Effects of variation in the structure of spermine on the association with DNA and the induction of DNA conformational changes

Hirak S. BASU,* Herman C. A. SCHWIETERT, Burt G. FEUERSTEIN and Laurence J. MARTON

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

The effects of spermine and spermine analogues on the *B*–*Z* transition of poly(dG-me⁵dC) and on the aggregation and ‘melting’ temperature of calf thymus DNA were studied by spectroscopic methods. The association constants of these polyamines with double- and single-stranded calf thymus DNA were calculated from their effects on the melting temperature. The effect of these compounds on the release of ethidium bromide (EB) from an EB–DNA complex were measured by a spectrofluorimetric method. This efficiency of the polyamine-induced *B*–*Z* transition strongly depended on the length of the central carbon chains of the compounds and on the functional groups attached to the carbon chains. Both the terminal primary amino groups and the length of the central carbon chain affected the aggregation of DNA. The affinity of the analogues for DNA increased as the number of *n*-butyl groups increased, but decreased with either an increase or a decrease in the length of the central carbon chain. The effect of spermine and spermine analogues on the release of EB from an EB–DNA complex did not always correlate with the affinities of analogues for calf thymus DNA. In particular, tetra-amines with more than one *n*-butyl group bound better to DNA than did spermine, but released bound EB and induced aggregation of DNA less well than did spermine. We postulate that either a bend and/or other localized conformational changes of DNA are responsible for the spermine-induced aggregation of DNA and the release of EB from the EB–DNA complex.

INTRODUCTION

The molecular mechanisms responsible for the biological functions of the polyamines putrescine, spermidine and spermine have been investigated intensively in recent years (for reviews, see Tabor & Tabor, 1984; McCann *et al.*, 1987; Pegg, 1988). Since the discovery that polyamines induce the left-handed *Z*-conformation in poly(dG-me⁵dC) under near-physiological conditions (Behe & Felsenfeld, 1981), attempts have been made to determine whether polyamine-induced changes in DNA conformation are related to the biological functions of polyamines. Results of physico-chemical studies of the effects of polyamines on the conformation of various polynucleotides *in vitro* (Bloomfield & Wilson, 1981; Feuerstein *et al.*, 1986; Marquet *et al.*, 1987; Basu & Marton, 1987; Basu *et al.*, 1987, 1988) have suggested possible mechanisms for polyamine functions *in vivo* (Basu & Marton, 1987). Testing these hypotheses *in vivo* is difficult, however, because of the presence of highly developed polyamine-biosynthetic pathways in living cells that maintain endogenous polyamines at levels needed for cell growth and proliferation. Several agents that deplete intracellular levels of polyamines have been tested (for a review, see McCann *et al.*, 1987); unfortunately, some of these compounds are polyamine analogues and mimic some of the physico-chemical properties of natural polyamines *in vitro* (Basu & Marton, 1987; Vertino *et al.*, 1987), and also appear to support growth in tissue culture (H. S. Basu, unpublished work). We found that small changes in polyamine structure produce major changes in both physico-chemical properties and the ability to support growth (Basu *et al.*, 1989; Vertino *et al.*, 1987). We speculated that polyamine analogues with these minor structural modifications may be recognized by cells as natural polyamines with respect to cellular

uptake and/or effects on polyamine-biosynthetic enzymes, but may not induce the conformational changes in nucleic acids caused by natural polyamines.

Ethidium bromide (EB) is an organic cation that interacts strongly with nucleic acids. This interaction produces a large increase in the fluorescence quantum yield of EB (for reviews, see Berman & Young, 1981; Neidle & Abraham, 1984). At high ionic strength (> 0.5 M-NaCl), the phenanthrene ring of EB intercalates between the bases of double-stranded DNA, whereas at lower ionic strength (< 0.1 M-NaCl) EB can also interact electrostatically with the phosphate groups of nucleic acids (Lepecq & Paoletti, 1967; Bresloff & Crothers, 1975, 1981). EB binds better to right-handed *B*-DNA than to left-handed *Z*-DNA and induces a *Z*- to *B*-transition *in vitro* (Pohl *et al.*, 1972; Walker *et al.*, 1985; Lamos *et al.*, 1986). The crystal structure of an EB–dinucleotide complex (Tsai *et al.*, 1977; Jain *et al.*, 1977; Jain & Sobell, 1984a,b) suggests that the phenanthrene ring of EB intercalates between DNA bases, but that the hydrophobic phenyl and ethyl residues probably reside at the minor groove of *B*-DNA. Release of EB from the EB–DNA complex causes a decrease in fluorescence quantum yield that can be used to monitor the interaction of other intercalating agents with DNA (Cain *et al.*, 1978) and quite recently has been used to study the association of polyamines with calf thymus DNA *in vitro* (Stewart, 1988).

