The interaction of spermine and pentamines with DNA

Hirak S. BASU and Laurence J. MARTON*

Brain Tumor Research Center of the Department of Neurological Surgery and the Department of Laboratory Medicine, School of Medicine, University of California, San Francisco, CA 94143, U.S.A.

We studied the effects of spermine, two naturally-occurring pentamines isolated from the thermophile *Thermus thermophilus* and one synthetic pentamine on the aggregation and 'melting' temperature of calf-thymus DNA and on the B-to-Z transition of poly(dG-me⁵dC). All pentamines caused aggregation of DNA at much lower concentrations than that of spermine. Concentrations that increased the melting temperature of DNA and induced the B-to-Z transition in poly(dG-me⁵dC) were different for each pentamine, but were comparable with the concentration of spermine needed to cause these effects. Our results suggest that both the total charge and the distance separating the charge, which is a function of the length of the carbon chains between amino groups, are important for the induction of conformational changes in DNA. The biological role of pentamines in *T. thermophilus* appears to be related to their ability to promote DNA condensation at high temperature.

INTRODUCTION

In 1982, Oshima reported the isolation and characterization of an unusual pentamine ('caldopentamine') from the extreme thermophile *Thermus thermophilus* grown at 80 °C. Later, several unusual tetramines, a second pentamine ('homocaldopentamine') (Oshima & Kawakata, 1983) and a hexamine were isolated from the same organism (Oshima & Senshu, 1985). Because of the different numbers and lengths of the interposed alkyl groups, the structures of caldopentamine [1,15-diamino-4,8,12-triazapentadecane (NH₂-[CH₂]₃-NH-[CH₂]₃-NH-[CH₂]₃-NH-[CH₂]₃-NH₂), hereafter called '3-3-3-3-3'], homocaldopentamine [1,16-diamino-4,8,12-triaza-hexadecane (NH₂-[CH₂]₃-NH-[CH₂]₃-NH-[CH₂]₃-NH-[CH₂]₄-NH₂), hereafter called '3-3-3-4'], and the synthetic pentamine 1,17-diamino-4,9,14-triazaheptadecane (NH₂-[CH₂]₃-NH-[CH₂]₄-NH-[CH₂]₄-NH-[CH₂]₃-NH₂, hereafter called '3-4-4-3') are different from the structure of spermine (NH₂-[CH₂]₃-NH-[CH₂]₄-NH-[CH₂]₃-NH₂, hereafter called '3-4-3'). These pentamines are therefore convenient analogues for the study of the effects of polyamine structure on DNA conformation.

The polyamines spermine and spermidine promote aggregation of DNA, increase its melting temperature (*T*_m), and induce the B-to-Z transition (reviewed by Feuerstein & Marton, 1987). To our knowledge, similar effects of pentamines on DNA or polynucleotides have not been reported. We have studied the effects of the pentamines on the aggregation and *T*_m of calf thymus DNA and on the B-to-Z transition of poly(dG-me⁵dC) and compared these effects with those caused by spermine.

MATERIALS AND METHODS

Calf thymus DNA, sodium cacodylate (reagent grade; Sigma, St. Louis, MO, U.S.A.), poly(dG-me⁵dC) (Pharmacia/P-L Biochemicals, Milwaukee, WI, U.S.A.) and spermine (reagent grade; Calbiochem, La Jolla, CA,

U.S.A.) were obtained from commercial suppliers; quartz-double-distilled water was used to prepare buffers. The pentamines were generously given by Professor Keiji Samejima, Josai University, Saitama, Japan. Spermine and the pentamines were dried overnight in a vacuum dessicator, weighed, and dissolved in 50 mM-NaCl/1 mM-sodium cacodylate buffer, pH 7.0, at various concentrations. Calf thymus DNA and poly(dG-me⁵dC) were dissolved in the same buffer containing 0.1 mM-EDTA and dialysed extensively against the buffer without EDTA. Concentrations of nucleic acids were determined from their u.v. absorbances and the reported molar absorption coefficients (McConnell & von Hippel, 1970; Inman & Baldwin, 1964).

