Interactions with Natural Polyamines and Thermal Stability of DNA. A DSC Study and a Theoretical Reconsideration

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Abstract: The effects of different amounts of four natural polyamines on the thermodynamics of the thermal denaturation of calf thymus and herring sperm DNA have been studied by means of differential scanning calorimetry. Enthalpy changes and the temperature of the maximum heat effect were determined. The stability of the double helix increases by increasing the polyamine/phosphate ratio and the number of positively charged groups on the polyamine molecule. A combination of Manning's polyelectrolyte theory and McGhee and von Hippel's multiple-site exclusion approach has been demonstrated to give a very good reproducibility of experimental results.

Introduction

"Polyamines" is the generic name of a group of small aliphatic polycationic compounds which are found in all living organisms.[1] In animal cells these usually consist of putrescine, spermidine, and spermine which together form a simple biosynthetic pathway.[1] Putrescine and spermidine are also found in prokaryotes, but spermine appears to be synthesized only by eukaryotes.[1] Their concentration varies during the cell cycle and they are thought to play a role in cell proliferation and differentiation, DNA replication, protein synthesis, membrane stabilization, and activity of several enzymes, including kinases, topoisomerases, and those enzymes involved in their own metabolism.[2−7] Exogenous polyamines and their analogues are readily transported into the cell, and their concentration becomes particularly elevated in biological fluids and tissues in a number of disease states such as cancer, psoriasis, and sickle cell anemia.[8−10] Despite the importance of such compounds in various processes involved in cell growth, their primary role in cell biology and mechanisms of their action are not clear yet. They are positively charged at physiological ionic conditions, so they are believed to interact electrostatically with organic anions in a manner that might contribute to the tertiary structure and/or integrity of macromolecules.[11] The negatively charged phosphate groups of the DNA and RNA are therefore the prime targets for interaction; however, despite the extended literature, no real amelioration of the model proposed by Liquori[12] about the precise position and orientation to which a polyamine binds to DNA has been reported, nor is it understood how the geometric constraints of the binding-mode influence the structural and mechanistic behavior of the system.[13] Polyamines have been shown to stabilize DNA against thermal[11,14,15] and alkaline denaturation,[16] enzymatic degradation,[17] shear breakage,[18] radiation damage, and intercalation of aromatic dyes.[19] They may be important in maintaining cellular DNA in a compact state, facilitating packaging of DNA into phage heads.[20−23] They have been shown to cause the condensation and aggregation of native DNAs,[23−29] as does the inorganic $Co(NH_3)_4^{3+}$ cation,[30] and they may prove a useful model for biologically important packaging processes, such as the structural organization of chromosomes.[31] The structural specificity of polyamines and their acetylated derivatives is an important feature in the induction and stabilization of left-handed Z-DNA.[11,32−34] So they are able to induce, like their acetyl derivatives, conformational transitions of DNA such as the B

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to Z transition of poly(dG-m^{5}dC)·poly(dG-m^{5}dC).[33,35,36] Lowering the charge density can both favor DNA triple helix formation and stabilization[36−38] and also stabilize bends and loops in t-RNA[22,39,40] and in ribosome.[41] It seems likely that the binding of polyamines will influence conformational dynamics (i.e., bending torsion and transient base unstacking) of DNA.[42] The effect of polyamines on the thermal stability of the duplex DNA is governed by a wide number of physicochemical parameters such as ionic charge, pH, and ionic concentration of the medium. The aim of this paper is to provide a quantitative description of the effects of polyamines on DNA thermal stability, taking into account the effects of both their inherent electrostatic forces and conformational restraints imposed by their size, shape, and nature. The binding of polyamines to DNA was interpreted by the counterion condensation binding theory of polyelectrolyte, as developed primarily by Manning.[43−45] Such theory has become widely used presumably because of its simplicity and its ability to predict with reasonable accuracy the entire spectrum of biopolyelectrolyte behavior.[46−49] We extended our consideration of the multiple-site exclusion binding theory of McGhee and von Hippel[50] to incorporate multivalent ligands, obtaining an accurate estimation of the extent of binding of the counterions to DNA and a complete description of the trend of the denaturation temperatures. The experimental trend of melting temperatures was then compared with the theoretical one. In our work, polyamines are of interest primarily as model ligands for the investigation on the electrostatic component of the interactions of large molecules, with well separated charges on their surface (as proteins) with nucleic acids.