We studied the effect of spermine and a variety of spermine analogues on the structure and conformation of calf thymus DNA and polynucleotides by u.v. and c.d. spectroscopy. The affinity of spermine and spermine analogues for calf thymus DNA was studied by measuring the effects of these analogues on the *T_m* of DNA and on their ability to release EB from a EB–DNA complex.

Abbreviations used: EB, ethidium bromide; *T_m*, DNA ‘melting’ temperature; DFS, $\beta\beta$ -difluoro-*n*-butylspermine.

* To whom correspondence and reprint requests should be sent, at the following address: c/o The Editorial Office, 1360 Ninth Avenue, Suite 210, San Francisco, CA 94122, U.S.A.

MATERIALS AND METHODS

Calf thymus DNA, sodium cacodylate and EB (reagent grade) were obtained from Sigma (St. Louis, MO, U.S.A.), poly(dG-me⁵dC) was obtained from Pharmacia/P-L Biochemicals (Milwaukee, WI, U.S.A.), and spermidine and spermine (reagent grade) from Calbiochem (La Jolla, CA, U.S.A.). Polyamine analogues (Table 1) were generously given by Dr. Philippe Bey of the Merrell Dow Research Institute (Cincinnati, OH, U.S.A.), Professor Raymond J. Bergeron, University of Florida (Gainesville, FL, U.S.A.) and Professor Keijiro Samejima, Josai University (Sakado, Saitama, Japan). Compounds were dried overnight in a vacuum desiccator, weighed, and dissolved in

50 mM-NaCl/1 mM-sodium cacodylate buffer (pH 7.0) prepared in deionized water. Stock solutions of calf thymus DNA and poly(dG-me⁵dC) were prepared in the same buffer, dialysed, and concentrations of the stock solutions were determined spectroscopically as described by Basu & Marton (1987).

Aggregation and melting temperature (T_m) of DNA were studied with a Perkin-Elmer Lambda 4c u.v./visible spectrophotometer equipped with multicell transporter, electrical heating system and an IBM-AT compatible personal computer using SOTWAYS (Moreno Valley, CA, U.S.A.) data-collection software. Aggregation was monitored by observing the increase in the absorbance of DNA (approx. 0.5 A_{260} unit) at 320 nm and the T_m was determined by heating DNA solutions (approx.

