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Application of the Poisson Boltzmann polyelectrolyte model for analysis of thermal denaturation of DNA in the presence of Na^+ and polyamine cations

Nikolay Korolev, Alexander P. Lyubartsev, Lars Nordenskiöld*

Division of Physical Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden

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Abstract

The Poisson Boltzmann (PB) cell model of polyelectrolyte solution has been used for calculation of the electrostatic free energy difference, ΔG^{el}, between double- and single-stranded DNA. The calculations have been performed for conditions relevant to describe the DNA helix-coil transition in NaCl solution in the presence of the natural polyamines putrescine^{2+}, spermidine^{3+}, spermine^{4+} and their synthetic homologs with different spacing between the charged amino groups, for which experimental values of the DNA 'melting' transition temperature (T_m) are available. Using the PB theory and the polyamine ion radius as an adjusting parameter provides quantitative agreement between experimental and theoretical T_m—salt concentration dependencies only by using physically unreasonable radii for the polyamine. Thus, modeling the linear and flexible polyamines as charged spheres within the PB cell model is an implausible oversimplification. We propose another explanation for the experimental observations, still within the frame of the 'primitive' PB polyelectrolyte theory. This explanation is based on an analysis of the ΔG^{el} dependence on the stoichiometry of polyamine-polyanion binding to double- and single-stranded DNA.

Keywords: Helix coil transition; DNA-charged ligand interactions; Free energy calculations; Spermine; Spermidine; Putrescine

1. Introduction

This work continues a series [1,2] of papers devoted to the application of polyelectrolyte theories for analysis of thermally induced ('melting') structural transitions in polynucleotides. In the preceding papers, we proposed an approach to the description of helix-coil transitions of polynucleotides, which is based on numerical calculations of the difference between the electrostatic free energy (ΔG^{el}) of double helix and single strand forms of DNA. The calculations of ΔG^{el} allowed us to use experimental values of the transition midpoint temperature (T_m) for evaluation of the salt independent non-electrostatic contribution to the free energy, enthalpy, entropy, and heat capacity changes of the helix-coil transition. As a result, our method avoids using thermodynamic parameters of the structural transition, namely melting enthal-

*Corresponding author. *Present address*: School of Biological Sciences, NTU—Nanyang Technological University, No. 1 Nanyang Walk, Blk. 5, Level 3, Singapore 637616, Singapore. Tel.: +65-6790-3737; fax: +65-6316-1964.

E-mail address: LarsNor@ntu.edu.sg (L. Nordenskiöld).

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py, entropy, and heat capacity, from calorimetric experiments. On the contrary, we could use calorimetry data for verification of the model and, in previous works [1,2], we have found good agreement between experiment and theory for thermodynamic parameters of structural transitions of DNA and RNA in the presence of monovalent salt. Additionally, unlike earlier theoretical analysis which has been limited to the description of salt dependence of T_m vs. concentration of monovalent cations, we have extended the application of our method to the analysis of structural transitions in DNA [1] and synthetic RNA [2] polynucleotides in the presence of both M^+ (M=K or Na) and the divalent (Mg^{2+}) cations. For this kind of system, experimental data have shown an anomalous destabilization of the double helix of DNA [5–7] or double- or triple-helical polyribonucleotides [8,9] upon addition of MCl. Earlier, attempts have been made to use counterion condensation (CC) theory to account for this phenomenon which resulted in only qualitative explanation of the experimental curves [10–13]. In the work [1], we have shown that the PB cell model gives satisfactory quantitative agreement with some deviations between the theoretical and experimental curves that can be reasonably explained by the influence of non-electrostatic component of Mg^{2+}–DNA interactions. Moreover, the PB polyelectrolyte theory allowed tracing the difference between DNA and RNA in interactions with Mg^{2+} [2].

In the present work, we continue testing the PB cell model and apply it to the experimental data [14] on the DNA melting in the presence of Na^+ and oligocationic organic polyamine ligands. We study the natural aliphatic polyamines putrescine^{2+}, spermidine^{3+}, spermine^{4+} as well as synthetic homologs of spermidine^{3+} with variable number of methylene groups between the central and one of the terminal amino groups of the triamine (Fig. 1). Experimental work by Thomas and Bloomfield [14] reported not only a decrease of the melting temperature of DNA upon addition of MCl (which is similar to the DNA [5–7] and RNA [8,9] melting behavior in the presence of Mg^{2+}), but also noticed an unusual dependence of T_m in the presence of spermidine^{3+} and its homologs. It was found that that in the presence

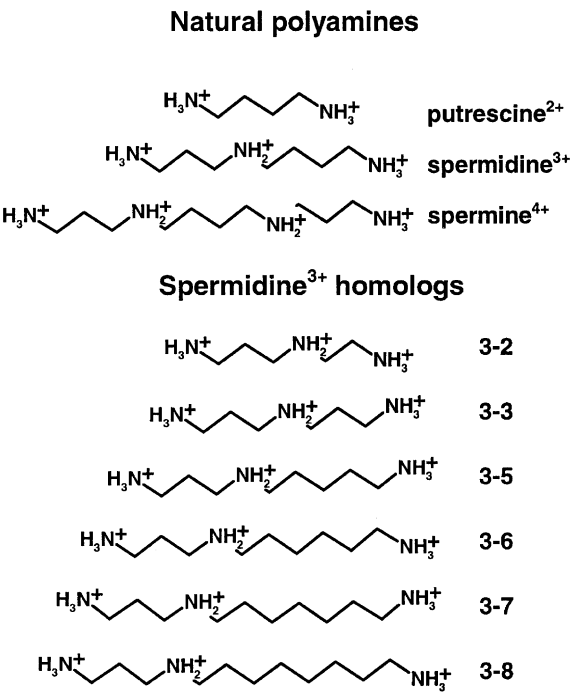

Fig. 1. Natural polyamines and synthetic homologs of spermidine^{3+} used by Thomas and Bloomfield [14] in studying DNA helix-coil transition.

