# SECONDARY STRUCTURE OF POLY-5-METHYLCYTIDYLIC ACID

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It was previously demonstrated that methylation of the $C_5$ position of the uracil residues in poly-uridylic acid (poly-U), resulting in formation of poly-ribothymidylic acid (poly-rT), leads to a marked increase in the secondary structure of the latter as well as in its ability to form a 1:1 complex with poly-adenylic acid (poly-A) of increased stability (Shugar and Szer, 1962; Szer et al., 1963). This is reflected by the mid-point of the helix-coil transition, or melting profile, $T_m$, which is $36^o$ for poly-rT as compared to $8.5^o$ for poly-U, both at pH 7 in the presence of 0.01M $MgCl_2$. For the 1:1 complexes with poly-A, at pH 7 in 0.15M NaCl and 0.015M sodium citrate, that with poly-U has a $T_m$ of $60^o$ as compared to $79^o$ for the one with poly-rT.

The foregoing suggested the advisability of synthesizing poly-5-methylcytidylic acid (poly-5-MeC) with a view to establishing whether the influence of a $C_5$ methyl in the pyrimidine ring is of a more general character; and whether it may be regarded as an apolar side chain, possessing free rotation, which affects the stability of the structure formed by the biopolymer in an aqueous environment.

5-methylcytidine was prepared as described by Fox et al., 1959, and converted to the 5'-pyrophosphate according to standard procedures (Tener, 1961; Moffat and Khorana, 1961). The polyribonucleotide was prepared with the aid of *Azotobacter vinelandii* polynucleotide phosphorylase under standard conditions (Basilio and Ochoa, 1963). The enzymatic reaction went to completion within 4-5 hours to give a 40-50% yield of polymer prior to its isolation.

Poly-5-MeC readily formed a 1:1 complex with poly-inosinic acid (poly-I). The temperature profiles for the twin-stranded complexes poly-(I + C) and poly-(I + 5-MeC) are exhibited in Fig. 1 from which it will be seen that, in 0.01M NaCl with 0.005M tris buffer pH 7.8, the latter has a $T_m$ which is 19.0° higher than the former (Fig. 1a). With an increase in NaCl concentration (Fig. 1b) this difference is only slightly reduced. Unlike the poly-(A + rT) twin-stranded complex, which possesses a higher temperature hyperchromicity than the corresponding poly-(A + U), the hyperchromicities of both complexes in this instance are practically identical. This is probably related to the behaviour of poly-5-MeC in neutral medium (see below).

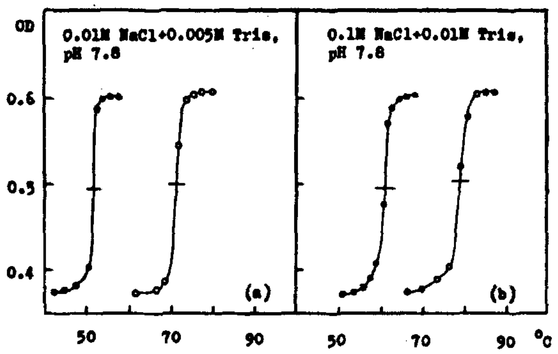

Fig. 1: Temperature profiles for the twin-stranded complexes of poly-I with poly-C and poly-5-MeC at 250 mμ; conditions as indicated in diagrams.
Poly-(I + C) ●–●–●, poly-(I + 5-MeC) o–o–o.

The increase in thermal stability of poly-(I + 5-MeC) with respect to poly-(I + C) is analogous, both qualitatively and quantitatively, to that prevailing between poly-(A + rT) and poly-(A + U). This suggests that the contribution of a $C_5$ methyl to the helix stability is identical for both uracil and cytosine residues; and may be taken to constitute reasonable evidence for stabilization of the helix as a result of the structure-forming influence of a methyl group on the surrounding water molecules (Hamaguchi and Geiduschek, 1962; Schachman, 1963).

