), alkaline phosphate (type III from *Escherichia coli*), and pyrophosphatase (type II from *Crotalus adamanteus* venom) were purchased from Sigma. HPLC was performed on an RPC-5 column in 0.02 M NaOH with a linear gradient of $NaClO_4$ (0–0.02 M over 30 min) at a flow rate of 1.0 ml/min. The eluent was monitored at a wavelength of 240 or 280 nm. The synthesis of the monomers I and II has been described previously (Van Vliet et al. 1994a). Oligomer II with a chain-length $n > 18$ was synthesized and isolated as described in an earlier paper (Van Vliet et al. 1994a).

Oligomerization Reactions in Aqueous Solution. All reactions were performed in Eppendorf tubes in a total volume of 10 μl. First solutions of oligomer II (37 μl, 0.027 M), $MgCl_2$ (4.0 μl, 1.0 M), and NaCl (1.0 μl, 1.0 M) were added to each tube. The mixtures were concentrated to dryness and the residues were dissolved in imidazole-HCl buffer (4.0 μl, 1.0 M, pH 6.5). Then a freshly prepared, cold solution of monomer I (6 μl, 0.17 M) was added at each tube at 0°C and the contents were mixed. After centrifugation the reaction mixtures were incubated at 1°C for several weeks. The reactions were quenched by addition of 2 equivalents EDTA per divalent metal ion, diluted with water to a total volume of 100 μl, and stored at a temperature of −25°C. Before HPLC analysis aliquots with a theoretical monomer content of 0.05 μmol were taken from the mixtures. Surviving imidazolides were hydrolyzed by incubation in sodium acetate (NaAc) buffer (pH 4.0, 0.1 M, 100 μl) for 1 h at 50°C. Reactions without oligomer II were incubated under identical conditions.

Oligomerization Reactions in Water-DMF Mixtures. Each reaction was performed in an Eppendorf tube. The following procedure was used for most experiments. Solutions of oligomer II, if required, and $MgCl_2$ were added to each tube, and evaporated to dryness. To the residues was added DMF followed by a freshly prepared solution of activated monomer in imidazole-HCl buffer (pH 6.5) at 0°C. The reactions were mixed thoroughly, centrifuged, and incubated at 1 or 37°C for various times. The reactions were quenched and prepared for HPLC analysis as described above. All reactions contained 0.4 M $MgCl_2$, 0.4 M imidazole-HCl buffer (pH 6.5), 0.1 μmol monomer I, and oligomer II (0, 1, 2, or 4 monomer equivalents) in 1:1 (v:v) water/DMF. The concentration of the monomer was reduced by increasing the volume of the reaction mixtures. Thus the volume of the reaction mixture was 10 μl with a monomer concentration of 0.01 M, 40 μl with 0.0025 M monomer, and 100 μl with 0.001 M monomer. Control experiments were conducted in which oligomer II was replaced by monomeric II, as the unphosphorylated bis-hydroxy compound (1, 2, and 4 equivalents). The whole series of reactions was also performed in the presence of the polynucleotide poly(U) (1, 2, and 4 monomer equivalents) using essentially the same procedure. Before HPLC analysis poly(U) was destroyed by ribonuclease digestion.

Enzyme Digestions and Chemical Hydrolysis. Ribonuclease digestions were performed on samples (0.05 μmol monomer equivalent) of the quenched reaction mixtures in Tris-HCl buffer (0.05 M, 100 μl, pH 7.6) containing 10 units of enzyme and incubated for 4 h at 37°C. Pyrophosphatase digestions were performed on isolated oligomers (0.05 ODU) in a Tris-HCl buffer (0.1 M, 100 μl, pH 7.2) containing 0.04 M $MgCl_2$ with 0.2 units enzyme. Incubation was for 5 h at 37°C. Alkaline phosphatase treatment was performed on samples in a Tris-HCl buffer (0.04 M, 100 μl, pH 8.0) containing $MgCl_2$ (0.02 M) with 0.2 units enzyme for 4 h at 37°C. After incubation with the enzymes EDTA (4 μl, 1.0 M, pH 9.0) was added and the mixtures were analyzed by HPLC. Cleavage of the pyrophosphate linkages was performed by treatment with $ZrCl_4$. To samples of isolated oligomers (75 μl, 0.05

Table 1. Product distributions in oligomerizations of monomer I (0.0025 M) in 50% DMF: effects of oligomer II and poly(U)[a]