of the most densely charged triamines, 3–2 and 3–3, double stranded (ds) DNA is less stable than in the presence of the homologs with longer distance between charged amino groups (see Fig. 1 for definition of the notions for the spermidine^{3+} analogs). At fixed Na^+ concentration below 100 mM, the values of T_m increase in the series 3–2>3–3>3–4 (spermidine^{3+})>3–5 with small changes in T_m for the higher homologs (from 3–6 to 3–8). This finding is counterintuitive, since it is expected that the densely charged ligands L^{Z+} would interact with the ds-DNA more strongly than with the denatured single stranded (ss) form, thus increasing the stability of the native DNA to a larger extent than similar ligands with larger distance between the charged groups.

Here we shall show that the PB theory can enable quantitative agreement between experimental and theoretical T_m–salt concentration dependencies by using the polyamine ion radius as adjusting parameter. However, the resulting values

of the fitted polyamine radii appears to be in principal contradiction with the observed trend in the experimental data: While experiment shows *increasing* stabilization of the DNA double helix with the increase of charge–charge distance in a polyamine, determined in the PB model, the 'optimal' values of polyamine radii have to be decreased with the size of the polyamine ion, in order to improve agreement between theoretical and experimental values of T_m. Thus, modeling the linear and flexible polyamines as charged spheres is an implausible oversimplification within the PB cell model. We propose another explanation for the experimental observations, still within the frame of the 'primitive' PB polyelectrolyte theory. This explanation is based on an analysis of the dependence of ΔG^{el} on the stoichiometry of the polyamine binding to double-helical and single-stranded DNA.

2. Methods

2.1. Calculation of thermodynamic functions

The thermodynamic approach and computational methods were described in detail in our previous papers [1,2]. Below, a brief account of our method underlining some differences in the technique we use in this work compared to the previous publications, is given.

The change in free energy of DNA melting (ΔG_m) is described as a sum of two terms, electrostatic, ΔG^{el}, and non-electrostatic, ΔG^{nel}. Assuming $\Delta V^{el}=0$, $\Delta G^{el}=\Delta E^{el}-T\cdot\Delta S^{el}$ where ΔE^{el} includes the electrostatic energy change ($\Delta E^{el}=E_d^{el}-E_n^{el}$) and ΔS^{el} is change in the electrostatic entropy ($\Delta S^{el}=S_d^{el}-S_n^{el}$), indices d and n are for denatured, single-stranded (ss) and native, double-stranded (ds) DNA states. We use the Poisson Boltzmann (PB) cylindrical cell model [15–19] for calculation of the terms contributing to ΔG^{el}.

In the PB model, the solvent is treated as continuum with a constant dielectric permittivity, which depends only on temperature. The polyions are approximated as infinitely long and uniformly charged cylinders of radius a with unit charge spacing b. For the ds- and ss-DNA, we use structural values successfully applied in the previous work [1,4] and listed in Table 1. The small mobile ions (including ligands L^{Z+}) are treated as impenetrable hard spheres with radii close to those determined for Na^+ and Cl^- [20,21] and using the radius of the polyamine cation L^{Z+} as an adjustable parameter (see Table 1). In the PB approach, the radii of the counterions and coions determine only the distance of closest approach of the small ions to the polyion, and do not produce any excluded volume effects near the surface of the cylindrical polyion.

Table 1
Parameters of DNA and mobile ions used in calculations

	Polyion charge density (b), e/Å	Polyion radius, Å
Double stranded DNA	1.7	9.5
Single stranded DNA	3.4	7
Radii of mobile ions, Å		
Na^+ (2.0)	Cl^- (2.0)	L^{Z+} (1–5)

The electrostatic internal energy per charged group of the polyelectrolyte, associated with introducing a polyion in the system, is calculated, within the mean field theory, by (see, e.g. [22]):

$$E^{el}/kT=-\frac{1}{2}|\psi(a)|+\frac{1}{2}\int_a^R 2\pi x\sum_\alpha[Z_\alpha\rho_\alpha(x)]\Psi(x)dx \qquad (1)$$

The electrostatic entropy contribution due to redistribution of ions around the charged cylinder is given by:

$$S^{el}=-k_B b_0\int_a^R 2\pi x\sum_\alpha\left[\rho_\alpha(x)\ln\left(\frac{\rho_\alpha(x)}{C_\alpha^0}\right)\right]dx \qquad (2)$$

In Eqs. (1) and (2), $\psi(x)=e\phi(x)/kT$ is the reduced electrostatic potential; a is a radius of cylindrical polyion; R is the radius of the cylindrical cell occupied by a polyion and defined by the polyion concentration; $\rho_\alpha(x)=C_\alpha^0\cdot\exp[-Z_\alpha\psi(x)]$ is the charge density function of α-species of ions with C_α^0 being the concentration of α ions at the at the outer cell boundary ($x=R$) where $\psi(x)$ is defined to be zero; $b_0=e^2/(\varepsilon kT)$ is so-called

Bjerrum length. Values of C_α^0 and electrostatic potential $\psi(x)$ as a function of coordinate can be easily calculated by iteration procedure for the given parameters of the polyion and concentration of species allowing determination electrostatic and entropy components of free energy by Eqs. (1) and (2).