Experimental Section

Materials. Calf thymus (ct-DNA) and herring sperm DNA (hs-DNA) sodium salts, with 41.9% and 42.2% dG-dC base pairs, respectively,[51,52] were purchased from Sigma and used without further purifications. We found a value of the A_{260}/A_{280} ratio of about 1.8, consistent with a low protein content.[53] The DNA solutions were prepared by dissolving the lyophilized DNA samples (≈3 mg/mL) in 1.0 mM Tris buffer, with 10 mM NaCl at pH 7.20 ± 0.01, for 48 h at 4 °C, then dialyzed exhaustively against the same buffer solution. The DNA concentration was determined spectrophotometrically at 260 nm by using molar extinction coefficient of 6600 cm^{-1} M^{-1} (expressed as molarity of phosphate groups).[54] The mean molecular weight of a nucleotide residue was assumed to be 330 g/mol. The polyamines putrescine ($NH_2(CH_2)_4NH_2$), cadaverine ($NH_2(CH_2)_5NH_2$), spermidine ($NH_2(CH_2)_3NH(CH_2)_4NH_2$), and spermine ($NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$) were purchased from Fluka and used without further purifications. These polyamines are present in the eukaryotic and/or prokaryotic cells, except for cadaverine which is a homologue of putrescine and is present only in some plants. The polyamines were dried over P_2O_5 in a vacuum desiccator for 12 h prior to use. We prepared fresh stock solution before each experiment in the same buffer as DNA. Samples for calorimetric measurements were prepared by mixing appropriate volumes of DNA and polyamine stock solutions at known concentration and diluting with the buffer solution to a standard volume (usually 5.0 mL). DNA concentration (c_p) was kept constant at 4.0 mM (in nucleotide monomer) for all experiments, while the amount of polyamine was varied in order to obtain solutions with polyamine/DNA phosphate concentration ratios (R_j) in a 0.005−0.5 range in which no significant variation of pH occurs. In our studies we also observed that the optical density of DNA solutions drops drastically at higher polyamine concentrations owing to some aggregation phenomena. The critical concentration of polyamines required to induce aggregation depends on the ionic strength.[23,42] Wilson and Bloomfield have determined, in light of Manning's theory of polyelectrolytes, the spermine concentration required to induce condensation of DNA at different ionic strengths. In all cases collapses occur when 89−90% of the DNA phosphate charges are neutralized by condensed counterions.[23]

Apparatus and Procedure. Calorimetric measurements were carried out on a second-generation Setaram Micro-DSC apparatus. The instrument was interfaced with a data translation A/D board for automatic data acquisition. A scan rate of 0.5 K/min was chosen for the present study. All analyses of the data were accomplished by using the software THESEUS developed at our laboratory.[55] The raw data were converted to an apparent molar heat capacity by correcting for the instrument's calibration curve and the buffer−buffer scanning curve and by dividing each data point by the scan rate and the number of moles of nucleotide in the sample cell, according to the Freire and Biltonen procedure.[56,57] Thermodynamic parameters as well as van't Hoff enthalpy were obtained by using a previously described treatment.[58−61]

For solving the nonlinear equations present in the theoretical section of this work we used the software MATLAB.

Results

In the Figure 1, a and b, the characteristic melting profiles of calf thymus DNA and herring sperm DNA are shown. The large differences in the two curves are reproducible and specific to the biological origin of each DNA. Heat capacity as a function of temperature is unique for a DNA sequence according to its base composition. The thermal melting profile of calf thymus DNA is completely different from that of herring sperm DNA, even though they have about the same base pair composition. While the melting curve of the herring sperm DNA is characterized by a large single peak, with a temperature of the maximum heat effect (T_{max}) centered at 342.3 K, the melting profile of calf thymus DNA near the principal maximum, centered at 340.4 K, shows three further "satellite" peaks at higher temperatures. A step-by-step calorimetric analysis of

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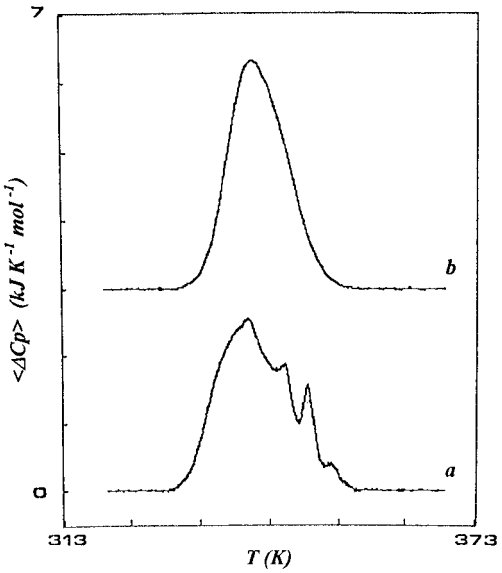

Figure 1. DSC thermal denaturation profiles of calf thymus (a) and herring sperm (b) DNAs. The buffer solution is 1.0 mM Tris, 10 mM NaCl, pH 7.2. The DNA concentration is 4.0 mM (in nucleotide residue). The heating rate is 0.5 K/min. The instrumental signal was corrected for the calibration curve and for the buffer−buffer reference baseline. Curve b is shifted on the ordinate axis by three units for illustrative purposes.

the ct-DNA melting profile also allowed us to show that the main melting peaks are independent of each other. The ct-DNA melting pattern is reproducible and identical with that obtained by Blake and co-workers by means of the differential UV-absorption technique.[62] Both experimental and theoretical approaches have attributed such "satellites" to the presence in the genome of highly repetitive short base pair sequences with higher content of dG-dC.[52,63−65] The portion of highly, moderately, or poor repetitive sequencies varies from the DNA of one species to those of others.[66,67] As can be seen, both the denaturation curves of ct-DNA and hs-DNA occur over a large temperature range of about 25°, even though the hs-DNA calorimetric profile is sharper than ct-DNA and the change of heat capacity from the native and the denatured state ($\Delta_d C_p$) is small for all the measurements. The denaturation of DNA is completely irreversible because the denaturation profile disappears after the first heating and a subsequent cooling at room temperature. However, this criterion for irreversibility could be too much restrictive.[68] In fact, the irreversibility arises essentially from the enormous number of pairing mistakes occurring when the two strands are rebuilding all the H-bonds among base pairs. Homopolynucleotides, as poly(dA-dT) lacking the specific sequences, or small oligonucleotides, have shown to be reversible in the reconstitution of the double helix.[53,61,64,66,67]

