EFFECT OF DI- AND POLYAMINES ON THE THERMAL TRANSITION OF SYNTHETIC POLYRIBONUCLEOTIDES

Włodzimierz Szer

Institute of Biochemistry & Biophysics, Academy of Sciences, Warsaw.

Received January 31, 1966

Certain widely distributed di- and polyamines are known to posses high affinity for binding polynucleotide phosphate (Felsenfeld & Huang, 1960). It was demonstrated that diamines NH_2-$(CH_2)_n$-NH_2 and spermine exhibit a specific effect on the thermal stability of native DNA, but not of RNA. This effect is positively correlated with the AT content of DNA; cadaverine (n=5) was shown to be more effective than the lower and higher diamines studied (Mandel, 1962; Mahler & Mehrotra, 1963). According to Mehrotra and Mahler (1964) diamines have no specific effect, as compared to divalent metal ions, on Tm of the 1:1 complexes of poly-(I + C), poly-(A + U) and on the "acid" form of poly-A.

A further refinement of prerequisites for the appearance of the effect induced by aliphatic amines seemed useful for clarifying their role. The relationship between the AT content and the extent of Tm enhancement suggested the advisability of examining the influence of these cations on the homopolymer of ribothymidylic acid (poly-rT); the latter is known to form an ordered state with a Tm 36° in 10^{-2}M Mg^{+2} (Szer et al., 1963). Investigations on the effect of diamines (n=2 to 6), spermidine NH_2-$(CH_2)_3$-NH-$(CH_2)_4$-NH_2 and spermine NH_2-$(CH_2)_3$-NH-$(CH_2)_4$-NH-$(CH_2)_3$-NH_2 on the thermal transition of poly-rT and other polyribonucleotides as well as on the 1:1 complex of the

former with poly-A, are the subject of this communication.

Experimental. The preparation and physical characteristics of poly-rT have been reported elsewhere (Swierkowski et al., 1965). The other polyribonucleotides employed were commercial products (Miles Chemical Co., Clifton, N.J.); they were further purified prior to use by deproteinization and exhaustive dialysis (Chamberlin et al., 1963). Stock solutions of recrystallized amine hydrochlorides were added directly to spectral cuvettes. Temperature profiles were run as previously described (Swierkowski et al., 1965).

Results and discussion. It is seen from the temperature profiles presented in Fig. 1 that all diamines tested are more effective than Mg^{+2} in rising Tm of poly-rT at an equivalent counterion-polymer phosphate ratio of 1:1, cadaverine being the most effective one. Thus, the regularity concerning diamine chain length and effectivity, as previously established for DNA, applies to poly-rT, pointing to thymine

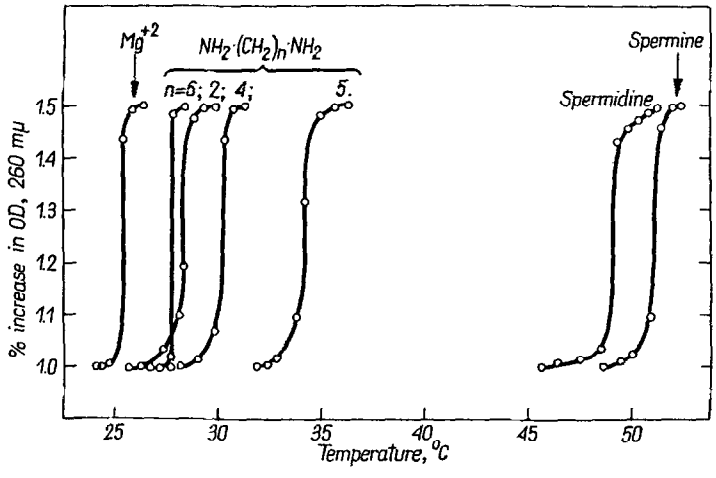

Fig. 1. Melting of poly-rT in the presence of Mg^{+2} and amines, as indicated in the diagram. Polymer concentration 4×10^{-5}M, as monomer, and equivalent counterion concentration. The transition observed was completely reversed on cooling. Identical curves were obtained in redistilled water and in 10^{-2}M phosphate buffer, pH 7.4.

as to the secondary binding site in DNA responsible for this interaction and indicating that the substitution of ribose for deoxyribose is not essential. Tm is substantially enhanced by polyamines; however, owing to the increased number of amine groups per molecule it is difficult to compare polyamines with divalent metal ions even at an equivalent concentration (Fig. 1).