The magnitude of the non-electrostatic term, ΔG^{nel}, is assumed to be independent of composition of the solution due to addition of salt. The temperature dependence of ΔG^{nel} can be written as equation:

$$\Delta G^{\mathrm{nel}}(T) = \Delta H^{\mathrm{nel}}(T_o) - T\cdot\Delta S^{\mathrm{nel}}(T_o) + \Delta C_p^{\mathrm{nel}}\cdot[T - T_{\mathrm{o}} - T\cdot\ln(T/T_o)] \quad (3)$$

Here ΔH^{nel}, ΔS^{nel}, and $\Delta C_p^{\mathrm{nel}}$ are changes of non-electrostatic enthalpy, entropy, and heat capacity respectively, and T_o is an arbitrary 'standard' temperature (we have chosen $T_o = 298.15$, and approximate $\Delta C_p^{\mathrm{nel}}$ as a constant independent of temperature).

The term ΔG^{nel} mainly represents the change of internal molecular properties of DNA during melting (i.e. ΔG^{nel} accounts for changes in hydrogen bonding, stacking interactions, internal motions, changes in hydration of bases, sugar and phosphate residues). It has been concluded [1,2] that the influence of alkali cations like Na^+ or K^+ on the thermodynamic parameters of polynucleotide transitions can be effectively calculated with the rather simple PB polyelectrolyte theory with all approximations that are inherent in this treatment. More subtle features of the ionic interactions do not seem to influence the helix coil transition characteristics noticeably (otherwise we would receive dependence of ΔG^{nel} on C_{Na}). These contributions are either small enough or, most likely, they balance each other in solutions of ds- and ss-DNA.

We use the following way to analyze the DNA melting in the presence of polyamines:

1. As in the previous work [1,2] we make use of the fact that $\Delta G^{\mathrm{nel}} = -\Delta G^{\mathrm{el}}$ at the middle of transition, and determine the temperature dependence of $\Delta G^{\mathrm{nel}}(T)$ in Eq. (3) from calculated values of $\Delta G^{\mathrm{el}}(T_m) = f(T_m, C_{\mathrm{M}}, C_{\mathrm{P}})$ in the absence of L^{Z+} and from experimental values of T_m at the same concentration of M^+ and DNA. Since experimental conditions used in DNA melting studies vary from laboratory to laboratory [23], it is desirable to use T_m values obtained in the absence of L^{Z+} determined for the same sample and under similar experimental conditions as the results found in the presence of L^{Z+}. The work [14] however, does not report these data. Therefore, we have applied an empirical formula proposed for the dependence of T_m on. $\log C_{\mathrm{M}}$ at different GC contents and based on numerous experiments with DNA from different sources [23,24]:

$$T_m = k\cdot\log C_{\mathrm{M}} + 0.41\cdot X_{\mathrm{GC}} + 81.5 \quad (4)$$

   For the calf thymus DNA used in ref. [14], the value of the GC-pair content $X_{\mathrm{GC}} = 0.41$, and $k = 17.5$ °C (this value of k is believed to be most reliable [23]). The fitted parameter values of non-electrostatic free energy term in Eq. (3) are the following: $\Delta H^{\mathrm{nel}} = 13.9$ kJ/mol, $\Delta S^{\mathrm{nel}} = 31.3$ J/(mol·K), and $\Delta C_p^{\mathrm{nel}} = 170$ J/(mol·K).
2. Next we calculate the total free energy change $\Delta G_m(T, C_{\mathrm{Na}}, C_{\mathrm{L}}, C_{\mathrm{P}}) = \Delta G^{\mathrm{el}}(T, C_{\mathrm{Na}}, C_{\mathrm{L}}, C_{\mathrm{P}}) + \Delta G^{\mathrm{nel}}(T)$ in the presence of L^{Z+} ions. Setting $\Delta G_m = 0$, we find the concentration dependence of the melting temperature $T_m = T_m(C_{\mathrm{Na}}, C_{\mathrm{L}}, C_{\mathrm{P}})$. We then plot theoretical curves of T_m vs. $\log C_{\mathrm{Na}}$ that can be compared with those determined experimentally in the presence of L^{Z+}.

Thus, any observed differences between experimental and calculated values of T_m can be either a measure of the reliability of the theoretical description, or it may be directly connected with salt effects on the non-electrostatic free energy in Eq. (3), e.g. due to the formation of hydrogen bonds between the DNA and polyamines, changes of hydration of DNA and L^{Z+}, and other non-electrostatic contributions. Within the 'primitive' PB cell model, used in this work the only possibility to take into account the change in structure of the charged ligand L^{Z+} is to vary the radius (more precisely, the closest distance between the ligand and the polyion axis, $a+\sigma$). We consequently use σ_{L} as an adjustable parameter changing it between 1 and 5 Å in calculation of the ΔG^{el} values.