Oligomer II (M)[b]	Poly(U) (M)[b]	Unreacted monomer (%)	Relative yield of oligomers of length n (%)				
			$n \geqslant 2$	$n \geqslant 3$	$n \geqslant 4$	$n \geqslant 5$	$n \geqslant 10$
—	—	56	44	12	4.9	2.0	
0.0025	—	55	45	15	8	3.8	0.3
0.005	—	55	45	23	13	8	0.7
0.01	—	53	47	26	16	10	0.8
—	0.0025	54	46	15	7	2.6	
—	0.005	56	44	14	7	2.4	
—	0.01	53	47	16	7	2.8	

[a] Conditions: 0.0025 M monomer I, 0.4 M $MgCl_2$ and 0.4 M imidazole buffer-HCl (pH 6.5) in DMF/water (1:1, v/v) for 5 weeks at 1°C
[b] Monomer equivalent

ODU) a solution of NaAc (13 µl, 3.0 M, pH 5.0) and $ZrCl_4$ (3 µl, 1.0 M) was added and the mixtures were incubated for various times at 50°C. The reactions were quenched by addition of EDTA (9 µl, 1.0 M, pH 9.0) and neutralized with NaOH (6.7 µl, 10 M). After filtration the mixtures were analyzed by HPLC.

Identification of Isolated Oligomers. Oligomers were isolated by HPLC on an RPC-5 column and neutralized with HCl (6 M). Oligomers of the preparative synthesis in water-DMF mixtures were isolated by Q-Sepharose with a linear gradient of TEAB. Isolated oligomers were degraded by $ZrCl_4$ and by pyrophosphatase to establish the length of the oligomers. For example, a pentamer was hydrolyzed with $ZrCl_4$, producing the tetramer, trimer, dimer, and monomer in increasing proportions with time. Similar results were achieved by digestion with pyrophosphatase. Terminal phosphate groups of isolated oligomers were removed by treatment with alkaline phosphatase, which resulted in the formation of a single peak on HPLC with a smaller retention time than the parent compound.

Results and Discussion

Table 1 summarizes the results of oligomerization experiments carried out with monomer I at a concentration of 0.0025 M. The results show that oligomerization of the monomer in 50% DMF is indeed stimulated by the addition of 1, 2, and 4 equivalents of oligomer II. Figure 3 compares the HPLC analyses of the products obtained with 0 and 2 equivalents of the oligomer. The yields of the longest products were most strongly increased. After 5 weeks of reaction at 1°C, for example, the yield of oligomers with lengths of 5 or more increased fourfold in the presence of two equivalents of oligomer II, although the total yield of all oligomers increased only from 44 to 45%. Inspection of the chromatograms suggests that it is primarily oligomers (dimers and longer) that condense to form longer products in the presence of the template (data not shown). Essentially the same results were obtained when the oligomerization was conducted at 20°C for 2 days.

Oligomer I is expected to be capable of forming a complex with two equivalents of oligomer II, as in Fig. 2A, although self-association of both analogues is also possible. The largest increase in the yield of the longest oligomers occurs after addition of two equivalents of oligomer II. The continued—although more moderate—increase in extent of oligomerization observed as the concentration of oligomer II is increased from 2 to 4 equivalents probably results from competition between self-structure of the template (Fig. 2B) and complex for-

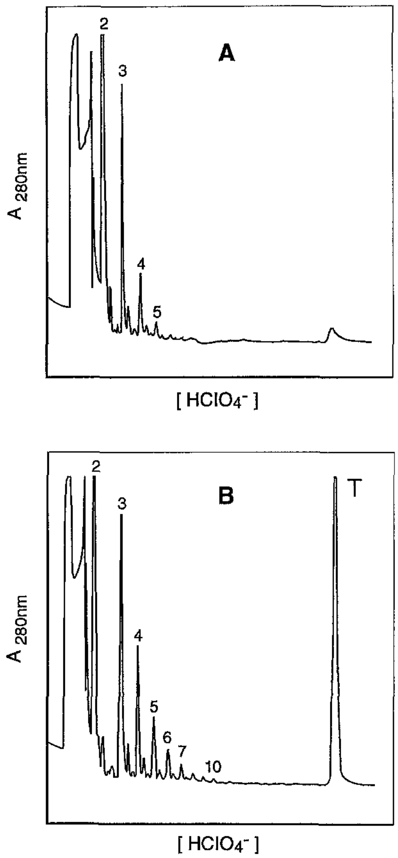

Fig. 3. HPLC chromatograms of oligomerization products of monomer I in DMF/H_2O (1:1, v/v). **A** Monomer I alone. **B** Monomer I in the presence of two equivalents of oligomer II. Conditions: 0.0025 M monomer, 0.4 M $MgCl_2$ and 0.4 M imidazole HCl (pH 6.5), 5 weeks at 1°C. The minor peaks visible between the labeled oligomers are due to the presence of a few percent of a biproduct produced during imidazolization of the monomer. The peak labeled *T* is due to elution of the template (oligomer II).