Tables 1 and 2 summarize the thermodynamic parameters that characterize the thermal denaturation process of the calf thymus and herring sperm DNA in the presence of the four polyamines. Each value represents the average of at least three experiments. The determination of melting temperature in all the experiments shows little deviation (±0.2 K) from the mean value even though different samples were used. The relative uncertainty of the value of the denaturation enthalpy is below 5%. Tables 1 and 2 also report the values of the thermodynamic parameters characterizing the ct-DNA and hs-DNA alone; such values are in good agreement with those found by Klump: T_{max} = 339.2 K, $\Delta_d H$ = 16.8 kJ/mol; T_{max} = 341.2 K, $\Delta_d H$ = 16.6 kJ/mol, respectively.[64]

Table 1. Thermodynamic Parameters of Calf Thymus DNA Denaturation in the Presence of Different Concentration Ratios between the Four Polyamines and DNA Phosphate[a]

R_j	T_{max} (K)[b]	$\Delta_d H$ (kJ·mol^{-1})[c]	<m>
	Putrescine		
0	340.4	17.0	28
0.05	343.4	17.5	24
0.1	346.2	18.3	23
0.15	348.6	18.9	25
0.2	350.8	19.7	26
0.25	352.6	20.4	25
0.35	355.0	21.3	26
0.5	355.8	21.7	26
	Cadaverine		
0.05	343.3	17.8	24
0.1	346.1	18.0	24
0.15	348.7	18.5	23
0.2	350.6	18.9	25
0.25	352.4	20.1	25
0.35	355.2	21.5	26
0.5	356.0	21.7	26
	Spermidine		
0.05	345.5	18.7	22
0.1	351.6	19.7	23
0.15	356.0	21.4	23
0.2	358.5	23.0	24
0.25	360.3		
0.35	362.7		
0.5	362.8		
	Spermine		
0.005	341.6	18.0	22
0.05	349.3	18.6	23
0.1	354.4	20.7	24
0.15	359.4	22.1	25
0.2	363.7		

[a] The cooperativity parameter <m> is equal to the ratio $\Delta H_{v.H.}/\Delta_d H$. The values of the thermodynamic parameters characterizing the ct-DNA alone are reported only in case of putrescine. [b] The error in T_{max} does not exceed 0.2 K. [c] The estimated (relative) uncertainties in $\Delta_d H$ are below 5% of reported values.

The stabilization of the double helix of DNA by polyamines is clearly shown by the increase of temperature of principal maxima at increasing the polyamine/DNA phosphate concentration ratio R_j for both DNAs. This stabilization increases at increasing the number of charged groups on the polyamine molecule. In fact, the diamines putrescine and cadaverine have the same effect on ct-DNA stability; the increment of maximum temperature is about 15 K for both polyamines in a R_j range of 0−0.5. For spermidine which has three positive charges, the stabilization in the same range is about 23 K. Finally spermine, which has four positive charges, shows the greater effect; the increase of temperature is about 24 K in the narrower concentration range. The same trend is observable for hs-DNA in the presence of the four polyamines. In Figure 2, the DSC profile of the ct-DNA itself and in the presence of different amounts of the two diamines, putrescine and cadaverine, are compared; in Figure 3, the thermal melting profiles of spermidine and spermine are reported. At increasing polyamine concentrations, a shift of the whole broad thermal denaturation peak is observed and its asymmetry is only slightly altered, while the fine

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Table 2. Thermodynamic Parameters of Herring Sperm DNA Denaturation in the Presence of Different Concentration Ratios between the Four Polyamines and DNA Phosphate[a]

R_j	T_{max} (K)[b]	$\Delta_d H$ (kJ·mol^{-1})[c]	$<m>$
	Putrescine		
0	342.3	16.7	34
0.05	345.1	18.1	30
0.15	350.9	18.9	29
0.2	353.0	19.7	30
0.25	354.7	20.1	30
0.35	356.9	20.5	31
0.5	357.5	21.3	32
	Cadaverine		
0.05	345.0	18.2	30
0.15	350.7	18.5	30
0.2	353.2	19.7	31
0.25	354.5	20.3	30
0.35	357.1	20.7	30
0.5	357.4	21.5	31
	Spermidine		
0.05	347.6	19.1	30
0.15	357.8	21.3	29
0.2	361.0	23.1	30
0.25	362.7	24.0	31
0.35	365.1	24.5	30
0.5	365.5	24.9	32
	Spermine		
0.05	352.8	18.7	29
0.1	358.2	19.8	29
0.15	361.3	21.1	31

[a] The cooperativity parameter $<m>$ is equal to the ratio $\Delta H_{v.H.}/\Delta_d H$. The value of the thermodynamic parameters characterizing the hs-DNA alone are reported only in case of putrescine. [b] The error in T_{max} does not exceed 0.2 K. [c] The estimated (relative) uncertainties on $\Delta_d H$ are below 5% of reported values.