Further experiments at higher diamine concentration demonstrated the existence of a linear relationship of the plot of Tm vs. logarithm concentration. The change in slope (Fig. 2) at about 10^{-4}M counterion may reflect a different extent of screening of the phosphate backbone charge.

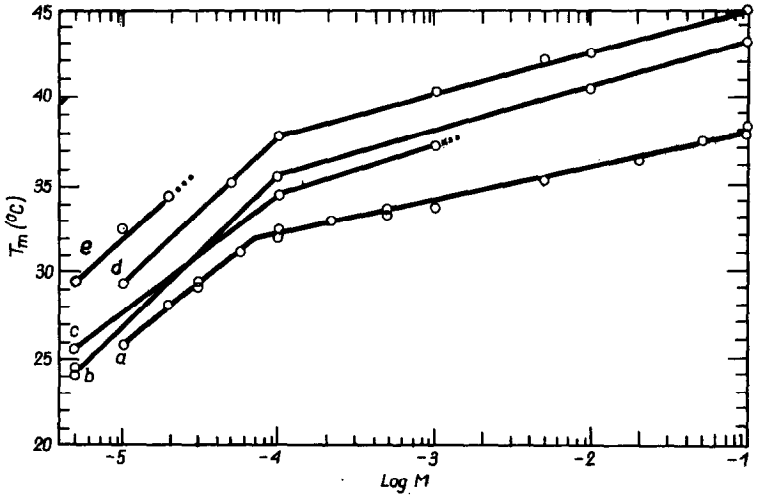

Fig. 2. Variation of Tm of poly-rT with the concentration of a) Mg^{+2} b) 1,2-diaminoethane, c) 1,6-diaminohexane, d) 1,4-diaminobutane, e) 1,5-diaminopeptane. Dotted lines indicate the appearance of absorbancy at 340 mμ.

In the case of cadaverine, spermidine and spermine at equivalent concentrations somewhat exceeding that of the polymer, measurements at 340 mμ revealed the appearance of turbidity; the resulting precipitate was shown to be a poly-rT - polyamine complex. Precipitation of this complex is a function of polymer-polyamine affinity rather than of

polyamine chain length; the poly-rT - 1,6-diaminohexane complex precipitates at a concentration exceeding 100-fold that of the polymer (Fig. 2).

Contrary to what might have been expected, none of the diamines tested proved to be more effective than Mg^{+2} in stabilizing the poly-(A + rT) complex at three various ionic strengths. In the presence of divalent cations at higher salt concentration (10^{-1}M Na^{+} + 10^{-3}M divalent cation) the melting of a triple-stranded structure was actually observed despite the 1:1 mixing ratio; however, at low salt (4×10^{-2}M Na^{+} + 10^{-4}M divalent cation), the lack of transition at 282.5 mμ indicated the melting of a double-stranded complex (cf. Stevens & Felsenfeld, 1964). The fact that diamines, particularly cadaverine and putrescine (n=4), enhance the thermal stability of the alternating sequence poly-dAT (Mahler & Mehrotra, 1964), and poly-rT, may be taken to imply that the presence of thymine in both strands is a factor. Alternate explanations are, of course, possible.

Poly-U was shown to exhibit some degree of ordered state near 0° and in the presence of at least 10^{-3}M Mg^{+2} (Lipsett, 1960). Fig. 3 reveals that all the diamines tested exhibit an additional stabilizing effect which is also apparently distinct from their activity as counter-ions. This stabilization differs from that found for poly-rT since it increases on reducing chain length from $C_{(6)}$ to $C_{(2)}$.

Unlike the poly-rT case formation of the ordered state of poly-U was observed at divalent ion concentration exceeding that of the polymer; it is presumably due to the low ability of poly-U to interact with itself. Again, as for poly-rT, a linear relationship exists of the plot of Tm vs. logarithm counterion concentration.