Table 1. Polyamine analogues used in the present study

Group	Abbreviation used here	Systematic (IUPAC) name	Chemical formula
I	Spermine	1,12-Diamino-4,9-diazadodecane	$\text{NH}_2-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_3-\text{NH}_2$
	4-4-4	1,14-Diamino-5,10-diazatetradecane	$\text{NH}_2-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_4-\text{NH}_2$
	3-3-3	1,11-Diamino-4,8-diazundecane	$\text{NH}_2-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_3-\text{NH}_2$
	3-2-3	1,10-Diamino-4,7-diazodecane	$\text{NH}_2-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_2-\text{NH}-(\text{CH}_2)_3-\text{NH}_2$
	3-8-3	1,16-Diamino-4,12-diazahexadecane	$\text{NH}_2-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_8-\text{NH}-(\text{CH}_2)_3-\text{NH}_2$
II	BESm	1,12-Bisethylamino-4,9-diazadodecane	$\text{C}_2\text{H}_5-\text{NH}-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_3-\text{NH}-\text{C}_2\text{H}_5$
	BE-4-4-4	1,14-Bisethylamino-5,10-diazatetradecane	$\text{C}_2\text{H}_5-\text{NH}-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_4-\text{NH}-\text{C}_2\text{H}_5$
	BE-3-3-3	1,11-Bisethylamino-4,8-diazundecane	$\text{C}_2\text{H}_5-\text{NH}-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_3-\text{NH}-\text{C}_2\text{H}_5$
	BE-3-2-3	1,10-Bisethylamino-4,7-diazodecane	$\text{C}_2\text{H}_5-\text{NH}-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_2-\text{NH}-(\text{CH}_2)_3-\text{NH}-\text{C}_2\text{H}_5$
III	DH-3-8-3	2,15-Dihydroxy-1,16-diamino-4,12-diazahexadecane	$\text{NH}_2-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_2-\text{NH}-(\text{CH}_2)_8-\text{NH}-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_2-\text{NH}_2$
	DM-3-8-3	2,15-Dimethyl-1,16-diamino-4,12-diazahexadecane	$\text{NH}_2-(\text{CH}_2)_2-\text{CH}(\text{CH}_3)-\text{NH}-(\text{CH}_2)_8-\text{NH}-\text{CH}(\text{CH}_3)-(\text{CH}_2)_2-\text{NH}_2$
	DFS	6,6-Difluoro-1,12-diamino-4,9-diazadodecane	$\text{NH}_2-(\text{CH}_2)_3-\text{NH}-\text{CH}_2-\text{CF}_2-(\text{CH}_2)_2-\text{NH}-(\text{CH}_2)_3-\text{NH}_2$
IV	4-4-4-4	1,19-Diamino-5,10,15-triazanonadecane	$\text{NH}_2-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_4-\text{NH}_2$
	3-2-2-3	1,13-Diamino-4,7,10-triazatridecane	$\text{NH}_2-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_2-\text{NH}-(\text{CH}_2)_2-\text{NH}-(\text{CH}_2)_3-\text{NH}_2$
	BE-3-2-2-3	1,13-Bisethylamino-4,7,10-triazatridecane	$\text{C}_2\text{H}_5-\text{NH}-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_2-\text{NH}-(\text{CH}_2)_2-\text{NH}-(\text{CH}_2)_3-\text{NH}-\text{C}_2\text{H}_5$

Table 2. Concentrations of polyamine analogues for the *B-Z* transition of poly(dG-me⁵dC) and aggregation of calf thymus DNA, their association constants with calf thymus DNA, and their ability to displace EB from EB-DNA complexes

Analogue	Concentration at the midpoint of the <i>B-Z</i> transition (μM)	Concentration at the onset of aggregation (mM)	$10^4 \times K_h$ (M^{-1})	$10^4 \times K_c$ (M^{-1})	Fraction of bound EB released by 30 μM -analogue
Spermine	2.0*	0.015*	5.70	0.01	0.228
4-4-4	4.2	0.082	43.8	0.11	0.165
3-3-3	4.1*	0.038	2.17	0.01	0.144
3-2-3	†	0.100*	0.73	0.01	0.046
3-8-3	4.0	0.390	8.6	0.05	0.154
BESm	1.4*	0.025*	2.06	0.02	0.090
BE-4-4-4	1.3	0.390	15.8	0.06	0.090
BE-3-3-3	8.0*	0.080*	2.13	0.02	0.120
BE-3-2-3	‡	0.200*	0.75	0.01	0.047
DH-3-2-3	3.4	0.250	2.15	0.01	0.198
DM-3-8-3	3.5	0.140	2.15	0.01	0.150
DFS	25.8	0.095	0.19	0.00§	0.025
4-4-4-4	1.0	0.020	50.4	0.24	0.569
3-2-2-3	†	0.057*	2.15	0.01	0.208
BE-3-2-2-3	†	0.170	0.20	0§	0.156

* Charge ratios calculated from these values have been reported (Basu *et al.*, 1989).

† Aggregation starts before completion of the *B-Z* transition.

‡ No *B-Z* transition before the onset of aggregation.

§ The small value is outside the limit of significant figures.

