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Analysis of High Molecular Weight Material from the Polymerization of Adenosine Cyclic 2′,3′-Phosphate

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Abstract. Adenosine cyclic 2′, 3′-phosphate has been polymerized at room temperature in the dry state using 1, 2-diaminoethane at alkaline pH as catalyst. The high molecular weight products have been analyzed by several different methods, including ion exchange and gel permeation chromatography and phosphate end-group analysis. Gel permeation chromatography showed that between 3.6% and 8.1% of material higher than the hexamer was formed in 3-, 15- and 40-day reactions. The material from a large-scale, 3-day reaction mixture was first fractionated by ion exchange chromatography and the highest molecular weight material then further fractionated by gel permeation chromatography. End-group analysis on the fractions obtained by this procedure showed that 0.67% of the total reaction mixture was polymer with an average chainlength of 13.4; a trace (0.15%) of higher polymer was also formed, but there was insufficient to determine its average chainlength. The prebiological relevance of these findings is discussed.

Key words: Adenosine Cyclic 2′, 3′-Phosphate — Polymerization — 1, 2-Diaminoethane — Oligonucleotides — Prebiotic — Gel Permeation — Ion Exchange.

Introduction

In an earlier paper (Verlander, Lohrmann and Orgel, 1973) we described the polymerization of adenosine cyclic 2′, 3′-phosphate in the solid state in the presence of various catalysts. The best yields of polymerized material were obtained with 1, 2-diaminoethane at alkaline pH's under very dry conditions. Analysis of the reaction mixture after three days by paper chromatography and enzymatic digestion showed that oligomers up to the hexamer were formed, and suggested that approximately 5% of higher molecular weight material was present. We have now undertaken a thorough analysis of the products obtained when larger amounts of adenosine cyclic 2′, 3′-phosphate are polymerized in the presence of 1, 2-diaminoethane, and have characterized the higher molecular weight material.

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Experimental[1]

Materials and general methods have been described previously (Verlander *et al.*, 1973). In this work we used two additional chromatographic systems: system V, n-butanol-acetic acid (2:1, v/v); and system VI, 95% ethanol-1 M ammonium acetate (pH 5.0) (7:3, v/v), made 2×10^{-3} M in EDTA. All six systems were routinely used for identification of products.

[8-^{14}C]-Poly (A) was synthesized from [8-^{14}C]-ppA (Schwartz) in an incubation mixture (2.2 ml) containing ppA (sp. act. = 0.25 mc/mmole), 0.2 mmole; NaOH, 0.2 mmole; $MgCl_2$, 40 μmole; Tris.HCl buffer (pH 9.0), 1.5 mmole; ApApA (as primer), 0.1 μmole; dithiothreitol, 5 μl, and polynucleotide phosphorylase, 100 units. After incubation at 37° (48 h) the reaction mixture was loaded directly onto a 0.9×100 cm Biogel P-30 (Bio-Rad) column and eluted with 10^{-3} M ammonium bicarbonate. The excluded material was pooled and lyophilized. The yield of poly (A) was 45%.

Polymerization mixtures contained [^{33}P]-A>p (Verlander *et al.*, 1973) (sp. act. = 0.1–0.25 mc/mmole) and a 5-fold molar excess of 1, 2-diaminoethane that had been titrated to pH 9.5. They were dried in aliquots containing 2 μmole of A>p by continuous evacuation over phosphorus pentoxide at room temperature (Verlander *et al.*, 1973). Samples were allowed to react at room temperature for the specified time and then analyzed.

Gel Permeation Chromatography

a) The reaction mixtures (2 μmole A>p) described above were dissolved in water (1.5 ml) and the pH's were adjusted to 6.5. Twenty absorbance units of poly (A), 5 absorbance units of $(Ap)_5A>p$, 5 absorbance units of $(Ap)_2A>p$ or $(Ap)_3A>p$, and 2 μmole of A>p were added as cold markers. The mixtures were then applied to a 2.5×100 cm Biogel P-10 (Bio-Rad) column, maintained at room temperature with a water jacket. The column had been equilibrated with 10^{-3} M ammonium bicarbonate, and was developed with the same buffer. Fractions were checked for radioactivity by pipetting 1 ml aliquots into a liquid scintillation cocktail, containing Aquasol (New England Nuclear, 10 ml) and then counting in a Beckman scintillation counter (Model LS200). In addition, the optical densities of the fractions were measured at 258 nm on a Zeiss PMQ2 spectrophotometer. Fractions containing a given product were pooled and lyophilized.

b) The hydrolysis and depolymerization of poly (A) by 1, 2-diaminoethane at pH 9.5 was studied in dry reaction mixtures (200 μl) containing [8-^{14}C]-poly (A), 10 absorbance units (0.25 μc), and 1, 2-diaminoethane, 5 μmole. The reaction mixtures were treated as in (a). Aliquots were checked after 3, 6 and 15 days by chromatography in systems I and II (Verlander *et al.*, 1973).

Finally, after 40 days, an aliquot was chromatographed on Biogel P-10, as described in a), with the inclusion of cold poly (A), $(Ap)_5A>p$, $(Ap)_2A>p$ and A>p markers. Fractions were checked for radioactivity and optical density in the same way as in a).

The same Biogel P-10 column was used for the analysis of all the experiments described in a) and b).

Ion Exchange Chromatography

We used ion exchange chromatography to achieve a preliminary separation of lower molecular weight material from the desired product. A reaction mixture (10 ml)

1 Abbreviations: A>p = adenosine cyclic 2′, 3′-phosphate; Ap = adenosine 2′(3′)-monophosphate; ppA = adenosine 5′-diphosphate; $(Ap)_nA>p$ = cyclic oligoadenylates; poly (A) = polyadenylic acid.