Structural parameters of DNA and mobile ions used in the PB calculations are listed in Table 1. In a number of works [1,3,4,25], it has been shown that this set of structural parameters give the best agreement between experimental data and the results of PB theory.

3. Results and discussion

3.1. Variation of the theoretical T_m values with the charge and radius of the ligand L^{Z+}

In agreement with results of the previous work [1,2], the function $\Delta g^{el} = \Delta G^{el}/RT$ displays a clear independence on the temperature in the range 20–100 °C for all values of the parameters both in the absence and in the presence of the polyamines (data not shown).

We assume in this work that the melting of DNA is a first order phase transition between two states, which means that the cooperative unit in the process is equal to the full polynucleotide molecule. If this assumption does not hold and redistribution of ions between different structural forms of a polyion can proceed during the transition, this would influence the calculation of the theoretical T_m curves [16,17,26,27]. This exchange of ions may be of importance for small values of the ratio $r = C_L/C_P$ and its influence on the DNA melting parameters has been analyzed in our previous work [1,2]. Under the conditions applied in the work [14], the polyamines were present in excess over the phosphate groups of DNA for the majority of the experimental data. Solution of the PB equation shows that when the ligand L^{Z+} is presents in excess, conditions which could lead to redistribution of the polyamines between ds- and ss-DNA within the interval of the structural transition (different concentration of L^{Z+} at the cell borders and/or absence of the saturation with the ligand at the surface of the polyion), are not present (data not shown).

In our previous work [1] we compared abilities of different polyelectrolyte theories to explain an anomalous behavior of melting temperature of DNA on Na^+ concentration in the presence of Mg^{2+} (addition of NaCl leads to the decrease of thermal stability of ds-DNA). We found that the

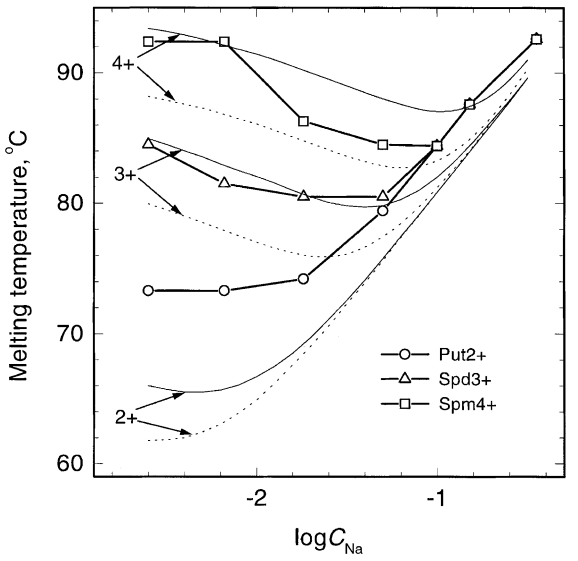

Fig. 2. Comparison of experimental data with the PB theory predictions of the T_m values of DNA helix-coil transition in the presence of polyamines and Na^+-ions. Experimental dependencies (thick lines with points) for putrescine^{2+}, (Put^{2+}), spermidine^{3+}, (Spd^{3+}), and spermine^{4+}, (Spm^{4+}), were taken from ref. [14] (Fig. 2 of the cited paper; $C_P = 0.04$ mM, $r = C_L/C_P = 0.5$). Theoretical curves were calculated for the ligand charge indicated in the graph and radius equal to 2 Å (thin solid lines) or 3 Å (thin dotted lines).

PB theory correctly predicts the position of the minima in the T_m–logC_{Na} dependences. The PB theory also describe reasonably well the magnitude of the Mg^{2+} stabilization of the DNA double helix and its dependence on Mg^{2+}, DNA and Na^+ concentrations. This good agreement between experimental data and theoretical predictions was observed with the assumption of delocalized purely electrostatic binding of 'bulk' hydrated Mg^{2+} ($\sigma_{Mg} = 3$ Å) and Na^+ ($\sigma_{Na} = 2$ Å) with ds- and ss-DNA approximated as uniformly charged cylinders. Observed differences between experimental and theoretical T_m values can be reasonably explained by the expected and relatively small non-electrostatic contribution due to preferential Mg^{2+} binding to the ds-DNA.

In Fig. 2, experimental data for the DNA melting in the presence of putrscine^{2+}, spermidine^{3+}, and spermine^{4+} (sold lines with points) are compared with the theoretical values (thin curves)

obtained from the solution of the PB equation according to the procedure described above. In Fig. 3, the results obtained for the DNA melting in the presence of spermidine^{3+} homologs are compared with the theoretical curves built for different values of the radius of the cation L^{3+} used in the PB cell model as an adjustable parameter.