0.5 A_{260} unit in 50 mM-NaCl/1 mM-sodium cacodylate, pH 7.0) between 60 and 100 °C at a heating rate of 0.5 °C/min as described by Basu & Marton (1987). Association constants of these ligand–nucleic acid systems were calculated from these plots as described (Basu & Marton, 1987) using the equation (Porschke & Jung, 1982):

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

where T_m (T_m^0) is the melting temperature in the presence (absence) of ligand at concentration L , R is the gas constant and K_h (K_c) is the binding constant with the helix (coil) form with binding stoichiometries n_h (n_c) at standard temperature and pressure. The value of ΔH [−33.5 kJ (−8.0 kcal)/mol] for base-pair melting was taken from Neumann & Ackermann (1969). Because of the large number of parameters, iterations were carried out with different guess values and strict constraints on n_h and n_c . The quality of the fit was checked at each convergence point. Convergence is assumed to be achieved when the difference between the sum of the squares of the residues in two successive iterations was < 0.001. K_h and K_c values from the best fit are listed in columns 4–7 of Table 2. These numbers are approximate, and good convergence may be achieved with K_h and K_c values within 20 % of the values listed in Table 2. Values for the spermine–DNA system from our earlier studies (Basu & Marton, 1987) are included for comparison. The *B*-to-*Z* transition of poly(dG-me⁵dC) (approx. 0.3 A_{256} unit) was determined by heating the sample to 60 °C for 5 min in the same buffer with various concentrations of the analogues using an upgraded Jasco UV-5 spectropolarimeter as described by Basu & Marton (1987) and Behe & Felsenfeld (1981).

The release of EB from the EB–DNA complex was monitored fluorimetrically as described by Cain *et al.* (1978) and Stewart (1988). Calf thymus DNA (0.2 A_{260} unit) was added to 3 ml of buffer (50 mM-NaCl/1 mM-sodium cacodylate, pH 7) containing 2.0 μ M-EB. At this EB/DNA ratio, over 95 % of EB was bound, as determined from fluorescence-enhancement studies (results not shown). The fluorescence quantum yield of the EB–DNA complex was monitored at an excitation wavelength of 546 nm and at an emission wavelength at 595 nm using a SPEX spectrofluorimeter coupled with an IBM-XT-compatible personal computer for data collection and storage. The complex was titrated by adding small volumes (μ l) of concentrated solutions of spermine or its analogues at room temperature. Data were recorded 60 s after the addition of each analogue. Under our conditions the fluorescence intensity was essentially the same at 60 s and 20 min after addition (results not shown), in agreement with data from another laboratory (Stewart, 1988). The fluorescence values were corrected for the dilution of the complex caused by the addition of ligand. None of the compounds had any observable effect on the fluorescence of EB in the absence of DNA (results not shown).

RESULTS AND DISCUSSION

The polyamine analogues used in the present study, listed in Table 1, can be divided into four groups. Group I consists of spermine, 4-4-4, 3-3-3, 3-2-3 and 3-8-3, which are tetra-amines with carbon chains of different lengths that have two primary and two secondary amino groups. Group II consists of BESm, BE-4-4-4, BE-3-3-3 and BE-3-2-3, which are tetra-amines with ethylated terminal amino groups. Group III consists of DH-3-8-3, DM-3-8-3 and DFS; in the first two compounds, the outer alkyl groups are either hydroxylated or methylated, and in the third compound the central chain is fluorinated. Group IV consists of the penta-amines 4-4-4-4, 3-2-2-3 and BE-3-2-2-3.

Representative c.d. spectra of poly(dG-me⁵dC) at various

Fig. 1. C.d. spectra of approx. 0.3 A_{254} unit of poly(dG-me⁵dC) in 50 mM-NaCl/1 mM-sodium cacodylate, pH 7.0, in the presence of 0 mM—(—), 3 mM—(....) and 8 mM—(---) 3-3-3

