OLIGOMERIZATION OF DEOXYNUCLEOSIDE-BISPHOSPHATE DIMERS: TEMPLATE AND LINKAGE SPECIFICITY*

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Abstract. Evidence is presented that a poly(U) template selectively favors the oligomerization of the activated, 3′–5′ pyrophosphate-linked dimer pdAppdAp, in comparison with the 3′–3′ and 5′–5′ linked dimers. In the absence of poly(U), the 5′–5′ linked dimer is the most reactive, and chains are formed which are more than 60 monomer units in length.

1. Introduction

2′-Deoxynucleoside-3′,5′-bisphosphorimidazolides containing adenine or guanine oligomerize to produce long, pyrophosphate-linked oligomers in the presence of the complementary polynucleotide template (Schwartz and Orgel, 1985). In the absence of a template, oligomerization still occurs, but is much less efficient (Schwartz *et al.*, 1987). In order to provide information on the ability of the polynucleotide template to direct the mode of linkage of the products, we have synthesized the 3′–3′, 5′–5′ and 3′–5′ linked dimers of pdAp and investigated their oligomerization in the presence and in the absence of a poly(U) template.

2. Experimental

Alkaline phosphase (type III from *E. coli*), bovine pancreatic ribonuclease (type I-A), and poly(U) were purchased from Sigma Chemical Company. EDAC was purchased form Janssen Chimica. The dimers of pdAp linked by 3′–3′, 3′–5′ or 5′–5′ pyrophosphate bonds were synthesized as described by Woerd *et al.* (1987). Imidazolation of the free phosphates of the dimers was carried out in solution as follows. Dimers (0.05M) were reacted with 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride (EDAC, 1.34M) and imidazole (1.34M) at pH 6.5 and 0° C. After two hours the formation of the bis-phosphoimidazolides was at least 90% complete, as determined by HPLC and ^{31}P-NMR (^{31}P-NMR shifts relative to phosphoric acid in D_2O: 3′–5′ $(pdAp)_2$: 3.5, 3.2, –8.6, and –9.5 ppm; bis-phosphoimidazolide of 3′–5′ $(pdAp)_2$: –6.4, –7.3, –9.5, and –10.0). After activation, 2.5 µl portions of this solution were added to Pyrex tubes containing 2 µmol $MgCl_2$, 1 µmol NaCl and, when required, 0.5 µmol poly(U) (monomer-equivalents). Water was added to produce a final volume of 10 µl. The final

* Nucleic Acid-Like Structures V. For the previous paper in this series see Visscher and Schwartz (1988).

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concentrations were 0.0125M dimer-bisphosphoimidazolide, 0.2M $MgCl_2$, 0.1M NaCl, approximately 0.3 imidazole and, if added, 0.05M poly(U) (monomer-equivalents). Reactions were carried out at 4° C and pH 6.5 for two weeks. At the conclusion of the reaction, 2.5 µl of KEDTA (1M, pH 9.0) and 7.5 µl of $K_4P_2O_7$ (1M, pH 9.0) were added and the reaction mixture was stored at –25° C. Analytical techniques have been described previously (Schwartz *et al.*, 1987). Thus, prior to analysis poly(U) was destroyed by digestion with pancreatic ribonuclease, and surviving imidazolides were destroyed by hydrolysis at pH 4.0. Analysis was by HPLC on RPC-5 in 0.02M NaOH with a linear gradient of $NaClO_4$ (0 to 0.04M over 60 min) at a flow rate of 1.0 ml/min. Peak detection was by absorbance monitoring at 254 nm. Identification of the cyclic dimers was aided by treatment with alkaline phosphatase. Identification of other oligomers of pdAp was aided by coinjection with previously identified oligomers obtained form ImpdApIm.

3. Results and Discussion

Of the three dimers of pdAp studied, only the 3′–5′ linked molecule showed a dramatic template effect (Figure 1 and Table I). Both overall yields as well as the production of the longest oligomers were markedly stimulated by the presence of poly(U). Although the 3′–3′ and 5′–5′ isomers showed some increase in yields in the presence of template, the effect was largely confined to oligomers less than 20 monomer units in length. The oligomerization in the absence of template was very different. The longest oligomers as well as the highest overall conversions of dimer to oligomers were produced in the order: 5′–5′ > 3′–3′ > 3′–5′.

Remarkably, the presence of the template seemed to *inhibit* production of the longest oligomers from the 5′–5′ isomer. It seems a reasonable hypothesis that the most stable structure formed by template and product oligomers is that in which the axial distance between base-pairs along the chain remains constant. The 3′–5′ linked dimer can be ordered in this manner by the template. The 3′–3′ and 5′–5′ dimers, however, can obviously only form staggered structures. In line with this reasoning, it is likely that the strong enhancement in yields of the longest oligomers that is caused by the presence of a poly(U) template in the oligomerization of the monomer ImpdApIm, also reflects the preferred formation of 3′–5′ linkages (Schwartz *et. al.*, 1987).

