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Triple Helices Formed by Polyuridylic Acid with Some Adenosine Derivatives

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Summary. We have prepared a variety of derivatives of adenosine which, at neutral pH's, carry protonated amine functions. These derivatives form stable helical structures with polyuridylic acid, but the melting points are not substantially higher than those of helical complexes formed by adenosine derivatives lacking cationic groups.

Key words: Polyuridylic Acid - Triple-Helix Formation - Melting Temperatures - Nucleoside Phosphoramidates - Prebiotic

INTRODUCTION

Polyuridylic acid (poly U) forms stable triple-helices with a variety of adenosine derivatives (Howard et al., 1966; Davies & Davidson, 1971). If an activated derivative of adenosine 5'-phosphoric acid, for example the 5'-phosphorimidazolide of adenosine, is incorporated into such a triple-helix, it undergoes template-directed condensation to yield short oligonucleotides in up to 50% yield (Weimann et al., 1968). The pyrimidine nucleosides and their derivatives do not form stable helices with the complementary purine homopolynucleotides. Consequently template-directed synthesis of the oligomers of uridylic and cytidylic acid does not occur.

Abbreviations used: Poly(U), Polyuridylic acid; A, Adenosine; pA, Adenosine 5'-phosphate; H_2NpA, Adenosine 5'-phosphoramidate; SdpA, Spermidine-N-(5'-phosphoadenosine); SpA, Spermine-N-(5'-phosphoadenosine); EnpA, 1,2-Diaminoethane-N-(5'-phosphoadenosine); PrnpA, 1,3-Diaminopropane-N-(5'-phosphoadenosine); BnpA, 1,4-Diaminobutane-N-(5'-phosphoadenosine); PnpA, 1,5-Diaminopentane-N-(5'-phosphoadenosine); HnpA, 1,6-Diaminohexane-N-(5'-phosphoadenosine).

As part of a program designed to find conditions under which pyrimidine derivatives would condense together on a complementary template we have searched for monomeric nucleotide derivatives that form unusually stable organized helices. This paper describes our initial studies with certain adenosine derivatives that incorporate one or more cationic groups in their structure. We hoped that the interaction of these positively charged groups with the negatively charged phosphates of the polynucleotide backbone would lead to the formation of stable complexes.

MATERIALS AND METHODS

Materials

Poly (U) was prepared by a modification of a published procedure (Steiner & Beers, 1958). 2'(3')-Glycyl-,2'(3')-lysyl- and 2'(3')-phenylalanyl esters of adenosine 5'-phosphate (Gottikh et al., 1970), adenosine 5'-phosphoramidate (Chambers et al., 1957), spermine-N-(5'-phosphoadenosine) and spermidine-N-(5'-phosphoadenosine) (Burton et al., 1974) were prepared by published procedures. Adenylyl-(5'→N)-glycine was synthesized by a new method (Lohrmann, unpublished results).

Compounds of the type $^{+}H_3N-(CH_2)_n-NHpA$ were obtained by a modification of the method described by Burton (Burton et al., 1974). Here we report the synthesis of 1,4-diaminobutane-N-(5'-phosphoadenosine). The other compounds were made by parallel procedures. The yields were between 40 and 60% in different preparations. The compounds were characterized (a) by their electrophoretic mobilities in System C, which demonstrated their zwitterionic character at neutral pH (b) by their giving a color reaction with ninhydrin reagent which indicated that at least one amino group was free, and (c) by their hydrolysing into pA and diamine under acidic conditions (pH<3).

Synthesis of P^1-[adenosine-5']-P^2-diphenylpyrophosphate ($\phi_2$ppA) (Michelson & Wold, 1962). Anhydrous adenosine 5'-phosphoric acid (0.486 g) was dissolved in a mixture of 0.53 ml of tri-n-octylamine and 6.7 ml of anhydrous dimethylformamide by gentle heating. After the solution had been cooled to room temperature, 1 ml of tri-n-butylamine, 6.7 ml of dioxane and 0.5 ml of diphenylphosphoryl chloride were added. The resulting mixture was allowed to stand at room temperature for three hours, and then concentrated to about one-third of the original volume in vacuo (0.1 mm Hg). Dioxane was added to

the resultant solution until it became turbid. Finally, the turbid solution was added dropwise to 500 ml of ether, and the fluffy white precipitate of $\phi_2$ppA which formed was collected by centrifugation, washed with ether, and dried in vacuo over P_2O_5.