stages of the *B* to *Z* transition in the presence of different concentrations of one of the analogues, 3-3-3, are shown in Fig. 1. The midpoint of the *B*–*Z* transition for each compound was determined by plotting the decrease of the molar ellipticity at 292 nm as a function of ligand concentration (Behe & Felsenfeld, 1981); results are listed in column 2 of Table 2. Because aggregation of DNA changes the c.d. spectra, we could not use this method to observe the complete *B*–*Z* transition by 3-2-3, 3-2-2-3 and BE-3-2-2-3, all of which induce aggregation before the *B*–*Z* transition is complete; we have shown (Basu *et al.*, 1989) that the *B*–*Z* transition could not be observed in the presence of BE-3-2-3 before the onset of aggregation. The concentrations of most of the analogues required for the onset of aggregation of calf thymus DNA or poly(dG-me⁵dC) are 2–20-fold higher than those required to induce a complete *B*–*Z* transition in poly(dG-me⁵dC). Therefore analogues that do not induce a complete *B*–*Z* transition before the onset of aggregation are less efficient for the induction of this transition than analogues for which the transition is complete before the onset of aggregation. Aggregated DNA has a characteristic c.d. spectrum that is distinct for either *B*- or *Z*-DNA (Becker *et al.*, 1979). Therefore aggregation, which in most instances takes place at a much higher concentration of analogue than that needed to induce the *B*-to-*Z* transition (see below), could be observed by c.d. spectroscopy.

The rate at which polyamines induce the *B*–*Z* transition is low, and all samples were heated to 60 °C for 5 min and cooled to ambient temperature before c.d. spectra were recorded (Behe & Felsenfeld, 1980). Because the exact mechanism of the *B*–*Z* transition is not known, the alteration of temperature forbids any direct correlation between polyamine–DNA affinity and the efficiency of induction of the *B*–*Z* transition. The enthalpy values of the polyamine–DNA system, however, are so small that the difference in the effects of analogues at various steps of the *B*–*Z* transition, and the effect of heating on these effects, are unlikely. This is further confirmed by the shape of the transition profiles (Basu & Marton, 1987). Therefore the midpoints of the transition give a fairly accurate description of the relative abilities of analogues to induce conformational changes.

Among all the unmodified tetra- and penta-amines classified in Group I and Group IV (Table 1), the tetra-amine spermine and the penta-amine 4-4-4-4 induced the *B*–*Z* transition most efficiently (column 2 of Table 2). The efficiency decreased with either an

increase (3-8-3) or a decrease (3-3-3, 3-2-3 and 3-2-2-3) in the length of the central carbon chain. The bisethyl derivatives of polyamines classified in Group II (Table 1) induced the Z-structure more efficiently than did the parent polyamines, if the parent compound had one or more n-butyl groups (compare spermine with BESm, and 4-4-4 with BE-4-4-4 in column 2 of Table 2). For polyamines with shorter carbon chain lengths, however, the bisethylated derivatives were relatively weaker than the parent polyamines (compare 3-3-3 with BE-3-3-3, and 3-2-3 with BE-3-2-3 in column 2 of Table 2) for induction of the Z-form. Among the chemically modified analogues (Group III of Table 1), the dihydroxy and dimethyl derivatives of 3-8-3, modified at both terminal n-propyl groups, were more efficient than 3-8-3 for the induction of the B-Z transition, but the $\beta\beta$ -difluoro-n-butylspermine (DFS) showed a marked decrease in its ability to induce the B-Z transition. The importance of the central n-butyl group of spermine for DNA conformational changes has been suggested by us (Basu & Marton, 1987) and by others (Thomas & Messner, 1988). On the other hand, an increase in the number of n-butyl groups from spermine to 4-4-4 slightly decreased the abilities of Group I compounds containing free N-termini to induce the Z-form, but did not significantly affect similar properties of bisethylated Group II derivatives.

Representative u.v. spectra of calf thymus DNA at various stages of aggregation in the presence of increasing concentrations of 3-3-3 are shown in Fig. 2. The increase in A_{320} that characterizes aggregation of DNA (Basu & Marton, 1987; Gosule & Schellman, 1978) was plotted against ligand concentration. These plots are sigmoidal, and concentrations of different analogues at the onset of aggregation (the start of the increase of the values of A_{320}) are listed in column 3 of Table 2. The ratio of the concentrations of positive/negative charges at the midpoint of the B-Z transition and at the onset of aggregation for spermine, 3-3-3, 3-2-3, BESm, BE-3-3-3, BE-3-2-3 and 3-2-2-3 calculated from these concentrations have been reported (Basu *et al.*, 1989).