Comparison of the experimental T_m vs. logC_M curves obtained for the DNA melting in the presence of Mg^{2+} [7] and with similar experimental data collected in the presence of the polyamines [14] reveals the following features:

1. For the equally charged ligands, Mg^{2+} and putrescine^{2+}, under similar conditions (equal C_P and $r=C_L/C_P=0.5$ and 1.0), the 'shallow' minimum on the T_m–logC_{Na} curves is observed at similar Na^+ concentration around log$C_{Na}\approx -2.5\div -1.8$ being shifted to higher C_{Na} at $r=1.0$ compared to the data at $r=0.5$. The values of T_m determined in the presence of either Mg^{2+} or putrescine^{2+} are also reasonably close to each other (68–75 °C at the minimum) provided the differences in DNA source and experimental procedures.
2. Melting curves obtained in the presence of spermidine^{3+} and its homologs as well as spermine^{4+} also display decrease of the T_m values upon addition of Na^+ in the range logC_{Na} from −2.6 to −1.2. For similar C_P and r, the ligands L^{3+} and L^{4+} show stronger stabilization of the ds-DNA (higher T_m). They also have minima in the curves T_m vs. logC_{Na} shifted towards higher salt concentration, compared to the curves obtained for putrescine^{2+} or Mg^{2+}.
3. DNA melting in the presence of spermidine^{3+} homologs reveals the following trend in the T_m dependence on the type of the triamine: at low Na^+ concentration, more densely charged polyamines (3–2, 3–3) makes the ds-DNA less resistant to the thermal denaturation compared to the similar DNA solutions with added spermidine^{3+} and its high molecular weight homologs from 3–5 to 3–8. This dependence is quite unusual because one would expect that the more densely charged ligand would bind to

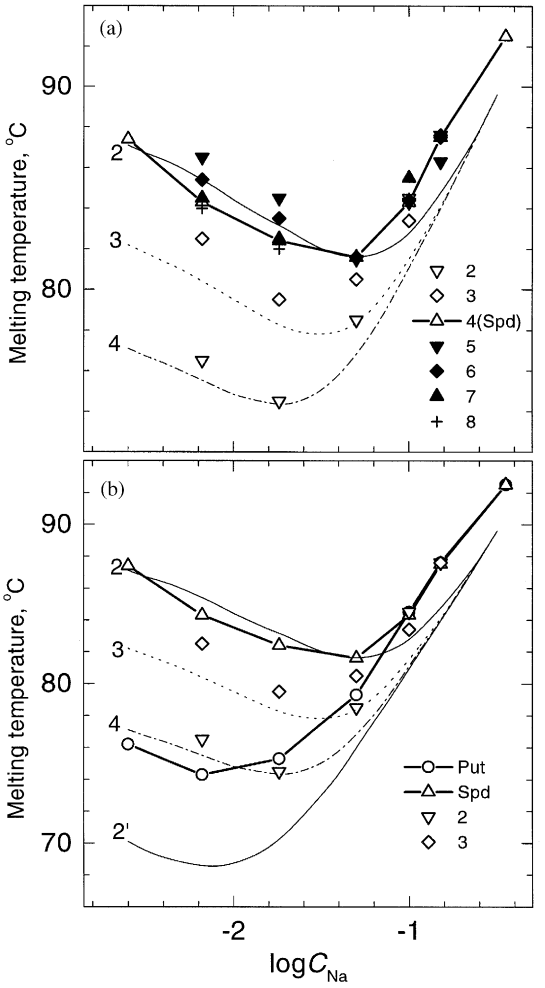

Fig. 3. Salt dependence of melting temperature of DNA in the presence of spermidine homologs. Experimental data were taken from ref. 14 (Figs. 1 and 3 of the cited paper; $C_P=0.04$ mM, $r=C_L/C_P=1.0$). (a) All polyamines have a charge +3 and a 1,3-diaminopropane group with varied number of methylene groups between central and second terminal amino group (numbers of the CH_2-groups are shown in figure). Points obtained for natural polyamine, Spd^{3+}, are connected by a solid line. Theoretical curves (thin lines) were calculated for the L^{3+} radius indicated near the lines. (b) Similar as (a) but the experimental results for only three shortest spermidine homologs are shown. Additionally, experimental (Put^{2+}, empty circles with solid line) and theoretical (thin line, L^{2+} radius is 2 Å) data on DNA melting in the presence of divalent cations are shown.

and stabilize the ds-DNA to a larger extent than the polyamines with larger charge–charge separations which may be considered not as L^{3+} particles but rather as a $(2+1)$ cation.

Comparison of the experimental and theoretical curves in Fig. 2 shows that for the DNA melting in the presence of spermine^{4+} and spermidine^{3+} one can obtain very good agreement between measured and predicted values of T_m applying reasonable values of the radius for the ligand L^{Z+} between 2 and 3 Å with calculations of the ΔG^{el} values using Eqs. (1) and (2). The theoretical curves reproduce both the absolute values of T_m and position of minima of the experimental dependences. For the DNA melting in the presence of putrescine^{2+}, similar values of σ_L do not give agreement with the experiment: the PB theory underestimates the stabilization influence of putrescine^{2+} on the ds-DNA. Use of a smaller and rather unrealistic radius ($\sigma_L = 1$ Å) for calculation of ΔG^{el}, brings calculated values of T_m closer to the experimental ones (not shown in Fig. 2), but worsen the shape of the T_m vs. logC_{Na} curve (the minimum on the curve becomes deeper and is shifted to the higher cation concentration, that is not observed in the experimental dependence).