The differences in secondary structure between poly-U and poly-rT have already been shown to be reflected in their activities as synthetic messengers in the cell-free amino acid incorporating system (Szer and Ochoa, 1964); and it would undoubtedly be of interest to extend such studies to poly-5-MeC. Nonetheless the present findings, taken in conjunction with those previously reported for poly-rT, point to the potential significance of these derivatives as minor constituents in some s-RNA species. Both of them would be expected to confer increased stability on helical regions in s-RNA as well as on interactions with complementary bases in which they may be involved during the process of information transfer. Similar considerations may be expected to apply in the case of DNA containing 5-methylcytosine. The influence of a $C_5$ methyl is thus seen to be quite different from that of methyl groups on the ring or amino nitrogens in some of the rare bases encountered, for example, in s-RNA.

Poly-C itself is known to possess some ordered structure in buffered medium at neutral pH (Ts'o et al., 1962; Helmkamp and Ts'o, 1962) and is believed to form a regular helix at acid pH (Langridge and Rich, 1963; Akinrimisi et al., 1963). Poly-dC exhibits similar properties (Inman, 1964). The properties of poly-5-MeC were found to differ somewhat from those of poly-C, but the differences are not as clear as in the case of the twin-stranded complexes with poly-I. At neutral pH the temperature hyperchromicity of poly-5-MeC in the range 20-85° is 15%, the temperature profile does not exhibit a sharp melting zone, while there is a red shift in $\lambda_{max}$, all similar to the behaviour of poly-C. The residual hyperchromicity is, however, much higher than for poly-C; on hydrolysis to mononucleotides, the values for poly-5-MeC and poly-C are, respectively, 52% and 38% (see Table I). While little can as yet be said about the configuration of poly-5-MeC in aqueous medium, the data in Table I suggest that, following "melting out" at 85°, a higher degree of order remains in poly-5-MeC as compared to poly-C.

Table I

Molar absorbancy of poly-5-methylcytidylic acid and its constituent mononucleotide in 0.1M buffers.

| | Temp. °C | pH | $\lambda_{max.}$ | $E_{max.}$ | $\Delta E_{max.}$ | $\%^{x}$ $\Delta E_{max.}$ % poly-C$^{xx}$ |
|---|---|---|---|---|---|---|
| 5-methylcytidine-5'-phosphate | 25 | 7.8 | 278.5 | 8.8 | | |
| | 85 | 7.8 | 278.5 | 8.6 | | |
| | 25 | 4.0 | 284 | 10.7 | | |
| | 85 | 4.0 | 282 | 8.2 | | |
| Poly-5-MeC | 25 | 7.8 | 275 | 5.8 | 52 | 38.5 |
| | 85 | 7.8 | 277 | 6.65 | 29 | 17.5 |
| | 25 | 4.0 | 280 | 6.7 | 58.5 | 50 |
| | 85 | 4.0 | 281.5 | 8.65 | 5.2 | 4.5 |

$$^{x}\ \%\ \Delta E_{max} = \frac{E_{polymer} - E_{monomer}}{E_{polymer}} \cdot 100$$

[xx] Data in this column were obtained from comparative experiments, run simultaneously with those of poly-5-MeC. They are in agreement with values given by Ts'o et al., 1962.

In acid medium the differences between poly-5-MeC and poly-C are not very pronounced. At pH 4, the point of maximum stability of the two polymers, poly-5-MeC exhibits a somewhat higher temperature hyperchromicity. Its melting profile is similar to that poly-C, but its $T_m$ is 79° as compared to 82° for poly-C. On hydrolysis to mononucleotides at 85°, the residual hyperchromicities are almost identical for both polymers. This is in agreement with the conclusions of Fasman et al., 1964, derived from optical rotatory dispersion studies on poly-C, that the contribution of hydrophobic forces to the stability of the acid form is negligible.

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