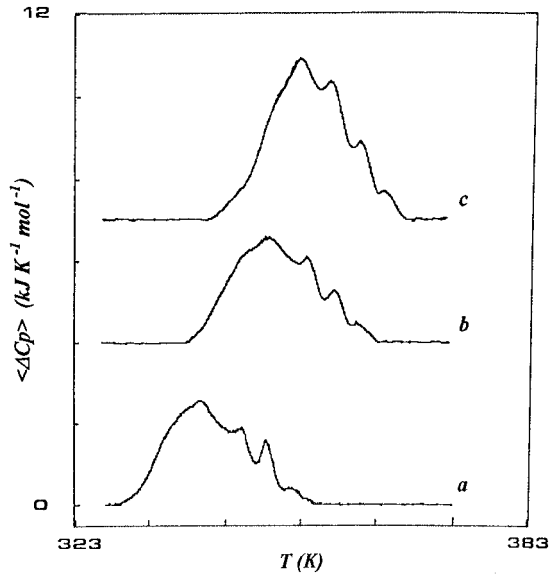

Figure 2. Melting of calf thymus DNA in the absence or in the presence of different amounts of two diamines. The amount of polyamine is indicated as R_j corresponding to the ratio between polyamine and DNA (expressed in moles of nuclotide per liter) concentrations: (a) $R_j = 0$, (b) $R_j = 0.15$ for putrescine, and (c) $R_j = 0.35$ for cadaverine. Curves b and c are shifted on the *y*-axis for illustrative purposes.

structure is still evident. This, with the observation that the trend of melting temperatures is the same for both the DNAs, could indicate that the interaction with polyamine does not depend on the composition or sequence of DNA. The same behavior is, in fact, observed for the DSC profile of hs-DNA in the presence of polyamines in Figures 4 and 5.

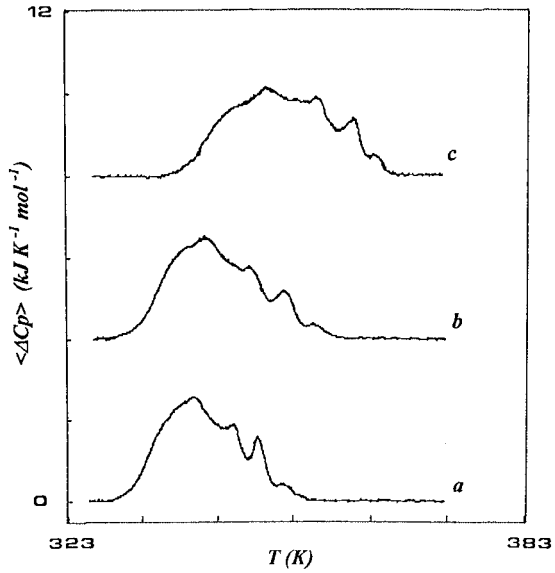

Figure 3. DSC thermal denaturation profiles of calf thymus in the absence or in the presence of spermine and spermidine at different R_j ratios: (a) $R_j = 0$, (b) $R_j = 0.05$ for spermine, and (c) $R_j = 0.15$ for spermidine. Curves b and c are shifted as in the Figure 2.

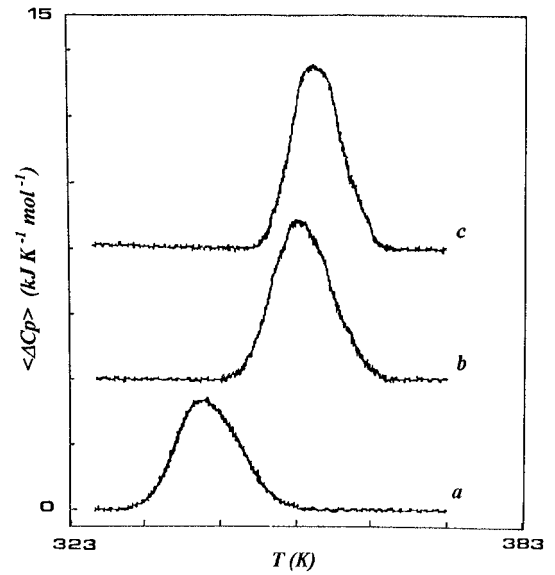

Figure 4. DSC profiles of herring sperm DNA in the absence and in the presence of different concentrations expressed in R_j ratio of spermine and spermidine: (a) $R_j = 0$, (b) $R_j = 0.1$ for spermine, and (c) $R_j = 0.25$. Curves b and c are shifted as in the Figure 2.

The calorimetric enthalpy increases with an increase in the concentrations ratio R_j for all the polyamines and for both DNAs. The $\Delta_d H$ values reported as a function of T_{max} increase quite linearly for all polyamines. Since $\Delta_d C_p$ is equal to the temperature derivative of $\Delta_d H$, it seems that the contribution to $\Delta_d C_p$ for the thermal denaturation of DNAs induced by the presence of studied polyamines is not zero. The calorimetric enthalpy seems also to be linked to the number of charged groups present on the polyamine molecule for both DNAs. The induced stabilization by spermidine is higher than those observed for putrescine and cadaverine in the same explored concentration range.

