EFFECT OF PARTIAL METHYLATION ON PROPERTIES OF POLYADENYLIC ACID*

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The methylation of bases in nucleic acids has been reported for S-RNA (1), ribosomal RNA (2), and DNA (3). The biological function served by the insertion of methyl groups into nucleic acids remains obscure, although various proposals have been made (4). We are studying this problem by the use of methylated homopolymers. Experimental findings are presented here which indicate that methylation of approximately one-seventh of the adenine residues of polyadenylic acid (poly A) produces a polymer which possesses considerable helical structure in neutral solutions, and which forms only a slightly more stable double helix with polyuridylic acid (poly U). In addition, the ability of poly A to form a poly (A + 2U) triple helix is lost on partial methylation of poly A.

Poly A and poly U were purchased from Calbiochem. Methylation of poly A in the N-1 position of adenine was carried out as described by Ludlum et al. (5). The extent of methylation was determined to be 14.9% and no significant amounts of methylated bases other than 1-methyl adenine were detected on paper chromatograms of the acid hydrolysate. Methylated poly A (Me-poly A) was dialyzed against 0.01 M EDTA, 0.1 M NaCl and several changes of deionized water. Polymer solutions were made up in 0.15 M NaCl plus 0.015 M Na citrate (SSC), pH 7.0, unless

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otherwise specified. $S_{20,w}$ values of 3.9 and 9.0 were obtained for unmethylated and methylated poly A respectively in the Spinco model E ultracentrifuge, using U-V optics. Mixing and melting curves were measured with the Zeiss PMQ II spectrophotometer which was equipped with a heatable cell holder.

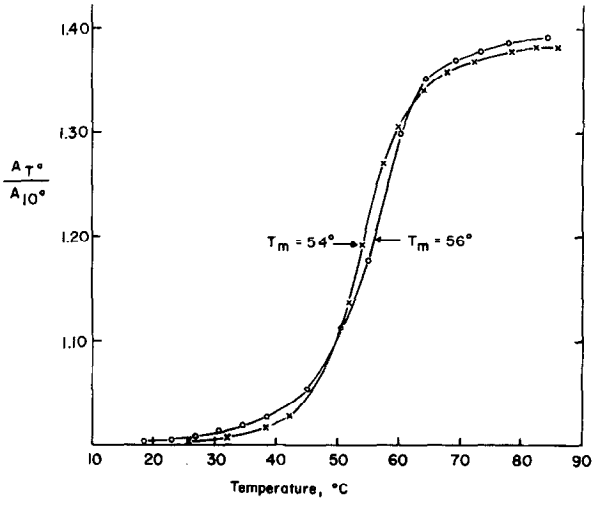

Fig. 1 - Melting curves for Me-poly A (X——X) and Me-poly A plus poly U (O——O) in SSC, pH 7.0.

As can be seen in fig. 1, the transitional profile of Me-poly A displays a well-defined symmetrical melting curve with a T_m of about 55°C. Poly A, by comparison melts over a considerable range of temperature and shows little evidence of a highly ordered secondary structure (fig. 2 and (6)).

Striking differences are observed when melting curves are run on equimolar (on a mononucleotide basis) mixtures of Me-poly A and poly U, as compared with poly (A + U). These results are given in figs. 1 and 2, where each mixture is shown in comparison with methylated or unmethylated poly A alone. Poly (A + U) exhibits a considerably sharpened melting profile. The T_m of 63° is in accord with results observed in other laboratories (5,7). In marked contrast, the melting curve of an

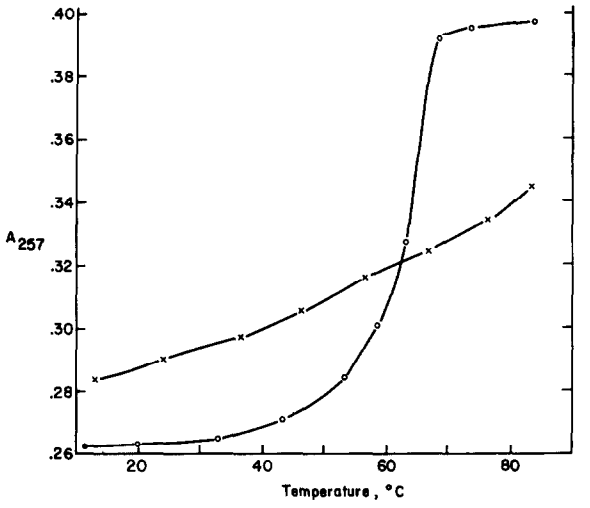

Fig. 2 - Melting curves for poly A (×——×) and poly (A + U) (o——o) in SSC, pH 7.0.