As found for the ability to induce the B-Z transition, the DNA-aggregating properties of Group I and IV compounds were strongest for spermine and 4-4-4, but weakened with either an increase (3-8-3) or a decrease (3-3-3, 3-2-3 etc.) in the length of the central carbon chain (column 3 of Table 2). For Group III

compounds, both dihydroxy and dimethyl derivatives were better than the parent (3-8-3), and the difluoro derivative was worse than the parent (spermine), in their abilities to aggregate DNA (column 3 of Table 2). Either an increase in the length of the central carbon chain (3-8-3) or an increase in the number of n-butyl groups (4-4-4), however, caused a marked decrease in the ability to aggregate DNA, but had only a slight effect on the ability to induce Z-structures. Unlike the trend found for induction of Z-structures, the aggregating abilities of bisethyl derivatives (Group II) were much weaker than those of the parent polyamines and were affected markedly by a change in the number of n-butyl groups; BESm was the most efficient and BE-4-4-4 was the least efficient compound for the aggregation of DNA. Thus, apart from the length and the structure of the central carbon chains, the terminal primary amino groups of polyamines are very important for DNA aggregation, and derivatization of these primary amino groups impairs the ability to aggregate DNA.

The T_m of DNA was calculated from cross-over points of the second derivatives of the melting profiles. Representative plots of T_m against increasing concentrations of some of the analogues are shown in Fig. 3. Because of the onset of aggregation of DNA, the T_m at $> 10 \mu\text{M}$ 4-4-4 and $> 15 \mu\text{M}$ spermine could not be determined. The highest concentration of 4-4-4 that could be used for the determination of T_m is less than the concentration needed for the induction of aggregation at room temperature (column 3 of Table 2), presumably because of the relatively higher efficiency of penta-amines for the induction of DNA aggregation at elevated temperatures (Basu & Marton, 1987).

A different correlation was observed when the affinities of spermine and the analogues for double-stranded (helix form) DNA were determined from T_m . For most of the compounds the affinity for DNA paralleled the ability to aggregate DNA (columns 4 and 5 of Table 2), and increased with the number of n-butyl groups. 4-4-4-4 and 4-4-4, however, had higher affinities for DNA than did spermine, a result that was not expected based on the results of the aggregation studies, which showed spermine to be the most efficient compound. With the exception of BE-3-3-3 and BE-3-2-3, for which no significant differences in affinities were observed, the affinity for double-stranded DNA decreased

Fig. 2. U.v. spectra of approx. 0.5 A_{260} units of calf thymus DNA in 50 mM-NaCl/1 mM-sodium cacodylate, pH 7.0, in the presence of 0 mM (—), 40 mM (····) or 100 mM (---) 3-3-3

Fig. 3. Melting temperature (T_m) of approx. 0.5 A_{260} unit of calf thymus DNA in 50 mM-NaCl/1 mM-sodium cacodylate, pH 7.0, in the presence of increasing concentrations of (i) 4-4-4-4 ($\square$), (ii) 4-4-4 ($\triangle$), (iii) BE-4-4-4 ($\circ$), (iv) spermine ($\bullet$) or (v) BE-3-2-3 ($\blacktriangle$)

The error in the determination of each point is $\pm 0.1^\circ\text{C}$.

Fig. 4. Release of EB from its complex with calf thymus DNA by (i) 4-4-4 (○), (ii) spermine (●), (iii) 4-4-4 (△), (iv) BE-4-4-4 (▲) or (v) BE-3-2-3 (□) in 50 mM-NaCl/1 mM-sodium cacodylate, pH 7.0, at room temperature

The error in the determination of each point is ± 0.02 .

with derivatization of the primary amino groups (column 4 and 5 of Table 2).

There was no clear correlation between the Z-forming and aggregating properties of the analogues and their affinities for DNA, except that either a decrease in the central carbon length or fluorination of the β -carbon of the central n-butyl group weakened all three. The loss of these properties may be the result of a change in the pK_a of one or both of the central secondary-amino groups of the analogues that may decrease the total positive charge. This hypothesis cannot be confirmed until the pK_a values of the analogues are known. Nevertheless, for spermidine analogues it is known that a decrease in the length of the central carbon chain to less than four carbon atoms or fluorination of the β -carbon of the central n-butyl group decreases the pK_a of the secondary amino groups to well below 7.0 (Delfini *et al.*, 1980; Kimberley & Goldstein, 1981; Thomas & Messner, 1988), which would cause the compounds to be less positively charged than spermidine at pH 7.

