# THE UNIQUE CONFORMATIONAL STABILITY OF POLY 2'-*O*-ETHYLADENYLIC ACID

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Received 18 September 1972

## 1. Introduction

Considerable effort has been made to elucidate the role of 2'-OH groups on the stability and ordered conformation of RNA. Earlier it had been postulated that intramolecular hydrogen bonding between the 2'-OH moiety and nearby groups in the polymer chain contributed to this stability [1, 2]. However, the evidence derived from a study of the physical characteristics of poly 2'-*O*-methyladenylic acid (poly Am) indicated that 2'-*O*-methyl groups did not impair the stability of single- or double-stranded polymers [3]. This implies that hydrogen bonding involving the 2'-OH group is not a major factor in the stabilization of ordered structures of polyribonucleotides. Among various other factors to be considered, the steric environment at the 2'-position may influence the conformation of polynucleotides, perhaps through an altered conformation of the pentose ring. In order to investigate this possible steric effect, a polymer containing the bulkier 2'-*O*-ethyl group (poly 2'-*O*-ethyladenylic acid) was synthesized.

The temperature absorbance profile of poly 2'-*O*-ethyladenylic acid indicates that it has an even greater tendency for ordered structure than poly rA (polyadenylate) or poly 2'-*O*-methyladenylic acid, thus suggesting that the bulkier group does indeed enhance conformational stability.

## 2. Materials and methods

Descending paper chromatography was carried out on Whatman No. 1 paper in the following solvent systems: (A) isopropyl alcohol–$NH_4OH$–0.1 M boric acid (7:1:2), (B) ethyl acetate–propyl alcohol–$H_2O$ (4:1:2, upper phase), (C) butyl alcohol–$NH_4OH$–$H_2O$ (86:5:14), (D) ethyl alcohol–1 M ammonium acetate, pH 7.5 (7:3), (E) propyl alcohol–$NH_4OH$–$H_2O$ (55:10:35) and (F) saturated ammonium sulfate–$H_2O$–isopropyl alcohol (78:18:2).

*Micrococcus luteus* polynucleotide phosphorylase (type I) was purchased from P-L Biochemicals, Inc.

### 2.1. *2'-O-Ethyladenosine*

The synthesis was carried out by a modification of the method used for the preparation of 2'-*O*-methyladenosine [4]. Two grams of adenosine in 80 ml of water was heated to 80° and mixed with a solution of diazoethane [5] (generated from 40 g of nitroethyl urea) in 200 ml of 1,2-dimethoxy ethane. After stirring overnight at room temperature, the crude reaction mixture was evaporated to dryness, redissolved in 40% ethanol and chromatographed on Bio-Rad AG 1-X2 ($OH^-$), 200–400 mesh. Elution with 40% ethanol gave pure 2'-*O*-ethyladenosine in 12% yield.

The purity of 2'-*O*-ethyladenosine was checked in solvent systems A, B, C and D. The product was further characterized by perchloric acid hydrolysis and determination of the liberated ethanol by gas chromatography [6]. Mass spectral analysis also indicated that the product was 2'-*O*-ethyladenosine [7].

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Table 1
Paper chromatography of ribose-, 2′-*O*-methylribose-, and 2′-*O*-ethylribose-containing nucleosides and nucleotides.

| Compound | $R_f$ Solvent A | $R_f$ Solvent D |
|---|---|---|
| Adenosine | 0.41 | 0.65 |
| Am | 0.63 | 0.77 |
| Ae | 0.71 | 0.81 |
| AMP | 0.04 | 0.19 |
| AmMP | 0.18 | 0.30 |
| AeMP | 0.25 | 0.41 |
| ADP | 0.03 | 0.08 |
| AmDP | 0.15 | 0.17 |
| AeDP | 0.20 | 0.35 |

### 2.2. *2′-O-ethyladenosine 5′-phosphate*

The phosphorylation of 2′-*O*-ethyladenosine was carried out by the method of Yoshikawa [8] using $POCl_3$ in triethyl phosphate. Pure 2′-*O*-ethyladenosine 5′-phosphate was isolated by chromatography on Bio-Rad AG 1-X2 (formate), 200–400 mesh. The monophosphate was homogeneous in solvent systems A, C and F and was obtained in 65% yield from the nucleoside.

### 2.3. *2′-O-Ethyladenosine 5′-diphosphate*

The diphosphate was prepared by the displacement of its morpholidate derivative with the tri-*n*-butylamine salt of orthophosphate [9]. The pure diphosphate was isolated by chromatography on Bio-Rad AG 1-X2 ($Cl^-$), 200–400 mesh using a gradient of 0–0.2 M LiCl in 0.01 M HCl. The diphosphate was further purified on DEAE-cellulose by elution with a 0–0.3 M ammonium bicarbonate gradient to give the pure diphosphate in 66% yield from the monophosphate. The product was homogeneous in solvent systems A and D (table 1) and upon complete acid hydrolysis released 1.92 moles of inorganic phosphorus/mole of nucleotide.

### 2.4. *Poly 2′-O-ethyladenylic acid*

The polymer (poly Ae) was synthesized in > 40% yield from the diphosphate using polynucleotide phosphorylase. The rate of polymerization was followed by determination of liberated inorganic phosphate. A typical 100 μl reaction mixture contained: 2′-*O*-ethyladenosine diphosphate, 25 mM; Tricine, pH 8.5, 150 mM; EDTA, 0.4 mM; $MnSO_4$, 10 mM; dithiothreitol, 6 mM; sodium azide, 1 mM; and 2.24 mg of polynucleotide phosphorylase. After incubation at 37° for 90 hr, the reaction mixture was deproteinized by shaking repeatedly with a mixture of isoamyl alcohol and chloroform and the polymer was purified by chromatography over Sephadex G-50. Elution with 0.1 M ammonium bicarbonate in 20% ethanol gave the pure polymer which was lyophilized several times to remove the bicarbonate.