The stabilization produced by spermine is comparable with that produced by spermidine in the R_j range 0−0.2. We cannot obtain a value of $\Delta_d H$ for thermal denaturation of ct-DNA at R_j values higher than 0.2 in the presence of spermidine because the denaturation profiles occur outside the temperature range

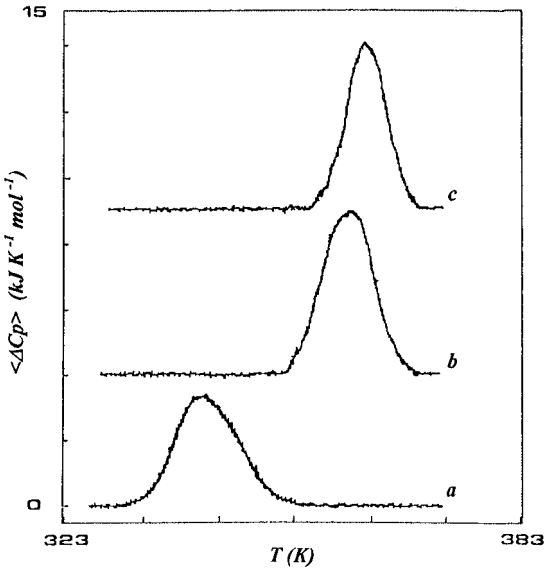

Figure 5. Melting profiles of herring sperm DNA itself (a) and in the presence of putrescine (b) at a concentration ratio $R_j = 0.35$ and cadaverine at $R_j = 0.5$ (c). Curves b and c are shifted as in the Figure 2.

experimentally accessible. The same happens for ct-DNA in the presence of spermine at $R_j = 0.2$. It was not possible to evaluate the thermodynamic parameters at R_j ratios higher than 0.15−0.2 for hs-DNA and ct-DNA, respectively, in the presence of spermine, because of the aggregation of DNA in solution.

A quantitative comparison of the calorimetrically measured enthalpies with the van't Hoff enthalpies gives information on the cooperativity of the transition as well as on the breadth of the denaturation profile. The ratio $\Delta H_{\text{v.H.}}/\Delta_{\text{d}}H$ is a parameter, indicated here by $<m>$, correlated with the mean number of nucleotides that melt as single thermodynamic entities; in other words, it gives an index of the relative, not absolute, size of the mean cooperative unit.[61,64,69,70] The values of the cooperativity parameter $<m>$ are reported in the fourth column of the Tables 1 and 2. As can be seen, this parameter is slightly decreased with respect to the DNA itself but it increases by increasing polyamine concentration. There is no large alteration of the size of the cooperative unit, but the effect on transition breadth on first addition of polyamine and the reduction in breadth with further addition can readily explained in line with the observations by Record[71] and Berestetskaya *et al.*[72]

Polyamine cations are almost completely bound to the double helix at lower concentration. Partial denaturation of a helix releases some of them, which are free to bind elsewhere on the same helix or on another one, increasing the stability of unmelted regions, thereby broadening the transitions. At higher R_j ratios, the binding of cations is more nearly saturated and the potential for such differential stabilizing effect is reduced.[71,72]

Discussion

We undertook this study to understand the binding properties of polyamines to DNA and the electrostatic component of the interactions of polycations with nucleic acids which can be useful to clarify their biological roles. Our results suggest that the interaction of these cations with DNA is predominantly dependent upon charge, but their structures also play a noticeable role. The approach here, developed for obtaining a theoretical trend of the thermal denaturation temperatures in the presence of different counterions, starts initially from the analysis of DNA thermal stability as a function of sodium and magnesium concentration, as performed by Krakauer, Manning, Record, and Wyman in several works.[43,71,73−77] Their approach is based on the assumption that a stoichiometric amount of released counterions accompanies and drives the denaturation process. Considering the effects of polyamine ligands on the melting temperature of a nucleic acid conformational transition, we assume that no anions participate in the interaction and that all solute species are sufficiently diluted in order to consider the water activity as unitary. The equilibrium between helix (*h*) and coil (*c*) states of a nucleotide residue in DNA can be expressed thus:

$$h \leftrightarrow c + \Delta r_i'(\text{Na}^+) + \Delta r_j'(\text{pAm}^{n+}) \qquad (1)$$

where $\Delta r_i'$ and $\Delta r_j'$ represent the numbers of univalent cations (Na^+) and polyamine cations (pAm^{n+}) physically released from a residue as it is converted from helix to coil under specified conditions. The problem, hence, is to determine the extent to which polyamines bind preferentially to a polynucleotide system to calculate the number of counterions (i.e., univalent and multivalent) released during the thermal helix−coil transition of DNA. The development of a theory for the dependence of melting temperature of DNA on the ionic concentration requires, on the other hand, a correct description, in terms of the Gibbs energy, of the system. For evaluating the real extent of binding of nonspherical multivalent ligand both to DNA double helix and coil and calculate the total Gibbs energy of the system, we must introduce, besides the polyelectrolyte term, a contribution due to "nonpolyelectrolyte" effects.

In order to calculate the polyelectrolyte contribution to Gibbs energy, we follow the procedure of the so-called "chemical model" as first developed by Manning[78] and resumed by Paoletti and co-authors[79,80] by which the polyelectrolyte contribution to Gibbs energy of polyelectrolyte can be factorized into two main terms: a purely electrostatic term and a Gibbs energy of mixing of the mobile species [the calculation procedure is reported in a Theoretical Appendix as supplementary material]. In the basic scheme of the counterion condensation theory, the real polyelectrolyte chain is modelled like a linear uniform array of charges with two types of counterions in solution, *i* and *j*, with valences z_i and z_j, respectively. In the present case, we consider the univalent supporting salt cation (Na^+) as the counterion *i* and the polyamine cation (pAm^{n+}) as the counterion *j*; we will always have $z_i = 1$ and z_j will have a different value for each polyamine molecule. The ionic behavior of polyelectrolyte is defined not by valence, or total charge, but by a single nondimensional structural parameter proportional to the unitless charge density ξ. This parameter is given by the ratio between the Bjerrum length, depending on solvent dielectric constant and temperature, and the average axial charge spacing, that is, the contour length divided by the number of charged groups.