In Fig. 3a, experimental melting temperatures of the DNA obtained in the presence of equimolar amount of spermidine^{3+} and its homologs from 3–2 to 3–8, at $r = C_L/C_P = 1.0$, are compared with theoretical curves calculated for different values of σ_L (2, 3 and 4 Å). Values of T_m are lowest for the lowest molecular weight triamines, and for the most densely charged L^{3+}, 3–2, T_m fall close to the similar data obtained for the doubly charged polyamine, putrescine^{2+}, (see Fig. 3b). This low efficiency of the densely charged triamines in stabilization of the ds-DNA cannot be explained by a lower degree of protonation, which could take place in the low molecular weight homologs [14]. The authors [14] proposed that this unusual effect might be due to some favorable interactions of the more densely charged polyamines with the ss-DNA. However, this explanation has not been developed further.

One can see that the PB theory allows reaching quantitative agreement between experimental and theoretical T_m–salt concentration dependencies by using the polyamine ion radius as adjusting parameter. However, this fitting procedure is in principal contradiction with the observed trend in the experimental data: experiment shows increasing stabilization of the DNA double helix with the increase of charge–charge distance in a polyamine, whereas in the PB model, one needs to decrease the size of the polyamine ion in order to improve agreement between the theoretical and experimental values of T_m. Thus, modeling the linear and flexible polyamines as charged spheres is an implausible oversimplification within the PB cell model.

3.2. Possible explanation of T_m dependence on the size of the polyamine cation

The above conclusion is that the polyamines, which are thin flexible discretely-charged cations cannot be approximated as charged spheres. Therefore, one needs to use a more adequate model for description of the polyamine binding to the polyanions. All-atom models of biopolymers systems such as the model developed in the work by Sharp and co-workers [28,29] or molecular dynamics simulations of DNA interactions with polyamines [30,31] would be the most suitable. However, it seems that the present state of the theory is not ready to be applied to the analysis of DNA thermal transitions, since such features of the DNA as single-stranded DNA interactions with cations or the influence of temperature on the energetics of DNA-ligand binding have so far not been developed to sufficient extent. Application of more elaborated models for studying of the structural transformation of polynucleotides is a task for future work.

However, we will show below that the highly simplified PB polyelectrolyte theory is still capable to propose a qualitative explanation for the counterintuitive dependence of the DNA melting temperature on the charge–charge separation in spermidine homologs (Fig. 3). To explain this phenomenon, we analyze the case of non-stoichiometric binding of the ligand L^{Z+} to the native and denatured DNA polyions. Let us assume, that under condition of the ligand L^{Z+} being in excess (valid for the data in Fig. 3), that some part of

the ligand L^{Z+} is tightly bound to the DNA polyion (both native and denatured). The degree of this tight binding depends on the ligand size, namely a longer ligand occupies a larger part of the DNA polyion compared to its homolog with shorter separation between charged amino groups. We use the following additional assumptions: (a) the ligand L^{Z+}, having a fixed length l, is attached to a part of the DNA polyion carrying $N=l/b$ negative charges; (b) binding of the ligand L^{Z+} precludes further tight association of the remaining ligands to the position already occupied; (c) bound ligands occupy all the available place on both single- and double-stranded DNA, so that after formation of the [L·DNA] complex, the polyion remains uniformly charged with a somewhat reduced charge density (i.e. increased value of the effective linear charge spacing, b_{eff},) depending on the length of the ligands: $1/b_{\text{eff}}=1/b-Z/l$. Finally, we assume that the distribution of the remaining (non-bound) ligands, as well as the monovalent salt, can be described within the same Poisson–Boltzmann model as before.

Now we analyze the change of the electrostatic free energy ($\Delta G^{\text{el}}_{\text{L·DNA}}$) associated with the change in the polyion–monovalent ion interactions caused by formation of the $[\text{L·DNA}]^{Z-N}$ complex:

$$\Delta g^{\text{el}}=\Delta G^{\text{el}}_{\text{L·DNA}}/\text{NRT}=g^{\text{el}}_c-g^{\text{el}}_f \qquad (5)$$

Here g^{el}_f and g^{el}_c are the electrostatic free energies (in RT units and per unit charge) of the free DNA (without tightly bound ligand, as considered in the previous section) and of the ligand-DNA complex. In computations of this section, we kept constant (3 Å) the ligand radius (which defines the distance of closest approach in the PB equation) and varied the length of the polyamines l. Increase of the length of the ligand leads to the size occupied by the polyamine on the DNA polyion, becoming larger, and the effective charge density of the polyion-complex increases. This also leads to the formation of non-stoichiometric (with respect to phosphate/amino-group ratio) polyamine DNA complexes, where complexes formed by the native DNA retain a larger part of the negative charge than those of the denatured ss-DNA. In Fig. 4, the values of Δg^{el} calculated for the ds- and ss-DNA