Representative fluorescence titration profiles of the EB-DNA complex with several of the analogues are shown in Fig. 4. Because of polyamine-induced aggregation of DNA, complete release of EB from the complex could not be achieved under the conditions used. A sharp increase in the release of EB was observed in the presence of 4-4-4-4 at concentrations above 12 μ M. The fraction of EB released from the complex by 30 μ M-spermine or the analogues (DNA phosphate/analogue, 1:1) are listed in column 8 of Table 2. Note that neither spermine nor 4-4-4-4 could aggregate the EB-DNA complex at concentrations at which they aggregate DNA alone (column 3 of Table 2).

In contrast with results reported recently by Stewart (1988), we found that spermine and the analogues could not release all bound EB from the EB-DNA complexes before aggregation of DNA occurred. This may be related to the higher ionic strengths used in our studies. Because the association of polyamines with DNA is more sensitive to change in the ionic strength than is the association of EB with DNA (Lepecq & Paoletti, 1967; Bresloff & Crothers, 1975; Braunlin *et al.*, 1982), the concentration of polyamines required to release all the EB from DNA under the conditions used in our experiments is too high to be used without causing DNA aggregation. We found that lowering the Na^+

concentration from 50 mM to 10 mM enhances the amount of EB released from 30 to 60% at identical spermine concentration (H. S. Basu, unpublished work).

For certain analogues we found a correlation between the release of bound EB from DNA (column 8 of Table 2) and the affinities for double-stranded calf-thymus DNA determined from T_m studies (column 4 of Table 2). The analogues 4-4-4-4 and DFS have the highest and lowest affinities for DNA and are the strongest and weakest analogues for the release of EB respectively. The bisethyl derivatives of spermine, 4-4-4 and 3-2-2-3 associated with DNA and released bound EB less well than did the parent polyamines. This correlation, however, was not found when the affinities of analogues of different chain lengths were compared with the ability to release bound EB. Thus 4-4-4, BE-4-4-4 and 3-8-3 have higher affinities for DNA than does spermine, but released EB less well; BE-3-2-2-3 has an affinity for DNA similar to that of DFS, but released EB more efficiently. These results suggest that simple polyamine-DNA association is not entirely responsible for the release of EB from DNA under the conditions used in these experiments. We believe that the release of EB is related to a conformational change in the DNA structure caused by interactions of spermine or its analogues. Although we cannot adequately explain the observed increase in the release of EB above 12 μ M-4-4-4-4, it is possible that binding of 4-4-4-4 to DNA above a certain analogue/DNA ratio may cause conformational changes that facilitate the release of bound EB.

The results reported here suggest that both the number of positive charges on polyamines and the distribution of charge on the surface of the molecule have profound effects on its ability to induce DNA conformational changes. Testing these analogues in tissue culture should shed light on the correlation between the structure of polyamines and their biological functions and may increase our understanding of how to modify these compounds, which may have potential as therapeutic agents. Our results should assist the search for polyamine analogues that will affect cell function and inhibit growth but will not produce the conformational changes in DNA caused by natural polyamines. Appropriate analogues may have therapeutic value and could be used to study the mechanisms involved in the biological functions of naturally occurring polyamines.

This work was supported in part by N.I.H. Program Project Grant CA-13525, National Co-operative Drug Discovery Group Grant CA-37606 (to L.J.M.), N.I.H. Grant CA-41757 (to B.G.F.) and the Aaron Silvera Cancer Research Fund. We thank Dr. Phillippe Bey, Professor Keijiro Samejima and Professor Raymond Bergeron for kindly providing analogues used in this study, Professor Richard H. Shafer for helpful discussions, and Mr. Neil Buckley for comments on the manuscript.