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The double helix of B-DNA has a value of the corresponding helix parameter $\xi_h = 4.2$,[45−49] while, assuming the coil state as a single stranded DNA, a value for the coil parameter $\xi_c = 1.8$ was evaluated by Manning.[81] Record *et al.*[82] confirmed this value, while Olson and Manning[83] have provided a configurational interpretation of this result.

We calculated all the values of the fraction of condensed cations by varying the concentrations ratio R_j, for both double helix and coil forms. From such values we can evaluate the fraction of condensed multivalent cation r_j which is the independent variable in the Scatchard plot, together with the dependent variable, K_j, the binding constant for a polyamine molecule. Such a plot is not linear, and the behavior interpreted as a "negative cooperativity" is only the simple result of the interplay of electrostatic interactions between ions of different valence.

Because of the large size and flexible conformation of the ligand, the binding interaction can have some "nonpolyelectrolytic" character, for which the employment of a theoretical scheme that takes into account ligand size and cooperativity is more appropriate. We can retain the more general formalism of the multiple-site exclusion binding model to analyze the interaction of polyamines with the nucleic acid lattice, this being mainly electrostatic in nature. Therefore, under more general conditions, it is possible to obtain a more correct estimation of the extent of binding of counterions by adding to the Gibbs energy a contribution due to "nonpolyelectrolyte" effects.

According to the McGhee and von Hippel theory, if the ligand binding is idealized as a site binding to a homogeneous lattice, the expression for minimization of the Gibbs energy in the absence of polyelectrolyte effects can be deduced by the multiple-site equilibrium expression:[50]

$$r_j'/[L]_f = K'_j(1 - n_j r_j')^{n_j}/[1 - (n_j - 1)r_j']^{n_j - 1} \qquad (2)$$

where n_j is the number of phosphates occupied by a bound molecule or otherwise excluded from the binding of other ligands, r_j' corresponds to the ratio of ligands bound per site, and $[L]_f$ is the concentration of free ligand. This equation defines K'_j the association constant in the absence of polyelectrolyte effects. As discussed by Friedman and Manning,[84] relationship 2 could also describe both multiple-site exclusion and polyelectrolyte effects: if the polyelectrolyte chemical equilibrium behavior of a ligand−polyion system is describable in terms of exclusion of n_j sites and if we ignore any alteration or influence due to the changes in the polynucleotide conformation determined by the binding of counterions, K'_j will not be constant because of the polyelectrolyte effect. In other words, if there is no variation of the parameter ξ with the binding fraction of multivalent counterions bound to the sites r_j', except by neutralization of the negative phosphates, we can incorporate the polyelectrolyte effects into eq 2 simply by replacing K'_j with K_j.[84] Lattice inhomogeneity, as well as multiple-site exclusion, is a source of anticooperativity in a binding equilibrium, but for our purposes the inhomogeneity in DNA was ignored. Specificity effects for DNA sequence were found to be minimal in studies that used synthetic homologues of natural polyamines for duplex stabilization, but, for simplicity and to reduce the number of parameters, we have assumed that ligand binding is not cooperative. Any cooperativity which might exist is thereby

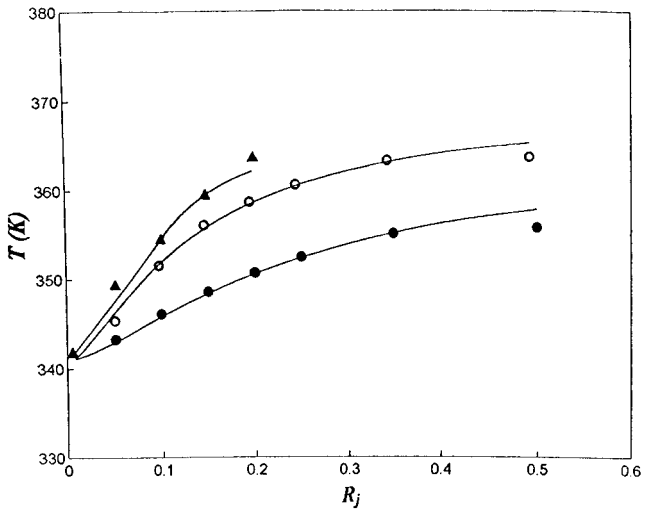

Figure 6. Comparison between the experimental thermal denaturation helix−coil transition temperatures of ct-DNA and the theoretical ones reported as a function of the ratio between the polyamine and DNA concentrations (expressed in number of moles of nucleotide) R_j. The continuous line is the theoretical trend of such temperatures in the presence of multivalent ligands, while the experimental data are reported with the symbols: (●) putrescine, (○) spermidine, and (▲) spermine. In this figure we do not give the melting temperature trend for the ct-DNA in the presence of cadaverine. The experimental data would have been superimposed onto the data regarding the system in the presence of putrescine, and the graph would have been unclear.

