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Catalysts for the Self-Polymerization of Adenosine Cyclic 2′,3′-Phosphate

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Summary. When adenosine cyclic 2′,3′-phosphate is evaporated from solution in the presence of simple catalysts such as aliphatic diamines at alkaline pH, and maintained in a dry state at moderate temperatures (25–85 °C), self-polymerization to give oligonucleotides of chainlength up to at least 6 is observed. The products contain an excess of [3′→5′]-linkages over [2′→5′]-linkages. The effects of different catalysts and reaction conditions on the efficiency of the reaction are described. The prebiological relevance of these reactions is discussed.

Key words: Polymerization — Phosphorylation — Adenosine Cyclic 2′,3′-phosphate — Oligonucleotides — Amines — 2-Aminoethanol — Amino Acids — Prebiotic.

Introduction

If nucleic acids were important during the earliest phases of the evolution of life, polynucleotides must have formed non-enzymatically on the primitive earth. Despite a considerable number of experimental studies, no plausible prebiotic synthesis of polynucleotides has been reported, although some progress has been made.

Short oligonucleotides are formed during the thermal phosphorylation of uridine (Moravek and Skoda, 1967; Österberg, Lohrmann, and Orgel, 1973) and when uridine 2′(3′)-phosphate is heated with uridine (Moravek, Kopecky, and Skoda, 1968; Skoda, Moravek, and Kopecky, 1969); cytidine cyclic 2′,3′-phosphate polymerizes to oligomers up to the hexamer at very high temperatures (Tapiero and Nagyvary, 1971). Imidazoles are claimed to catalyze the polymerization of thymidine 5′-monophosphate in aqueous solution (Ibanez, Kimball, and Oro, 1972), but the total yield of polymerized material is very low. None of these reactions yields subtantial amounts of polynucleotide material at moderate temperatures (0–70 °C).

The cyclic 2′,3′-phosphates are the major products of the urea-catalyzed phosphorylation of nucleosides at moderate temperatures in the solid state (Lohrmann and Orgel, 1971). Adenosine cyclic 2′,3′-phosphate polymerizes on a poly (U) template in aqueous solution, in the presence of amines, to give short oligonucleotides (Renz, Lohrmann, and Orgel, 1971). We have

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therefore investigated the direct (i.e. non-template-directed) synthesis of oligonucleotides from adenosine cyclic 2′,3′-phosphate in the solid state, in the presence of amines and related catalysts.

Experimental

Materials

Reagent grade chemicals were used throughout, unless otherwise specified. 1,2-Diaminoethane, 1,3-diaminopropane, and 1,5-diaminopentane (Aldrich) were distilled before use; spermidine and spermine (Sigma) were used without further purification. Catalyst solutions were generally made up as 1M aqueous stock solutions and titrated to the desired pH with HCl.

A and Ap were from Calbiochem and pA was from Schwartz; A > p was supplied by Sigma as the sodium salt, and converted to the ammonium form with Dowex 50 X-8 before use. [8-^{14}C] Ap (Schwartz) was cyclized to A > p by the method of Smith, Moffatt, and Khorana (1958) and purified by paper chromatography in System I (q. v.). ^{33}P-labelled A > p was prepared on a 1 mc scale by the following modification of the methods of Biebricher (1973) and Lohrmann and Orgel (1971) using $H_3{}^{33}PO_4$ (from ICN, supplied as a solution in 0.01 N HCl).

Reaction mixtures (A, 2.5 μmole; $NH_4H_2PO_4$, 1 μmole; urea, 10 μmole; $H_3{}^{33}PO_4$, 1 mc, and sufficient NH_3 to neutralize the HCl) were dried onto the surface of glass tubes by continuous evacuation (oil-pump) over phosphorus pentoxide (4 hrs) before heating at 100° and ambient humidity for 42 hrs. After dissolving in water (5 ml) and adjusting to pH 7 cold A > p marker (2 μmole) was added and the reaction products were applied to a 0.7 × 5 cm column of QAE Sephadex A25 (Pharmacia), bicarbonate form, and eluted with a linear ammonium bicarbonate gradient (0–0.5 M; 2 × 100 ml). The fractions with the highest ratio of optical density to radioactivity were pooled, lyophilized, reapplied to a similar column and then eluted with a less steep, linear bicarbonate gradient (0–0.2 M; 2 × 100 ml). The second peak eluting from this column was identified as A > p from its paper chromatographic and electrophoretic mobilities and also by digestion with ribonuclease T_2 and acid phosphatase (q.v.).

A^3pA was supplied by Sigma and the oligoadenylates, ApA > p through $(Ap)_5A > p$ by Miles Laboratories. Ribonuclease T_2 (1000 units/mg) was supplied by Worthington (stock solution, 1 mg/ml); polynucleotide phosphorylase (from *Micrococcus lysodeikticus;* 2000 units/ml) and acid prostatic phosphatase (from human semen; 500 units/ml) were a gift from Dr. C. K. Biebricher.

Chromatography and Electrophoresis

Paper chromatography was carried out on Whatman 3 MM paper by the descending technique. The solvent systems used were I, isopropanol-conc. ammonia-water (7:1:2, v/v); II, n-propanol-conc. ammonia-water (55:10:35, v/v). Mobilities are summarized in Table 1. Reaction products were identified by co-chromatography with authentic material in both systems.

Paper electrophoresis was carried out in a high voltage tank (at 3000 v) on Whatman 3 MM paper using Varsol as coolant. Buffer systems used were III, 0.03 M potassium phosphate, pH 7.1 and IV, 0.05 M formic acid, adjusted to pH 2.7 with ammonia.

Chromatograms were passed through a Baird Atomic RSC 363 scanner with integrator. Yields were calculated by summation of the total counts on the paper and,

Abbreviations: A = adenosine; A > p = adenosine cyclic 2′,3′-phosphate; Ap = adenosine 2′(3′)-monophosphate; pA = adenosine 5′-monophosphate; A^3pA = adenylyl-[3′→5′]-adenosine; $(Ap)_nAp$ = oligoadenylates; poly (u) = polyuridyic acid.