Aggregation of DNA was studied from u.v. spectra at different temperatures recorded on a Gilford model 2600 u.v./visible spectrophotometer fitted with an electronic heating block and a Hewlett-Packard model 7225B plotter. For *T*_m determination, ligands were added to DNA at 60 °C and the *A*₂₅₈ was recorded between 60 and 100 °C at a heating rate of 0.5 °C/min. C.d. spectra were recorded at 60 °C on a Jasco J-500 spectropolarimeter coupled with a DP-500 data processor and an IBM personal computer for data collection and storage.

RESULTS AND DISCUSSION

The aggregation of calf-thymus DNA induced by polycations was studied at 25 and 60 °C. The latter temperature was the closest to the optimum temperature for the growth of *T. thermophilus* that could be used under the experimental conditions without causing melting of calf thymus DNA. Plots of the increase in the value of *A*₃₂₀ of 75 μM-DNA phosphate (0.5 *A*₂₅₈ unit) against different pentamine/base-pair ratios at 25 and 60 °C are shown in Figs. 1(a) and 1(b). It is clear that pentamines cause DNA to aggregate at much lower concentrations than do the concentration of spermine needed to cause the same effect.

Abbreviations used: *T*_m, 'melting' temperature; Me⁵, 5-methyl; abbreviations for, and structures of, polyamines are given in the text.

Fig. 1. A_{320} of calf thymus DNA ($A_{258} = 0.5$) in 50 mM-NaCl/1 mM-sodium cacodylate buffer, pH 7.0, at (a) 23 °C and (b) 60 °C in the presence of various concentrations of spermine (x), 3-3-3-3 (Δ), 3-3-3-4 (∇) or 3-4-4-3 ($\square$)

The effect of pentamines and spermine on the B-to-Z transition was studied by measuring the increase in the ellipticity at 252 nm (θ_{252}) and the decrease in the ellipticity at 292 nm (θ_{292}) of 0.3 A_{258} unit of poly(dG-me⁵dC). Plots of τ_{252} and θ_{292} at various concentrations of pentamines (Figs. 2a and 2b respectively) show that 3-4-3, 3-3-3-4, and 3-4-4-3 induce a complete B-to-Z transition at comparable concentrations, but 3-3-3-3 could not induce a complete transition up to the concentration at which aggregation occurred. Under similar conditions, all other polycations, such as $\text{CO}(\text{NH}_3)^{3+}$, polyamines etc., induce a complete transition of poly(dG-me⁵dC) (Behe & Felsenfeld, 1981) before the onset of aggregation, if any. To our knowledge, 3-3-3-3 is the only cation with more than two positive charges that cannot effect a complete transition

and the only polyamine studied that does not have a tetramethylene bridge between the charged groups. These results suggest that the distribution of the charge along the backbone of long-chain polycations is important for their relatively higher efficiency in the induction of the B-to-Z transition compared with point charges. However, the position of the tetramethylene chain within the molecule seems to have little effect on the ability of pentamines to cause this transition.

Because pentamines isolated from *T. thermophilus* grown at high temperature might be expected to have a significant role in the stabilization of DNA at high temperature, we studied their effects on the T_m of calf thymus DNA. Plots of T_m against various concentrations of 3-4-3 and the pentamines are shown in Fig. 3. T_m increased with increasing concentrations of all com-

Fig. 2. Ellipticity (θ) of poly(dG-me⁵dC) ($A_{258} = 0.3$) in 50 mM-NaCl/1 mM-sodium cacodylate buffer, pH 7.0, at 60 °C at (a) 252 nm (θ_{252}) and (b) 292 nm (θ_{292}) in the presence of various concentrations of spermine (x), 3-3-3-3 (Δ), 3-3-3-4 (∇) or 3-4-4-3 ($\square$)

Fig. 3. T_m of calf thymus DNA ($A_{258} = 0.5$) in 50 mM-NaCl/1 mM-sodium cacodylate, pH 7.0, in the presence of various concentrations of spermine ($\times$), 3-3-3-3 (Δ), 3-3-3-4 (∇) or 3-4-4-3 ($\square$)

pounds, but the effects were different for each of them. T_m increased more or less linearly for 3-4-3 and 3-4-4-3. After an initial sharp increase, the T_m for 3-3-3-4 levelled off, indicating a relatively high affinity of this pentamine for DNA. The T_m profile for 3-3-3-3 is sigmoidal, which suggests that the association between this pentamine and DNA is co-operative.