absorbed into the parameter n_j, which is the effective size of the ligand and which could assume nonintegral values. Starting from relationship 2, we calculated the real extent of binding per phosphate of all the four polyamines to the native and the coil states of DNA. The fraction r_j' is lower than the value calculated taking into account only the polyelectrolyte effects (namely r_j), and as a consequence the extent of binding of monovalent ions of the supporting salt is higher. We have then calculated the theoretical trend of melting temperature of DNA solutions in the presence of multivalent cations and compared it to the experimental one in the case of ct-DNA and hs-DNA in the presence of the cationic polyamines using suitable values for the polyamine charge, z_j, and the parameter n_j. Such comparisons are reported in the Figures 6 and 7. There is not a large influence on the calculated denaturation temperatures supposing the presence of binding sites on the single strands; thus we did not consider any statistical effect on the binding of polyamines to the denatured form of the DNA but only the polyelectrolyte contribution to the binding. For the diamines we assumed a value for $z_j = 2$, and a good fit is obtained when we used a value for $n_j = 4$. Electrostatic interactions between the positively charged amino groups and the negative phosphates seem to be insensitive to the variation of the methylene groups. We were not able to obtain a correct fit for the thermal melting temperature of DNA in the presence of the triamine (spermidine) and the tetramine (spermine) by using values of $z_j = 3$ and 4, respectively. The obtained curves, in fact, gave melting temperatures higher than the experimental ones, even if we used higher values for n_j. The calculated theoretical trends represented in the Figures 6 and 7 are obtained supposing the values of $z_j = 2.5$ and $n_j = 5$ for the triamine, and $z_j = 3.5$ and $n_j = 5$ for the tetramine. As can be seen, there is good agreement between the calculated melting temperature and the experimental one. Conversely, Manning's theory of polyelectrolytes alone cannot correctly describe the polyamine−DNA interaction. In Figure 8, in fact, is reported the comparison between the right theoretical trend of thermal melting temperatures of DNA in

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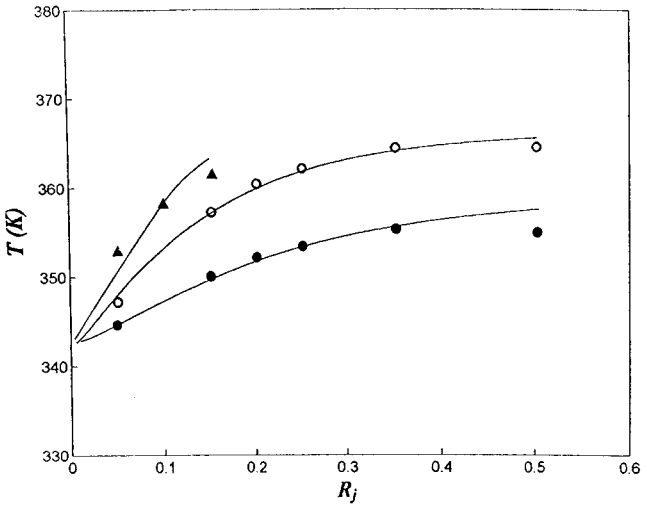

Figure 7. Comparison between the experimental thermal denaturation helix−coil transition temperatures of hs-DNA and the theoretical ones reported as a function of the ratio between the polyamine and DNA concentrations (expressed in number of moles of nucleotide). The continuous line is the theoretical trend of such temperatures in the presence of multivalent ligands, while the experimental data are reported with the symbols: (●) cadaverine, (○) spermidine, and (▲) spermine. In this figure we do not give the melting temperature trend for the hs-DNA in the presence of putrescine for the same reason as the Figure 6.

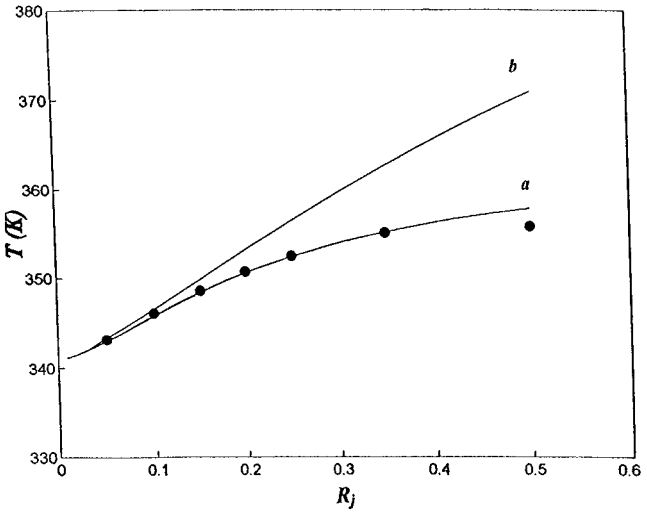

Figure 8. Comparison between the experimental trend of thermal melting temperatures for ct-DNA in the presence of putrescine (●) and the calculated helix−coil transition temperatures for a DNA in the presence of a diamine by using Manning's theory which incorporates McGhee and von Hippel neighbor exclusion effects (a) or by only using polyelectrolyte contribution to the binding (b).

the presence of a diamine, as shown in Figure 6, and that calculated only by using Manning's theory. This figure clearly shows that the binding of cationic polyamines to DNA could, in principle, be treated with counterion condensation theory, but the calculated values of the fraction of condensed cations are not correct. In other words, the estimation of the thermal denaturation temperatures trend does not agree, especially for spermine and spermidine, with the experimental ones. There is a slight agreement only for the two diamines and in the range of lower R_j values, where the polyelectrolyte effects give the major contribution.