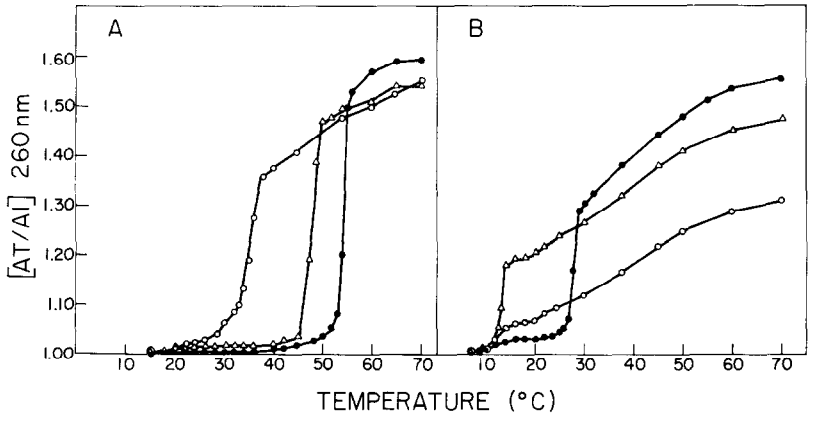


Fig. 1. (A) Absorbance–temperature profiles of poly rA (○—○—○), poly Am (△—△—△), and poly Ae (●—●—●) in 0.15 M NaCl, 0.01 M cacodylate at pH 5.7. $A_T/A_I$ is the ratio of absorbance at temperature T over absorbance at initial temperature. (B) Absorbance–temperature profiles of poly rA (○—○—○), poly Am (△—△—△), and poly Ae (●—●—●) in 0.15 M NaCl, 0.01 M cacodylate at pH 7.0.

## 3. Results and discussion

The results indicate that polynucleotide phosphorylase can utilize 2′-*O*-ethyladenosine 5′-diphosphate as a substrate although the rate of polymerization is even slower than that obtained with 2′-*O*-methyladenosine 5′-diphosphate [10]. The polymerization proceeded optimally at 37° and increasing the temperature in increments up to 55° led to a slower reaction. Similarly, varying the amount of $Mn^{2+}$ in the reaction mixture from 2.5 to 20 mM yielded a slower polymerization rate.

Chromatography of poly Ae on Whatman No. 1 in solvent E indicated that all of the UV absorbing material stayed at the origin along with standard samples of poly rA and poly Am. As expected, treatment of poly Ae with 0.1 M NaOH for 18 hr at 37° was without effect. Hydrolysis of the polymer was achieved by the combined actions of snake venom phosphodiesterase, micrococcal nuclease and *E. coli* alkaline phosphatase [10]. Subsequent chromatography of this reaction mixture in solvent system A and C showed only free nucleoside, i.e., 2′-*O*-ethyladenosine. The size of the polymer was determined by sucrose density gradient centrifugation using 5–20% sucrose in 0.1 M potassium acetate and 0.02 M Tris acetate, pH 9.0. The results indicated a heterodisperse polymer of greater than 14 S in length.

The absorbance–temperature (Tm) profile of poly Ae was determined at pH 5.7 and 7.0 and compared with that of poly rA and poly Am (fig. 1). Poly Ae showed a cooperative transition at pH 5.7 (fig. 1A), the Tm being 55°. Under identical conditions poly rA and poly Am also show a cooperative transition, but the Tm of the cooperative melt is 35° and 48°, respectively, which is significantly lower than that of poly Ae.

At pH 7.0 (fig. 1B) the temperature absorbance profiles show that poly rA melts non-cooperatively, while poly Am and poly Ae exhibit a partial cooperative transition with a Tm of 13.5° and 28°, respectively. This clearly indicates that an ethyl group at the 2′-position of an adenine-containing polymer has a greater stabilizing influence than a methyl group. Further confirmation of this stabilizing influence is provided by the determination of extinction coefficients at pH 7.0 for poly rA, poly Am and poly Ae. In 0.01 M cacodylate and 0.15 M NaCl the extinction coefficients for poly rA, poly Am and poly Ae at 260 nm and 25° are 9.1, 7.9 and $6.5 \times 10^3$ per mole of nucleotide phosphate, respectively. Preliminary experiments also indicate that poly Ae forms a stable duplex with poly rU.

The temperature absorption profiles of polynucleotides at pH 5.7 and pH 7.0 indicate that under identical conditions the 2′-*O*-ethyl-containing polymer possesses a greater conformational stability than poly rA and poly Am and consequently exhibits increased hyperchromicity which lies in the order poly Ae > poly Am > poly rA. Other 2′-substitutions in uracil-containing polymers such as 2′-Cl- [11], 2′-F- [12] and 2′-$NH_2$- [13] have been reported to decrease ordered structure in comparison to the corresponding ribopolymer, while 2′-methoxy [3] and 2′-azido [14] groups enhance ordered polynucleotide structure.

Although a complete explanation of these observations is not clear at the present time, it may be suggested that steric factors near the 2′-position of the ribose ring may play a prominent role in the stability and structure of polynucleotides, including those of RNA and DNA.

## Acknowledgements

This work was supported in part by Grant GB-20764 from the National Science Foundation and a special grant from Smith Kline and French Laboratories. Michigan Agricultural Experiment Station Article No. 5990.

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