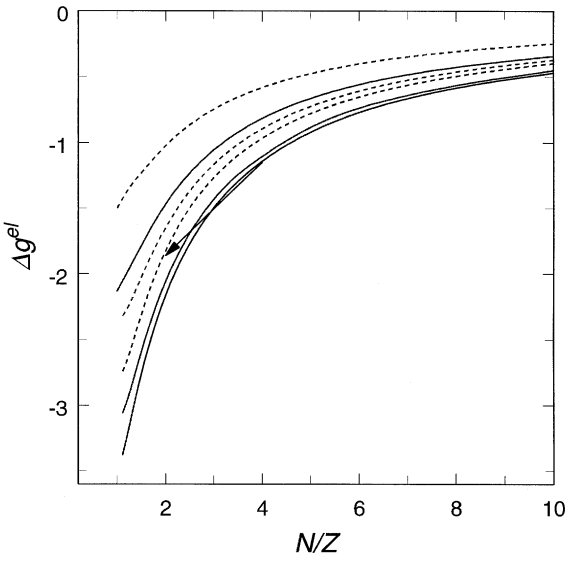

Fig. 4. Dependence of electrostatic energy of complex formation between DNA and cationic ligand, Δg^{el}, on the stoichiometry of ligand-polyion binding expressed as a N/Z ratio. Thick and thin lines are respectively for native and denatured DNA polyions; Na^+ concentration from bottom to top: 1.44, 12.7 and 113 mM. Other details are in the text.

polyions are drawn with the dependence on the size of the ligand-binding site measured as the N/Z ratio. Fig. 4 shows that increase of the ligand-binding site of the polyion leads to a reduction of the electrostatic energy gain due to the formation of the complex. Obviously, this effect is due to the decrease in neutralization of the polyion charge due to 'smearing' of the charge of the ligand over a larger and larger piece of the polyion (increase in N/Z ratio) This makes the g^{el}_c value in Eq. (5) closer and closer to the free energy of DNA in the absence of the ligand, g^{el}_f. One can also note that the change in free energy Δg^{el}, is more negative, in the case of the same N/Z ratio, for ds-DNA than for ss-DNA.

Now, take into account that ligands of the same size interact with different number of charged groups on the ds- and ss-DNA polyions. For example, when charge–charge separation in the ligand is equal to, say, 6.8 Å (this corresponds to the distance between charged amino groups separated by 4 methylene groups), then each amino

group of the polyamine is capable to cover a section of the ds-DNA carrying four negative charges (b_n=1.7 Å and N_{nat}/Z=4) but interacts tightly with only two phosphate groups of the denatured ss-DNA (b_d=3.4 Å, N_{den}/Z=2). Correspondingly, the N/Z ratio differs in the two sorts of polyamine-DNA complexes, being roughly two times higher for the native DNA. An example of comparison of the free energy changes is shown in Fig. 4 by an arrow connecting the values of Δg^{el} calculated for N_{nat}/Z=4 and N_{den}/Z=2 at a fixed Na^+ concentration of 1.3 mM. This demonstrates an unfavourable contribution to the stability of the double stranded form of DNA caused by the formation of polyamine–DNA complexes.

The dependences of the difference in Δg^{el} between ds- and ss-DNAs $\delta\Delta g^{el}=\Delta g^{el}_{den}-\Delta g^{el}_{nat}$, on the length of the polyamine ligand (expressed in terms of $N_{nat}/Z\approx 2\cdot N_{den}/Z$ ratio) is shown in Fig. 5 for different concentrations of Na^+. The curves in Fig. 5 display a minimum at $N_{nat}/Z\approx 2.5$ which corresponds to a charge–charge separation in the ligand L^{Z+} of approximately 4.2 Å. This value is close to the minimal separation between the charged amino groups in spermidine homologs with two methyl groups in between. Polyamine analogs with values $N_{nat}/Z<2.5$ have not been experimentally investigated and it remains to be seen if the steep increase in $\delta\Delta g^{el}$ at low values of the N/Z ratio can be traced in experimental studies of DNA helix coil transition in the presence of linear cationic ligands of high charge density.

One can see from Fig. 5, that changes in $\delta\Delta g^{el}$, due to the hypothesis of tight electrostatic binding of ligands to DNA, reflect the most essential features of the experimental dependence of T_m on the presence of different spermidine^{3+} homologs shown in Fig. 3, namely the steep change in T_m for the low molecular weight homologs (from 3–3 to 3–5) at low salt concentration and minor changes both in $\delta\Delta g^{el}$ and in T_m for the higher spermidine^{3+} homologs and at $C_{Na}>100$ mM. From Fig. 5 we find that the change in the N_{nat}/Z value from 2.5 to 6 gives rise to a magnitude of $\delta\Delta g^{el}$ approximately 0.35 at low Na^+ concentration ($\log C_{Na}=-2.88$, the lowest curve in Fig. 5), 0.15 at $\log C_{Na}=-1.85$ (second curve from the bottom), and almost nothing at higher salt concentration. The positive change in $\delta\Delta g^{el}$ means that increase of the charge–charge separation in the ligand result in stabilization of the double helical DNA which is reflected in experiment showing rise in the DNA melting temperature of approximately 8–10 °C in the low salt region ($\log C_{Na}=-2.2--1.5$) when the densely charged 3–2 triamine is substituted by its homologs 3–4, 3–5 and higher (Fig. 3). In the inset of Fig. 5, the change in electrostatic free energy between ds- and ss-DNA as a function of Na^+ concentration for different additions of Mg^{2+} is displayed ($r=C_{Mg}/C_P$ varies from 0 to 2.5). Comparing the scales of changes in T_m and $\delta\Delta g^{el}$ in the polyamine—DNA system with the analogous features observed for DNA melting in the presence of