Synthesis of 1,4-diaminobutane-N-(5'-phosphoadenosine). The $\phi_2$ppA prepared as described above was dissolved in a minimum volume of anhydrous dimethylsulfoxide (10-15ml), to which 0.882 g of 1,4-diaminobutane was added. The solution was allowed to stand at room temperature for 5 hours, and then poured into 300 ml of water. The aqueous solution was brought to pH 12 with 5N KOH, and then poured onto freshly prepared Dowex 2 column, which had previously been thoroughly washed first with 1N NaOH and then with water. The resin was washed free of excess amine with a large volume of water and was then removed from the column and titrated to pH 7.2 with 6N HCl to release the phosphoramidate. The resin was then returned to the column and re-extracted with water. The combined extract was lyophilized to give 311 mg (53.3%) of product.

In order to obtain an analytically pure sample, we rechromatographed our compound on Dowex 2 column and lyophilized and dried in vacuo over P_2O_5 at room temperature.

Anal.Calcd. for BnpA·H_2O ($C_{14}H_{24}N_7O_6P·H_2O$)C, 38.62; H, 5.98; N, 22.53; P, 7.13. Found: C, 38.50; H, 5.95; N, 22.64; P, 6.74.

Methods

Paper chromatography was performed by descending elution on Whatman 3MM paper with the following solvents:
A, isopropanol:conc. NH_4OH:H_2O (7:1:2)
B, 95% ethanol:1M ammonium acetate at pH 7.5 (7:3).
Paper electrophoresis was carried out at 3500 volts on Whatman 3MM paper in 0.03 M potassium phosphate at pH 7.1 (System C).

The chromatographic mobilities of compounds of the type $^+H_3N(CH_2)_n$-NHpA are assembled in Table 1. They did not migrate in System C.

Thermal transitions (Howard et al., 1966) of helical complexes were monitored by UV measurements made at 2600 Å, using a Cary 14 recording spectrophotometer attached to a Honeywell 560 XY recorder (Renz et al., 1971). Our cell had an optical path length of 12 μm.

All melting temperatures were measured at pH 7.5 in solutions 0.05M in poly (U), 0.2M in NaCl and 0.025M in the

Table 1

	R_f values	
	A	B
$^{+}H_3N(CH_2)_2$-NH-pA	0.34	0.64
$^{+}H_3N(CH_2)_3$-NH-pA	0.34	0.67
$^{+}H_3N(CH_2)_4$-NH-pA	0.34	0.71
$^{+}H_3N(CH_2)_5$-NH-pA	0.38	0.82
$^{+}H_3N(CH_2)_6$-NH-pA	0.44	0.86
A	1.00	1.00

adenylic acid derivative. When $MgCl_2$ or spermidine hydrochloride was also present, the concentration is specified in the appropriate figure.

DISCUSSION

The melting points of the triple-helices formed by poly (U) with compounds of the type

$$^{+}H_3N(CH_2)_n\text{-NH} - \overset{\overset{O^-}{|}}{\underset{\underset{O}{||}}{P}} - \text{OA}$$

are illustrated in Fig.1. Under our conditions we never observed the formation of precipitates (Ts'o & Huang, 1968;

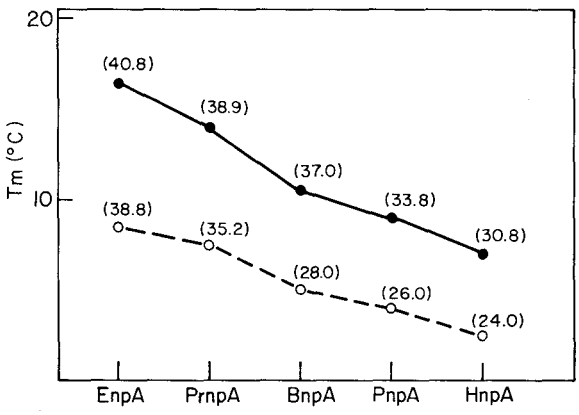


Fig.1. Effect of chain length in compounds of the type $^{+}H_3N\text{-}(CH_2)_n\text{-NHpA}$ on T_m for the complex between poly (U) (0.05M) and $^{+}H_3N(CH_2)_n\text{-NHpA}$ (0.025M) at pH 7.5, in the presence of 0.2M NaCl. ——, T_m in the presence of $MgCl_2$ (0.075M); - - -, T_m in the absence of $MgCl_2$. Numbers in parenthesis indicate hypochromicity