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equimolar mixture of Me-poly A and poly U shows little difference from that of Me-poly A alone. However, the sharpness of the melting profile and the extent of hyperchromicity on heating indicates that interaction occurred between Me-poly A and poly U. This was confirmed by examination of the spectra of Me-poly A, poly U, and a 1:1 mixture of the two, as shown in fig. 3, where the pronounced hypochromic effect on mixing

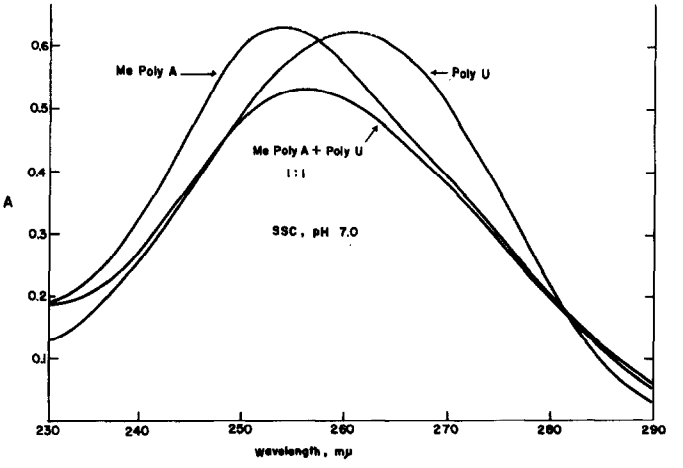

Fig. 3 - Spectral curves for Me-poly A, poly U, and a 1:1 mixture of the two.

is clearly evident. The lowered T_m for Me-poly A + poly U is consistent with the finding of Lazarus and Swartz (11) that the copolymer of deoxy 6-N-methyl adenylic acid and thymidylic acid melts with a T_m about 20° below the melting temperature of deoxyadenylic acid - thymidylic acid copolymer.

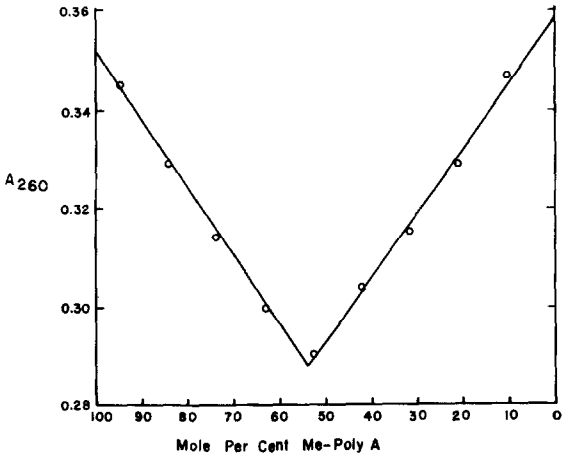

Fig. 4 - Mixing curve for Me-poly A plus poly U in SSC, pH 7.0. Similar curves were obtained for other solvents listed in the text. Mixtures were held at 25° for at least 2 hours prior to reading.

Mixing curves for Me-poly A and poly U were carried out in various solvents. The 1:1 mixing ratio of Me-poly A and poly U shown in fig. 4 was obtained not only in SSC, but in solvents where poly A and poly U form 1:2 triple helices. Melting curves were determined also in SSC, pH 7.0, which was 10^{-2}M in magnesium ion, and in SSC, pH 7.0, which contained 50% ethylene glycol. In the case of poly A-- poly U mixtures, 1:2 complexes are formed in these latter two solvents (8,9), but with Me-poly A - poly U mixtures, no change from the 1:1 ratio seen in fig. 4 was observed.

The reported effects can be explained as follows: Methylation in the 1-position of adenine converts the amine of carbon 6 to the imine, with a pK of 7.2 (10). The imine, which is largely in its protonated

state, is capable of forming a stronger hydrogen bond than the unprotonated amine, and methylation of poly A at the level reported here is sufficient to bring about a double helical structure which is comparable in its stability to Me-poly A plus poly U. The Me-poly A structure may be similar to poly A in acid solution, where hydrogen bonding occurs between the amine of one adenine and N_7 of an adenine in the opposite strand (12). Biologically, methylation of adenine at N_1 may alter base pairing, and affect the transcription of genetic information. This possiblity is under investigation.

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