The self-organizing properties of the 5′–5′ dimer of pdAp are quite remarkable. It should be noted that in spite of the low dimer concentration of 0.0125M, at which cyclization is strongly favored above oligomerization, the reaction in the absence of template produced oligomers with chain lengths exceeding 60 monomeric units.

4. Abbreviations

EDAC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; KEDTA, the potassium salt of ethylene diamine tetraacetic acid; poly(U), polyuridylic acid; dA, 2′-deoxyadenosine; pdA, the 5′-phosphate; dAp, the 3′-phosphate; pdAp, the bis-phosphate; pdAppdAp, the P^1, P^2 (3′–5′)-dinucleotide pyrophosphate (brackets between 3′– and 5′-phosphates indicate other isomers); ImpdApIm, the bis-phosphoimidazolide of dA.

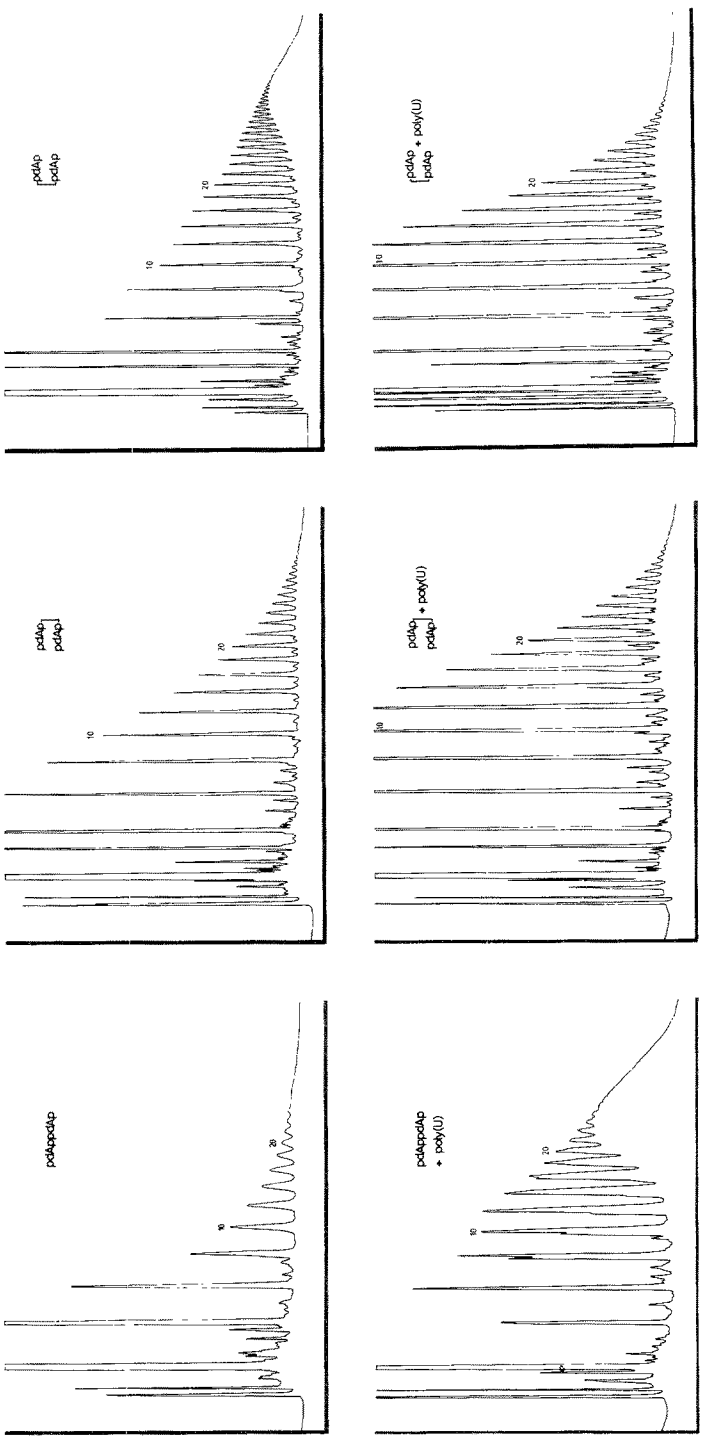

Fig. 1. HPLC separations of the products of oligomerizations of 3′–5′, 3′–3′ and 5′–5′ linked dimers of pdAp, after activation by conversion to diimidazolides. For conditions and product distributions see Table I.

TABLE I

Product Distributions in the Oligomerization of Dimers of pdAp*

Dimer	Template	Cyclization of Dimer (%)	Yield of Higher Oligomers of Length n (%)		
			$n \geq 4$	$n \geq 10$	$n \geq 20$
3′–5′	poly(U)	13	84	69	37
	–	74	21	6	1
5′–5′	poly(U)	41	55	34	12
	–	68	30	19	13
3′–3′	poly(U)	42	53	34	10
	–	73	26	13	6

* Reaction conditions: 0.0125 M Dimer-diimidazolide, 0.2 M $MgCl_2$, 0.1 M NaCl, pH 6.5 in imidazole-EDAC mixture (0.3M) at 4 °C for 2 weeks. See Experimental section for details of the imidazolation.

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