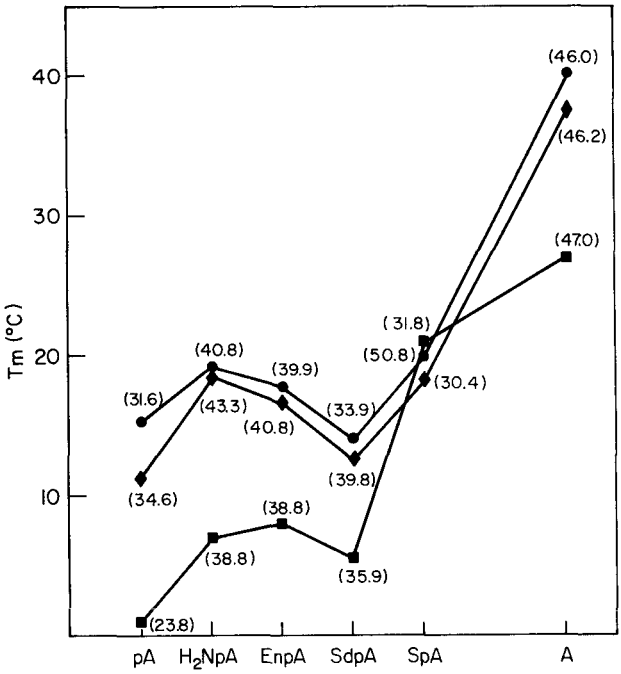
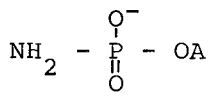


Fig.2. Effect of net charge on the T_m of the complexes between poly (U) (0.05M) and various pA derivative (0.025M) at pH 7.5, in the presence of 0.2M NaCl. ♦, T_m in the presence of $MgCl_2$ (0.075M), ●, T_m in the presence of spermidine hydrochloride (0.075M), ■, T_m in the absence of $MgCl_2$ or spermidine hydrochloride. Numbers in parenthesis indicate hypochromicity

Ts'o & Schweizer, 1968). Both in the presence and in the absence of Mg^{2+}, the 1,2-diaminoethane derivative forms the most stable helices, and the stability falls steadily as the length of the hydrocarbon chain increases. In no case is the stability as great as that of the helix formed by adenosine itself. Surprisingly, the simple phosphoramidate of pA, although it carries a net negative charge

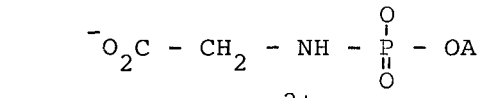


forms helices about as stable as those formed by the 1,2-diaminoethane derivative.

The melting points of a series of helices formed by phosphoramidate derivatives of adenosine carrying differing numbers of amino groups are illustrated in Fig.2. The melting points are not greatly changed when spermidine is substituted for Mg^{2+} in the solution. In the absence of polyvalent cations the complexes are much less stable, except in the case of SpA. The highest measured melting point in the series

was 20 °C, which is still substantially below the melting point of the simple adenosine: 2 poly (U) helix (37.5 °C).

One other phosphoramidate was studied, the glycine derivative



In presence of Mg^{2+} it formed a relatively unstable triple-helix that melted at 8 °C.

The helix formed by the 2'(3')-glycine ester of pA melted at 17.5 ° in the presence of Mg^{2+}. The 2'(3')-phenylalanyl ester of pA formed a triple helix melting at 14 °C. Surprisingly, the 2'(3')-lysyl ester formed a helix that melted at the low temperature of 8.5 °C, even though the amino acid residue carries two positive charges.

The results described above show that cationic groups incorporated into nucleoside derivatives do not stabilize the triple helices effectively. Presumably, the stereochemistry of the helices prevents the simultaneous formation of hydrogen-bonds between the bases, and close contacts between charged ammonium groups and backbone phosphates. This explains why complexes which carry only small negative charges (e.g. the spermidine-pA-containing helix) are still strongly stabilized by external Mg^{2+} ions or spermidine ions.

There is one exception to the above generalization. The spermine-pA containing helix melts at the relatively high temperature of 21 °C in the absence of external cations, and at slightly *lower* temperatures in the presence of Mg^{2+} or spermidine. This suggests that, of all the compounds studied, only the spermine derivative has a structure which permits the NH_3^+ groups to interact strongly with the phosphate groups of the polynucleotide chains. Unfortunately, we do not know whether the principle isomer present in the spermine derivative has an external or an internal phosphoramidate group.

Whatever the theoretical explanation of our findings, it is clear that the present approach does not seem likely to lead to a plausible prebiotic synthesis of pyrimidine oligonucleotides. In a forthcoming paper we shall describe template-directed reactions involving dinucleotides that contain pyrimidines.

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