ROLE OF THE 2'-HYDROXYL IN POLYNUCLEOTIDE CONFORMATION. POLY 2'-*O*-METHYLURIDYLIC ACID

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1. Introduction

The rather unexpected finding that polynucleotide phosphorylase can polymerize 2'-*O*-methyl nucleoside-5'-pyrophosphates made available two model polynucleotide systems for the direct investigation of the role of the ribose 2'-hydroxyl in nucleic acid conformation, viz. poly 2'-*O*-methyladenylic acid [1] and poly 2'-*O*-methylcytidylic acid [2], i.e. poly 2'-*O*-MeA and poly 2'-*O*-MeC. The properties of both these polynucleotide analogues demonstrated that involvement of the ribose 2'-OH as donor in an intramolecular hydrogen bond need not be invoked in the formation of either the neutral single-stranded stacked structures, or of the acid twin-helical forms, for either poly rA [3] or poly rC [4] as compared to poly dA and poly dC.

We now report preliminary data for a third system, poly 2'-*O*-MeU, which may be prepared by polymerization of 2'-*O*-MeUDP with polynucleotide phosphorylase under conditions analogous to those used for the preparation of poly 2'-*O*-MeC [2] or by deamination of poly 2'-*O*-MeC as previously described for deamination of poly dC [5]. As might have been anticipated, poly 2'-*O*-MeU proved to be of special interest inasmuch as poly dU is incapable of forming any ordered structure under conditions where poly rU exists in a helical form [5].

2. Experimental and results

The poly 2'-*O*-MeU sample reported on below was homogeneous in the ultracentrifuge with an $S_{20,w}$ of 9.0 in 0.01 M NaCl and 0.005 M phosphate buffer pH 7.8. Its absorption spectrum under these conditions, as expected, was like that of poly rU or poly dU. Hydrolysis with a mixture of snake venom phosphodiesterase, micrococcal nuclease and *E. coli* phosphatase [1, 2] in 0.1 M tris buffer pH 9.0 and 0.015 M $MgCl_2$ was accompanied by about 10% hyperchromicity at 260 nm and a shift of the absorption maximum from 260 nm to 262 nm.

Addition of NaCl or $MgCl_2$ to an aqueous solution of poly 2'-*O*-MeU was accompanied by pronounced hypochromicity, as with poly rU at low temperatures, but not with poly dU [5]. At 10° maximum hypochromicity was observed in the presence of 1.1 M Na^+ or 0.1 M Mg^{2+}. The resulting transition profiles, although not as sharp as those for poly rU [6, 7] (see fig. 1), are typical for the cooperative melting of a helical structure, and in marked contrast to the behaviour of poly dU, which forms no ordered structure under these or any other conditions.

Even more striking is the effect of polyamines. In the presence of spermidine (fig. 2, left), the transition profile for poly 2'-*O*-MeU, although still slightly broader than that for poly rU, exhibits an almost identical T_m value, whereas poly dU shows no effect whatever. In the presence of spermine (fig. 2, right) the T_m for poly 2'-*O*-MeU is even higher than that for poly rU, again under conditions where poly dU remains a random coil.

Poly 2'-*O*-MeU readily formed helical complexes with either poly rA or poly dA, as shown by the spectral changes and hypochromicities of the appropriate mixtures, and the resulting transition profiles.

With ratios of poly rA to poly 2'-*O*-MeU of 1:1 and 1:2, the corresponding temperature hyperchromicities

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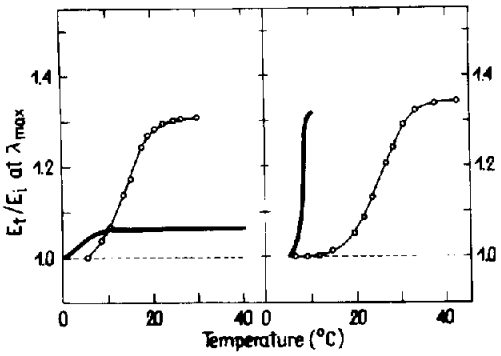

Fig. 1. Melting profiles at neutral pH of poly rU (——), poly dU (- - - -) and poly 2′-*O*-MeU (oooo) in the presence of 0.2 M NaCl (left) and 0.1 M $MgCl_2$ (right). E_t/E_i is ratio of absorbance at temp. t to that at initial temperature.

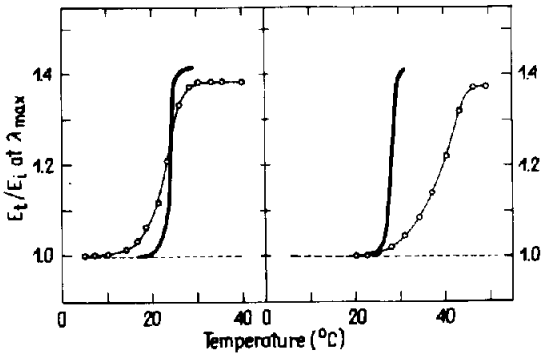

Fig. 2. Melting profiles for poly rU (— —), poly dU (- - - -) and poly 2′-*O*-MeU (oooo), each at a concentration of 0.035 M in 0.005 M phosphate buffer pH 7.4, in the presence of 0.25 M spermidine per mole polymer (left), and 0.35 M spermine per mole polymer (right). E_t/E_i as in fig. 1.