It will be noted that polyamines exhibit a considerable influence on the Tm of poly-U (Fig. 3). The shift in helix-coil transition from several degrees above 0° at 10^{-3}M Mg^{+2} to 25°-28° in the presence

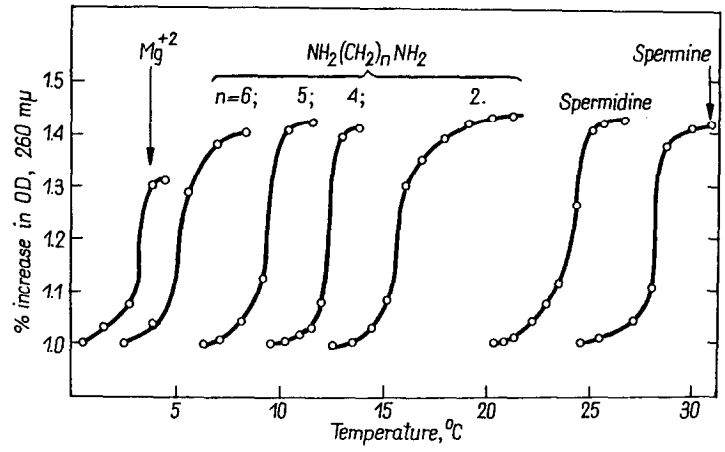

Fig. 3. Melting of poly-U in the presence of Mg^{-2} and amines, as indicated in the diagram. Polymer concentration 4 x 10^{-5}M as monomer. Concentration of divalent cations 10^{-3}M. Concentration of polyamines was equivalent to polymer phosphate. The transition observed was completely reversed on cooling. Identical curves were obtained in redistilled water and in 10^{-2}M phosphate buffer, pH 7.4.

of equivalent amounts of polyamines makes poly-U a suitable model for investigating the impact of secondary structure at convenient temperatures. At higher spermine molarity a spermine-poly-U complex precipitates, as observed earlier by Huang & Felsenfeld (1960); the concentration of spermidine may be raised to 10^{-4}M and Tm increases by a further 2.5°.

The melting behaviour of poly-C and poly-A which are known to possess broad melting profiles at neutral pH with no indication of co-operativeness was not affected by the presence of di- and polyamines. In a way, the influence of aliphatic amines on the ordered state of pyrimidine polyribonucleotides is analogous to that of a 5-methyl substituent. A 5-methyl group is without effect on the structure of the neutral form of poly-C (Szer, 1965), but it contributes to the further enhancement of the ordered state of poly-U (Szer et al., 1963), the latter being known to exhibit a co-operative type of transition (Lipsett, 1960). The aliphatic nature of both factors discussed, and the

requirement of a pre-existing regular structure for their manifestation, suggest the involvement of hydrophobic interactions.

A full account of the effect of various mono- and divalent metal ions, mono-, di-, and polyamines on the thermal transition of poly-rT will be published in Acta Biochimica Polonica.

The author wishes to thank Professor D.Shugar for encouragament and Dr B.Ames for a helpful discussion. The excellent technical assistance of Mrs. M.Dutkowska is acknowlegded.

This investigation has been aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.

References

Chamberlin M., Baldwin R.L. & Berg P., J.Mol.Biol., 7, 334 (1963).
Felsenfeld G. & Huang S.L., Biochim.Biophys.Acta, 37, 425 (1960).
Huang S.L. & Felsenfeld G., Nature, 188, 301 (1960).
Lipsett M.N., Proc.Nat.Acad.Sci., Wash., 46, 445 (1960).
Mahler H.R. & Mehrotra B.D., Biochim.Biophys.Acta, 68, 211 (1963).
Mandel H., J.Mol.Biol., 5, 435 (1962).
Mehrotra B.D. & Mahler H.R., Biochim.Biophys.Acta, 91, 78 (1964).
Stevens C.L. & Felsenfeld G., Biopolymers, 2, 203 (1964).
Swierkowski M., Szer W. & Shugar D., Biochem.Z., 342, 429 (1965).
Szer W., Biochem.Biophys.Res.Comm., 20, 182 (1965).
Szer W., Swierkowski M. & Shugar D., Acta Biochim.Polon., 10, 87 (1963).