Table 2. Product distributions in oligomerizations of monomer I (0.01 M) in 50% DMF: effect of poly(U)[a]

Poly(U) (M)[b]	Unreacted monomer (%)	Relative yield of oligomers of length n (%)				
		$n \geq 2$	$n \geq 3$	$n \geq 4$	$n \geq 5$	$n \geq 10$
—	31	69	41	16	1.8	0.14
0.01	25	75	47	22	4.4	0.9
0.02	24	76	45	22	4.2	0.9
0.04	24	76	46	23	4.7	1.0

[a] Conditions: 0.01 M monomer I, 0.4 M $MgCl_2$ and 0.4 M imidazole buffer-HCl (pH 6.5) in DMF/water (1:1, v/v) for 5 weeks at 1°C
[b] Monomer equivalent

mation between oligomers I and II. In a series of control experiments, we found that adding 1 to 4 equivalents of monomeric II (as the unphosphorylated bis-hydroxy compound) had no effect on oligomerization of monomer I. This observation supports the conclusion that the catalysis is due to a template effect exerted by oligomer II (i.e., the condensation being favored along the chain) rather than through the formation of two-dimensional hydrogen-bonded sheets similar to those observed for the complexes barbituric acid–triaminopyrimidine (Lehn et al. 1990) or cyanuric acid–melamine (Seto and Whitesides 1990). To support the view that the observed template catalysis in 50% DMF is a general effect, due to the decreased polarity of the solvent, and not specific to DMF, we also conducted experiments in 50% DMSO. The polarity of both solvents is similar and, as expected, similar results were obtained (not shown).

Because of the resemblance of uracil to the barbituric acid–like rings of oligomer II, we also conducted experiments in the presence of poly(U). In 50% DMF no stimulation of the reaction was observed at a monomer concentration of 0.0025 M (Table 1), but at a concentration of 0.01 M an effect was observed (Table 2). After 5 weeks at 1°C the total conversion of monomer to oligomer increased from 69 to 76% in the presence of poly(U), and the yield of oligomers with lengths of 10 and more increased by about twofold. In contrast to the experiments using oligomer II as template, addition of 2 or 4 equivalents of poly(U) produced no further stimulation of the reaction. This observation is in accord with the known properties of poly(U), for which self-structure is negligible. The rather moderate effect of the poly(U) template contrasts with earlier results obtained in the oligomerization of acyclic nucleoside analogues related to glycerol (Visscher and Schwartz 1988). However, oligomer I is structurally further removed from poly(U) than were the earlier analogues, and constraints in the backbones of both oligomers may disfavor a conformation in which a hydrogen-bonded complex is possible.

In organic solvents, where hydrogen bonds are many times stronger than in aqueous solution, a number of examples of self-organization and molecular recognition which depend solely on hydrogen bonds are known. (See, for example: Branda et al. 1994; Chang et al. 1991; Murray and Zimmerman 1992; Jorgensen and Severance 1991). However, there are relatively few reports of such complex formation being utilized to catalyze a chemical reaction. Catalysis of a bimolecular reaction has been reported (Kelley et al. 1989), and especially interesting are examples of self-replicating dimer formation (Tjivikua et al. 1990; Terfort and Kiedrowski 1992). A question which these studies as well as the present work raises is whether environments could have existed on the prebiotic Earth in which enhanced hydrogen bond formation would have been likely. At least two different kinds of environments seem possible. Vesicles and micelles are known to effectively concentrate reactants into apolar internal compartments, and in one case this effect has been demonstrated by the formation of hydrogen-bonded base pairs between derivatives of adenine and uracil (Nowick et al. 1993). An additional possibility is suggested by the observation that monolayers at an air–water interface display enhanced hydrogen bonding, and nucleotides and nucleic acid bases have been shown to bind to diaminotriazine-functionalized monolayers on water (Kurihara et al. 1991). The evolutionary importance of phase separation and compartmentalization has been emphasized repeatedly (Oparin 1957; Fox and Dose 1977; Eigen et al. 1981; Walde et al. 1994), and lipid-like molecules seem to be formed readily in prebiotic experiments and are present in meteorites (Deamer and Pashley 1989). It may therefore be worth entertaining the possibility that environments in which reduced solvent polarity led to enhanced hydrogen bonding may have played a role in prebiotic chemistry.

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