Polynucleotide phosphorylase forms polymers from an ADP analog in which the 5' oxygen is replaced by a methylene group

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RNA molecules in which particular nucleoside 5' oxygens are replaced by methylene groups would be useful in mechanistic studies on phosphodiester cleavage reactions that involve the breaking of the bond between the phosphorus and the 5' oxygen. Phosphodiester linkages containing this substitution will be resistant to cleavage by alkali, cyclizing ribonucleases, certain other endonucleases, and exonucleases that produce breakdown products with terminal 3' phosphate groups. Moreover, oligoribonucleotides possessing specific linkages of this type will be of value in studying the cleavage mechanisms employed by a number of ribozyme structures that have been recently described (1). We report here an observation that can lead to the synthesis of molecules incorporating such resistant linkages: in the presence of the primer A-A and an ADP analog (ppcA) containing a methylene group in place of the 5' oxygen, polynucleotide phosphorylase yields products of the form A-A(pcA)_n (where pcA represents the 5'-deoxy-5'-phosphonomethyladenosine moiety).

Synthesis of the ADP and ATP analogs ppcA and pppcA. The mononucleotide analog 5'-deoxy-5'-phosphonomethyladenosine (pcA) was prepared by the method of Jones and Moffatt (2), and the material was used to synthesize ADP and ATP analogs as potential substrates for polymerizing enzymes. The ATP analog pppcA was obtained by condensation of pcA with pyrophosphate, using the method developed by Hoard and Ott (3) for the conversion of AMP to ATP. A modification of this method, previously used for preparing nucleoside diphosphates from mononucleotides (4), was adapted for the reaction of pcA with the phosphate anion to give the ADP analog ppcA. These analogs were purified by paper chromatography on Whatman 3MM paper using the solvent system *n*-propyl alcohol-conc. ammonia-water (55:10:35, v/v).

Polymerization of ppcA. Enzymatic reactions were conducted in 100 mM Tris HCl (pH 9.0 at 25°C) and contained 1 mM A-A as primer, 5 mM ppcA, 10 U/ml primer-independent *M. luteus* polynucleotide phosphorylase, and 10 mM MgCl₂ (or MnCl₂). After incubation for 8 hours at 37°C, the mixture was heated at 85°C for 5 min., and any precipitate that formed was removed by centrifugation. Under these conditions more than 90% of the ppcA was converted into oligonucleotide products.

Separation of products. Reaction products were separated by

anion-exchange HPLC using a column (25×1 cm) of cross-linked polyethyleneimine-silica (5) with a solvent system consisting of 200 ml of aqueous 0.05 M KH₂PO₄–30% (v/v) MeOH containing a linear gradient of 0–0.6 M (NH₄)₂SO₄ at pH 6. The products were eluted at a flow rate of 1 ml/min. and were detected by their absorbance at 254 nm. The elution volumes (ml) measured for the starting materials A-A and ppcA were 24 and 66, while the volumes (ml) obtained for the series A-A(pcA)_n (where n = 1 through 10) were 41, 51, 63, 76, 90, 104, 117, 131, 144, and 156, respectively. These values differ only slightly from the series of elution volumes obtained for the corresponding set of oligomers (A-A_n-A, where n = 1 through 10) that was generated by a polymerization reaction carried out as described above, but with ADP in place of the analog ppcA.

Product characterization. The oligonucleotide fractions were desalted by dialysis and subjected to a number of chemical and enzymatic treatments to confirm their structures. Products from these experiments were identified by anion-exchange chromatography using the system described above. For example, spleen phosphodiesterase treatment of A-A(pcA)₂ gave equimolar amounts of adenosine 3'-phosphate and A(pcA)₂. The resistance to enzymatic cleavage exhibited by the two phosphonomethylene linkages is in agreement with the recorded inability to hydrolyze the chemically synthesized dinucleotide phosphate analogs UpcA and UpcU with the same enzyme (6). Exposure of A-A(pcA)₂ to alkali gave, as expected, the same trimer A(pcA)₂ together with a mixture of adenosine 2'- and 3'-phosphates. Degradation with snake venom phosphodiesterase or with S1 nuclease gave one equivalent each of adenosine and adenosine 5'-phosphate and two equivalents of pcA. Similar experiments with some of the larger oligomers formed in the polymerization gave degradation products consistent with their assigned structures.

Physical properties of oligomers. Absorbance-temperature measurements carried out on A-A(pcA)₈ over the range 0–80°C show that this decamer has about the same hyperchromicity as that observed for A-A₈-A, indicating that its adenine moieties undergo some form of base-stacking. Furthermore, an equimolar mixture of A-A(pcA)₈ and U-U₈-U gave a melting curve similar to that obtained from a mixture of A-A₈-A and U-U₈-U. This suggests that the presence of

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phosphonomethylene linkages does not interfere with the ability of these oligomers to adopt structural conformations like those occurring in normal base-paired duplexes.

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