EFFECTS OF DIAMINES ON THE THERMAL TRANSITION OF DNA

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Polyamines are present and injected into the host cell in association with the DNA of certain bacteriophages (Hershey 1955, 1957; Ames, Dubin, and Rosenthal, 1958; Ames and Dubin, 1960). They are capable of interacting with isolated nucleic acids (Keister, 1958; Razin and Rozansky, 1959) and with the helical poly A - poly U dimer (Felsenfeld and Huang, 1960), and of protecting π, the protoplast infecting agent derived from bacteriophage T2 (Fraser and Mahler, 1958) as well as intact bacteriophage T5 (Tabor, 1960) against thermal inactivation. We now wish to report a pronounced effect of aliphatic diamines of the general structure $H_2N(CH_2)_nNH_2$ on the melting (denaturation) curve, i.e. the transition helix → coil of certain nucleic acids, determined in standard medium (0.15M NaCl, 0.015M Na citrate, pH ~ 7; Doty et al., 1959): the addition of appropriate amines raises T_m, the denaturation temperature, by as much as 5°. This effect is a function of the chain length and the concentration of amine; it has been observed with DNA obtained from both calf thymus and T2.

EXPERIMENTAL

When 0.05M cadaverine (diaminopentane, n = 5) is added to DNA dissolved in the standard medium, and the absorbance-temperature profile (Doty et al., 1959) determined the curve is displaced $\sim 5^{\circ}$ towards higher temperatures (Fig. 1), i.e. there is a pronounced effect in T_m, the midpoint, but essentially no alteration of either the total extent, i.e. the height, or the compositional heterogenity, i.e. the width or dispersity of the transition. Mg^{++} at an equimolar concentration does not give rise to comparable changes.

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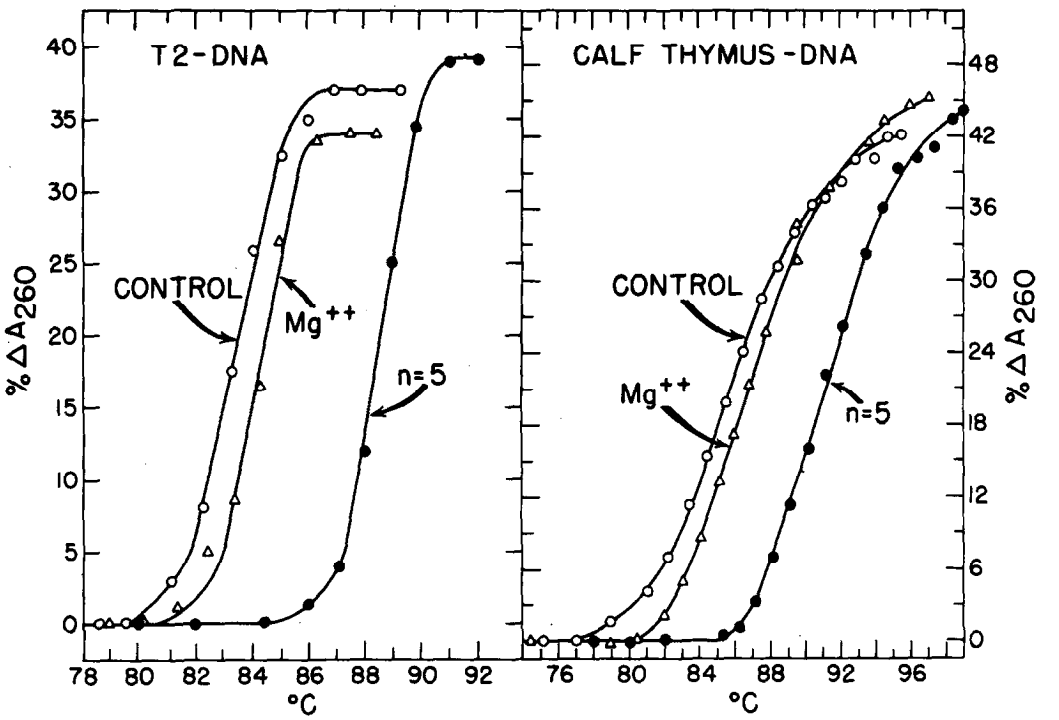