By using a non-linear regression program that follows the Marquardt-Levenberg algorithm, T_m data were fit with the equation developed by Porschke & Jung (1982):

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \frac{R}{\Delta H} \cdot \ln \left[\frac{(1 + K_h L)^{2/n_h}}{(1 + K_c L)^{2/n_c}} \right]$$

where T_m and T_m^0 are the melting temperatures for DNA with and without ligands respectively, K_h and K_c are the apparent association constants at 25 °C of the ligand with the helical and coiled form of DNA respectively, n_h and n_c are the numbers of nucleotides bound to each ligand molecule in the helical and coiled form respectively, L is the concentration of free ligand, and ΔH is the change in enthalpy during melting of a base-pair. Assuming a value of -33.6 kJ (-8.0 kcal)/mol for ΔH (Neumann & Ackermann, 1969), the K_h , K_c , n_h and n_c values were calculated from the fit using the value of L determined by successive approximation with the intermediate values of K_h and the initial concentrations of DNA and ligands. Because of the large number of parameters, iterations were carried out with different guess values, and the quality of the fit was checked at each convergence point. Convergence was assumed to be achieved when the difference between the sum of the squares of the residuals in two successive iterations was < 0.001 . Values for the best fit are listed in Table 1.

The K values for spermine with double-stranded DNA at 25 °C in the presence of 1–17 mM- Na^+ and 71–154 mM- Na^+ have been reported by Porschke (1984) and Braulin *et al.* (1982) respectively. Using these data, we plotted $\ln K$ versus $\ln[\text{Na}^+]$ and by extrapolation

Table 1. Association parameters for spermine and pentamines with calf thymus DNA at 25 °C

Symbols for parameters are defined in the text

Ligand	$10^4 \times K_h$ (M^{-1})	$10^4 \times K_c$ (M^{-1})	n_h	n_c	K_h/K_c
3-4-3	5.7	0.97	3.7	3.2	5.9
3-3-3-3	5.3	1.0	4.8	4.2	5.1
3-3-3-4	75.0	12.0	4.8	4.2	6.2
3-4-4-3	76.0	12.0	4.8	4.2	6.4

obtained a value for K of $5.98 \times 10^4 \text{ M}^{-1}$ at 51 mM- Na^+ , which is in excellent agreement with the value calculated from T_m data (Table 1). Moreover, the value of K_h/K_c determined from our melting studies is in good agreement with the K_h/K_c value calculated from the broadening of T_m profiles (Morgan *et al.*, 1986) using the independent-site binding model.

Values listed in Table 1 show clearly that the affinities of 3-3-3-3 with both helical and coiled DNA are markedly lower than those of the other pentamines, which emphasizes the importance of a four-carbon chain for DNA-polyamine association. These results are in accord with crystallographic observations (Drew & Dickerson, 1981) and theoretical modelling studies of the polyamine-DNA interaction (Feuerstein *et al.*, 1986), both of which show that the tetramethylene chain of spermine associates with specific sites in the major groove of B-DNA.

Because the extent of the increase in T_m induced by pentamines is very similar to those induced by spermine (3-4-3) at similar concentrations, it is difficult to postulate a straightforward hypothesis for the biological role of the pentamines in general and for 3-3-3-3 in particular as stabilizers of DNA at high temperature. It is possible that pentamines are required for the condensation of DNA at high temperatures at which conventional polyamines may not be effective, as observed from the aggregation studies (see Fig. 1).

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