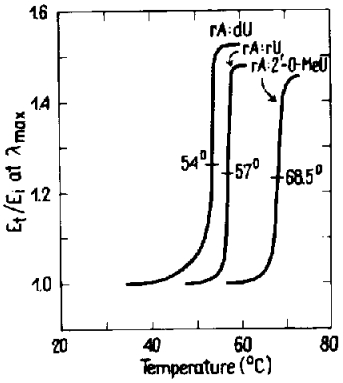

Fig. 3. Helix-coil transition profiles for the double-stranded complexes of poly rA with poly dU, poly rU and poly 2′-*O*-MeU, in 0.1 M Na^+ and 0.01 M phosphate buffer pH 7.8. All profiles measured at λ_{max} of complexes. E_t/E_i as in fig. 1. Temperatures indicated for each curve are the T_m values.

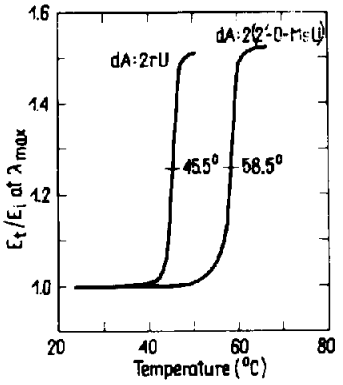

Fig. 4. Helix-coil transition profiles for melting of the triple-stranded helices poly dA : 2rU and poly dA : 2(2′-*O*-MeU) to homopolymer components, in 0.1 M Na^+ and 0.01 M phosphate buffer pH 7.8. E_t/E_i as in fig. 1. Temperatures indicated for each curve are T_m values.

in 0.03 M NaCl were, respectively, 46% and 30%, testifying to the formation of a double-stranded helix, regardless of the ratio of the components, and further confirmed by mixing experiments. In 1:1 mixtures, this double-stranded helix is formed over a range of Na^+ concentrations of 0.02 to 3.0 M. However, in a 1:2 mixture and at Na^+ concentrations above 0.45 M, formation of a triple-stranded helix could be observed at low temperatures; but this dismutated to the double-stranded helix and free poly 2′-*O*-MeU at about 20°. No melting of the triple-stranded helix to homopolymers could be detected. Fig. 3 exhibits the helix-coil transition for the double-stranded poly rA:2′-*O*-MeU as compared to poly rA:dU [5] and poly rA:rU [6] under the same conditions.

A corresponding examination of the 1:1 and 1:2 mixtures of poly dA with poly 2′-*O*-MeU over an Na^+ concentration range of 0.02 to 0.80 M demonstrated the formation of only the triple-stranded poly

dA:2(2′-*O*-MeU), which melted out directly to homopolymers. With poly rU, poly dA likewise forms only the triple-stranded poly dA:2rU [8], which also melts out directly to the constituent homopolymers. Fig. 4 presents the melting profiles for poly dA:2(2′-*O*-MeU) and poly dA:2rU in the presence of 0.1 M Na^+ at pH 7.8. Under these conditions, a direct comparison with poly dA:2dU is not feasible since the latter initially melts out to the double-stranded form [5]. However, at an Na^+ concentration greater than 1 M, poly dA:2dU does melt out directly to homopolymer components [5] and, under these conditions, the T_m values (in 1 M Na^+) for poly dA:2dU, poly dA:2rU and poly dA:2(2′-*O*-MeU) are, respectively, 71, 77 and 88°.

3. Discussion

Since poly 2′-*O*-MeU readily assumes a helical conformation, whereas poly dU does not, it follows that formation of a poly rU helix is not dependent on formation of an intramolecular hydrogen bond involving the 2′-*O*H as donor, thus supporting previous findings for poly rA [3] and poly rC [4]. The possibility that the lone electron pair on the 2′-*O*H oxygen acts as an acceptor seems most unlikely for steric reasons, particularly in the case of poly 2′-*O*-MeU, the more so in that the T_m for poly 2′-*O*-MeU is not less, and in some instances is even higher, than that for poly rU (figs. 1 and 2). This latter fact is in qualitative agreement with the finding, by means of CD spectroscopy, that the stacking of the uracil rings in 2′-*O*-MeUpU is more pronounced than in UpU [9].

On the other hand, it is rather puzzling that poly 2′-*O*-MeU requires 0.1 M Mg^{2+} in order to attain maximal ordered structure as compared to about 0.01 M for poly rU, and this is being further investigated.

As regards the helical complexes with poly rA and poly dA, it can be seen that poly 2′-*O*-MeU resembles, at least qualitatively, poly rU and not poly dU. Furthermore, whereas the complexes involving poly rU are somewhat more stable than the corresponding ones with poly dU (fig. 3 and last paragraph of Results), the replacement of the 2′-OH in poly rU by 2′-*O*-Me leads to a further and even more pronounced enhancement of stability (fig. 4 and last paragraph of Results). These results are certainly not compatible with hydrogen bonding of the 2′-*O*H groups of poly rU. It appears more likely that the source of these effects is related to modifications in conformation of the carbohydrate rings and, especially the effect of the solvent, since the ribose 2′-*O*H in poly rA:rU and rheovirus RNA are located on the surface of the large groove of the helices, hence in direct contact with solvent [cf. 3].

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