FIG. 1 ABSORBANCY-TEMPERATURE PROFILES OF CALF THYMUS AND T2 DNA determined by the method of Doty et al. (1959). Experimental conditions are given in the legend to Table 1. Data are expressed as $\%\Delta A_{280}$, the percent absorbancy increase, i.e. $[A_{280}(t) - A_{280}(t = 75°)/A_{280}(t = 75°)] \times 100$.

Similar curves have been obtained for the series n = 1 to n = 8; the results are compared in Table 1.

The T_m's determined in this manner are quite reproducible (standard deviation $\leq$ 0.2°), and except for n = 8 the ΔT_m appear to be essentially independent of the DNA used. They are also independent of the concentration of the latter, within the range tested (A_{280} between 0.1 and 1.5), but depend markedly on the concentration of amine; for n = 5 half maximal ΔT_m is obtained at $\sim 4 \times 10^{-3}$ M.

DISCUSSION

The results reported here mark the first instance of a chain-length specific effect of polyamines on a physicochemical property of isolated, highly purified DNA. In this they differ from those reported for π (Fraser and Mahler, 1958) which were structure-dependent but were based on biological activity,

TABLE 1

EFFECT OF DIVALENT CATIONS ON T_m OF CALF THYMUS AND T2 DNA IN STANDARD MEDIUM

Cation Added	Thymus DNA		T2 DNA	
	T_m(°C)	ΔT_m(°C)	T_m(°C)	ΔT_m(°C)
None	85.8	-	83.4	-
Mg^{++}	87.1	1.3	84.2	0.8
diaminoethane^{++}	87.8	2.0	85.6	2.2
diaminopropane^{++}	90.0	4.2	87.4	4.0
diaminobutane^{++}	90.6	4.8	88.4	5.0
diaminopentane^{++}	91.1	5.3	88.6	5.2
diaminohexane^{++}	89.6	3.8	87.6	4.2
diaminoheptane^{++}	88.2	2.4	86.2	2.8
diaminooctane^{++}	86.5	0.7	85.4	2.0

A_{260} vs temperature profiles and T_m's (mid point of the transition) were determined as described by Doty et al. (1959) (see also Fig. 1). The standard system contained 0.15M NaCl, 0.015 Na citrate, pH 7.2; to this was added calf thymus DNA (Worthington Biochemicals) A_{260} (22° to 75°)= 0.700, or T2 DNA (isolated by the method of Mandell and Hershey, 1960), A_{260}(22° to 75°) = 0.400. In the first set of experiments all salts (as chlorides) were 0.050M, in the second 0.043M. ΔT_m is defined as T_m (experimental) - T_m(control). Doty et al. (1959) report T_m = 86° for thymus DNA, T_m = 83.7 for T4 DNA.

and those cited for the poly A - poly U interaction (Felsenfeld and Huang, 1960) which were based on a physicochemical parameter, but appeared to be structure-independent; i.e. various diamines, monovalent and divalent cations, all were able to effect the formation of the double helix albeit at different concentrations, and therefore were simply regarded as counterions of varying effectiveness. The present studies all were conducted at high ionic strength to damp out any such non-specific effects (as evidenced by the small increment in T_m brought about by Mg^{++}), and might tentatively be interpreted as suggesting the formation of a configurationally or conformationally stabilized complex or aggregate between DNA and diammonium cation (Fraser and Mahler, 1958). It will be remembered that an increase in the guanine-cytosine content of DNAs and thus presumably an enhanced stability due to a larger number of hydrogen bonds in the duplex is similarly reflected by a rise in T_m (Marmur et al., 1959). Configurational or conformational stabilization by interaction of (presumably single stranded) RNA with divalent metal cations has been postulated by Fuwa

et al. (1960); that effect, however, is reflected by a change in the over-all shape and extent of the transition.

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