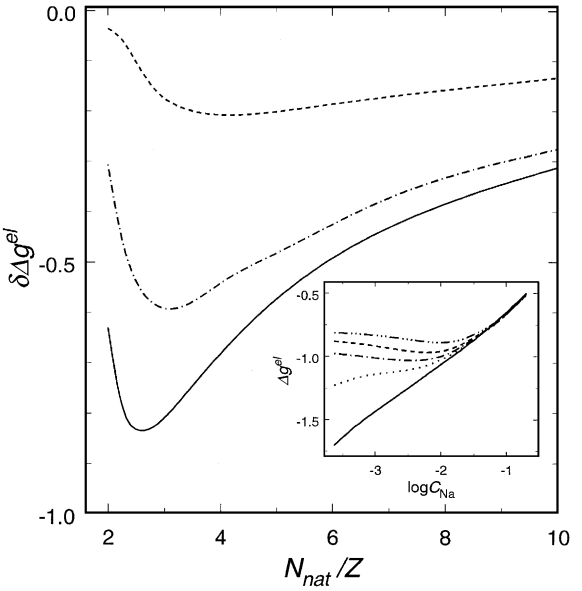

Fig. 5. Estimation of the change in free energy due to the different stoichiometry of DNA—oligocation complex of the ds- and ssDNA polyions, $\delta\Delta g^{el}$, in dependence of the stoichiometry of the ligand—ss-DNA complex (N_{den}/Z ratio); Na^+ concentration from bottom to top: 1.44, 12.7 and 113 mM. In the inset, dependence of $\delta\Delta g^{el}$ on Na^+ concentration is shown to compare the magnitude $\delta\Delta g^{el}$ change caused by the change of the polyamine charge density or addition of Mg^{2+}. Values of $\delta\Delta g^{el}$ were calculated for DNA solutions containing different amounts of Mg^{2+} ($r=C_{Mg}/C_P$ from bottom to top: 0.0, 0.25, 0.5, 1.0 and 2.5; C_P=0.1 mM; data were taken from our previous work [1]).

Mg^{2+}, one can notice a good quantitative correspondence between the magnitudes of changes in T_m and variation in the electrostatic free energy. For example, for the Mg/DNA system at $\log C_{Na} = -3$, the difference in Δg^{el} for $r = 1.0$ and 0.0 is approximately 0.5 and it corresponds to an increase in T_m of approximately 20°. For $\log C_{Na} = -2.5$, similar values for $\delta\Delta g^{el}$ and T_m are 0.3 and 15° [1]. Again, for the polyamine/DNA system, the change in N_{nat}/Z from 3 to 6.5 gives a smaller (than in the examples above) rise in $\delta\Delta g^{el}$ of respectively approxiamtely 0.25 and 0.15 (Fig. 5) that is reflected by the corresponding increase in T_m of approximately 6–10 °C (Fig. 3).

An important point of the proposed explanation is the statement that tight binding of the polyamine to different polyanions depends solely on their length and that the more densely charged ds-DNA cannot increase the degree of the polyamine complexation compared to the less densely charged ss-DNA. To support the view of the limiting ability of ds-DNA for close association with the polyamines, we can refer to data recently obtained by us in molecular dynamics (MD) simulations of the polyamine–DNA systems [30,31] and (Korolev et al., in preparation). In these papers, we study the influence of a number of the natural (putrscine^{2+}, spermidine^{3+}, spermine^{4+}) and one synthetic (diaminepropane^{2+}) polyamines on binding, dynamics, structure and other properties of DNA in conditions simulating real DNA crystals or fibers. The results from the cited works show that only a limited amount of the polyamine molecules are capable of forming distinct long-lived complexes in the minor groove or with the charged oxygen atoms of the phosphate groups of the DNA oligomers. In the MD simulations, we rarely observed simultaneous presence of two or more polyamine molecules in the minor groove or in the closest vicinity of other subgroups of DNA, because of polyamine molecules repelling each other. Additionally, charged amino groups of the polyamines compete with the water molecules and other cations for the presence near electronegative sites of the DNA even in the simulated DNA crystals, where the amount of water and ions is limited. In dilute water solutions and in the presence of NaCl (i.e. under the conditions applicable to DNA melting studies), exclusion of the polyamines from formation of close complexes with the DNA polyanions can only be enhanced compared with the conditions of the DNA crystals. One should also mention the well-established fact that binding of cationic ligands to polyanions is anticooperative, that is the presence of bound ligand precludes attachment of the next molecule L^{Z+} at the same or nearby location of the polyion [32].

Another possible origin of the T_m dependence on the polyamine length would that it is due to different degree of desolvation of the homologous polyamines upon association with DNA. However, experimental data on polyamine–DNA interactions do not support this explanation since no significant desolvation effects have been found in experiments on polyamine–DNA binding [33–35]. These experimental studies support the picture of mobile delocalized and structurally non-specific binding of the polyamines to DNA. However, this binding does not preclude situations, seen also in the molecular dynamics simulations [30,31], when a substantial part of the polyamine molecules is located in close vicinity of DNA, effectively screening the DNA charge from the rest of the ions [34].

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