REFERENCES

- Baillon, J. G., Mamont, P. S., Wagner, J., Gerhart, F. & Lux, P. (1988) *Eur. J. Biochem.* **176**, 237-242
- Basu, H. S. & Marton, L. J. (1987) *Biochem. J.* **244**, 243-246
- Basu, H. S., Shafer, R. H. & Marton, L. J. (1987) *Nucleic Acids Res.* **15**, 5873-5886
- Basu, H. S., Feuerstein, B. G., Zarling, D. A., Shafer, R. H. & Marton, L. J. (1988) *J. Biomol. Struct. Dyn.* **6**, 299-309
- Basu, H. S., Feuerstein, B. G., Deen, D. F., Lubich, W. P., Bergeron, R. J., Samejima, K. & Marton, L. J. (1989) *Cancer Res.* **49**, 5591-5597
- Becker, M., Misselwitz, R., Damaschun, H., Damaschun, G. & Zirwer, D. (1979) *Nucleic Acids Res.* **6**, 1297-1309
- Behe, M. & Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1619-1623
- Berman, H. M. & Young, P. R. (1981) *Annu. Rev. Biophys. Bioeng.* **10**, 87-114

- Bloomfield, V. A. & Wilson, R. W. (1981) in *Polyamines in Biology and Medicine* (Marton, L. J. & Morris, D. R., eds.), pp. 183–206, Marcel Dekker, New York
- Braunlin, W. H., Strick, T. J. & Record, M. T., Jr. (1982) *Biopolymers* **21**, 1301–1314
- Bresloff, J. L. & Crothers, D. M. (1975) *J. Mol. Biol.* **95**, 103–123
- Bresloff, J. L. & Crothers, D. M. (1981) *Biochemistry* **20**, 3547–3553
- Cain, B. F., Baguley, B. C. & Denny, W. A. (1978) *J. Med. Chem.* **21**, 658–668
- Delfini, M., Segre, A. L., Canti, F., Barbucci, R., Barone, V. & Ferruti, P. (1980) *J. Chem. Soc. Perkin Trans. 2*, 900–903
- Feuerstein, B. G., Pattabiraman, N. & Marton, L. J. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5948–5952
- Gosule, L. C. & Schellman, J. A. (1978) *J. Mol. Biol.* **121**, 311–326
- Jain, S. C. & Sobell, H. M. (1984a) *J. Biomol. Struct. Dyn.* **1**, 1161–1177
- Jain, S. C. & Sobell, H. M. (1984b) *J. Biomol. Struct. Dyn.* **1**, 1179–1194
- Jain, S. C., Tsai, C.-C. & Sobell, H. M. (1977) *J. Mol. Biol.* **114**, 317–331
- Kimberly, M. M. & Goldstein, J. H. (1981) *Anal. Chem.* **53**, 789–793
- Lamos, M. L., Walker, G. T., Krugh, T. R. & Turner, D. H. (1986) *Biochemistry* **25**, 687–691
- Lepecq, J.-B & Paoletti, C. (1967) *J. Mol. Biol.* **27**, 87–106
- Marquet, R., Wyart, A. & Houssier, C. (1987) *Biochim. Biophys. Acta* **909**, 165–172
- McCann, P. P., Pegg, A. E. & Sjoerdsma, A. (1987) *Inhibition of Polyamine Metabolism*, Academic Press, Orlando
- Neidle, S. & Abraham, Z. (1984) *CRC Crit. Rev. Biochem.* 73–121
- Neumann, E. & Ackermann, T. (1969) *J. Phys. Chem.* **73**, 2170–2178
- Pegg, A. E. (1988) *Cancer Res.* **48**, 759–774
- Pohl, F. M., Jovin, T. M., Baehr, W. & Holbrook, J. J. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3805–3809
- Porschke, D. & Jung, M. (1982) *Nucleic Acids Res.* **10**, 6163–6167
- Stewart, K. D. (1988) *Biochem. Biophys. Res. Commun.* **152**, 1441–1446
- Tabor, C. W. & Tabor, H. (1984) *Annu. Rev. Biochem.* **53**, 749–890
- Thomas, T. J. & Messner, R. P. (1988) *J. Mol. Biol.* **201**, 463–467
- Tsai, C.-C., Jain, S. C. & Sobell, H. M. (1977) *J. Mol. Biol.* **114**, 301–315
- Vertino, P. M., Bergeron, R. J., Cavanaugh, P. F. & Porter, C. W. (1987) *Biopolymers* **26**, 691–703
- Walker, G. T., Stone, M. & Krugh, T. R. (1985) *Biochemistry* **24**, 7462–7471

Received 13 March 1989/4 January 1990; accepted 7 February 1990