The analysis of noncompetitive and competitive binding isotherms by the multiple-site exclusion equation contains the implicit assumption that the polyamines and other oligomeric cationic ligands bind to a discrete site on DNA, even though the existence of discrete binding sites for such molecules is questionable. Competitive, as well as noncompetitive binding experiments with polyamines were already analyzed according to the multiple-site exclusion theory by Braunlin and colleagues,[85] but the extent to which these oligocations are localized at individual phosphate binding sites or delocalized on the DNA molecule is currently not known.

The entropy change in electrostatic binding reactions is predicted to be the driving force, with a negligible enthalpic contribution.[82] The dependence of the binding constant with temperature for such reactions should be small. Ross and Shapiro found that the interaction between spermine and DNA occurs with no detectable change of heat content.[86] Since the interaction between this polyvalent cation and DNA occurs spontaneously, the very small value of the observed binding ΔH suggested that this phenomenon is primarily entropic in origin. The small value for $\Delta H_{\text{v.H.}}$ found by the measurements of Braunlin of the binding of spermine and spermidine over the range 4−37 °C confirms this prediction.[85] They were only able to say that the binding enthalpy of these cations to DNA could not be more than a few kilocalories.[85]

Nevertheless, the effects of polyamines on the thermal stability of nucleic acids could reflect trends in their interaction with either double or single stranded DNA, or both, and do not necessarily provide information exclusively concerning the interaction with the double helix. Since double stranded DNA is more rigid than, and has a geometry different from single stranded DNA, an equality in interaction would be surprising, but, in the absence of direct binding measurements on both forms, it cannot be ruled out. From this viewpoint it is not surprising that, in order to obtain a correct thoretical trend of thermal melting temperatures of DNA, the values of z_j of 2.5 for the triamine and 3.5 for the tetramine have been used; the binding of polyamines to single strand DNA could in some way alter the polynucleotide conformation, varying both the value of the charge density parameter ξ and the extent of binding of polycations to single strand. Of course, it would have been difficult to take in account these changes, and thus we do not expect that such parameters have a deep physical meaning. However, they could help in a clarification of the binding mechanism of such a molecule to DNA given their near invisibility in solution to any structural technique (including NMR).[13]

Some information concerning double helix and polyamines are only available by X-ray investigations. In fact, from inspection of the various crystal structures of DNA sequences in the presence of spermine, it appears that this molecule can adopt a wide variety of binding modes.[87−90] In his model, Egli, for example, has shown a mode of complex interactions involving the binding of the amino groups of spermine in the major groove of DNA,[91] while Feuerstein supposed spermine docked into the major groove, spontaneously inducing a bend in B-DNA.[92]

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Molecular modeling and molecular mechanical calculations on polyamine−DNA interactions have suggested a binding mode involving the docking of polyamines either in the major or minor grooves of DNA.[93] In the valuable work of Liquori,[12] confirmed by other studies,[93−95] spermine bridges the minor groove of DNA to give a complex in which the binding is between the positive amino and imino groups of polyamine and the negative phosphate groups of DNA, by electrostatic interactions and directional hydrogen bonds, as well as hydrophobic interactions between the nonpolar sides of nucleotide and polyamines.

It is likely, though there is as yet no proof, that a given polyamine and a given DNA may have a number of competing binding modes, each of which correlates with different polyamine functions. Indirect support for this hypothesis comes from the fact that no correlation has been observed between the binding strength of different polyamines and any of biological activity[96] or efficiency with which they induce, for example, the B → Z transition, DNA aggregation, or condensation. Anyway, a complete rationalization of our data on double helix thermal stability would require further measurements of the binding of our polyamines to the single-stranded form of DNA over a wide range of ionic strengths.

Conclusion

The great number of biological processes involving polyamines makes these compounds difficult to classify. Nevertheless, they are included in the growth substances class because of all their roles in the growing processes, in the development of organisms, and in metabolism and because they are active at relatively low concentration. Their aliphatic structure together with their capability to interact strictly with certain essential metabolisms make the polyamines very peculiar substances.

The data presented here provides a measure of the effects of some natural polyamines on the thermal stability of the double helix of DNA as measured by the temperatures of the maximum heat effect, T_{max}, and denaturation enthalpies, $\Delta_d H$. We have analyzed, by using the binding formalism of McGhee and von Hippel and the hypotheses of Manning's theory on polyelectrolyte contributions, the interaction of spermine, spermidine, putrescine, and cadaverine with DNA. The temperature dependence of binding of these ligands is consistent with a predominantly electrostatic interaction of entropic origin driven by the release of counterions, but there are noticeable structural effects that must be taken into account for obtaining correct calculated trends of thermal melting temperature. The adopted model, which can generally be used for the binding of polybases to DNA, suggests stimulating ideas about the arrangement of polyamine molecules on DNA and on the real extent of the binding of these oligocations. We hope that our findings will help to clarify the biological roles of such molecules and provide a model system for the electrostatic component of the interactions of protamines and specific proteins with nucleic acids.

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Supporting Information Available: Theoretical Appendix for calculating the polyelectrolyte contribution to Gibbs energy (12 pages). See any current masthead page for ordering and Internet access instructions.

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