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David Pastré · Olivier Piétrement · Fabrice Landousy
Loïc Hamon · Isabelle Sorel · Marie-Odile David
Etienne Delain · Alain Zozime · Eric Le Cam

A new approach to DNA bending by polyamines and its implication in DNA condensation

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Abstract Polyamines are known to induce dynamical bending of DNA molecules. This mechanism is very important since many DNA binding proteins (DNAse, transcription factor, etc.) exert their action by their ability to bend DNA. We propose an analytical model which describes the dynamical bending of DNA by polyamine ions in highly diluted DNA solutions. The bending probability depends on the entropy loss of polyamines due to their localization. This localization is facilitated by the electrostatic repulsion between multivalent counterions condensed on DNA, which reduces the entropy loss in counterion localization. Therefore DNA bending by polyamines depends on the competition between monovalent counterions and polyamines. We find that the bending probability is weak for a low binding ratio of polyamines (i.e. number of bound polyamines per base pair), whereas a high bending probability can be reached at large polyamine binding ratio. In addition, we describe a new mechanism of DNA bending. It occurs with the help of thermal agitation, which initiates the bending and favours the polyamine localization. This model provides further insights into DNA bending by polyamines and its implication in DNA condensation. A qualitative estimation of the DNA bending probability is obtained by measuring the cleavage efficiency of DNA by bleomycin versus spermidine concentration. Indeed, a local helix distortion by polyamines results in an amplification of the double-strand cleavage by bleomycin. The measurement of the bleomycin amplification is performed by analysing images of DNA molecules with atomic force microscope. Some features of the dynamical bending indicate that condensation and bending are interrelated.

Keywords DNA bending · DNA condensation · Polyamines · Bleomycin · Atomic force microscopy

D. Pastré and O. Piétrement have contributed equally to the work.

D. Pastré (✉) · L. Hamon · I. Sorel · M.-O. David · A. Zozime
Laboratoire d'Etude des Milieux Nanométriques,
Université d'Evry, Rue du Père Jarlan,
91025 Évry Cedex, France
E-mail: d.pastre@iut.univ-evry.fr
Tel.: +33-1-64470303
Fax: +33-1-69470105

O. Piétrement · F. Landousy · E. Delain · E. Le Cam
Laboratoire de Microscopie Moléculaire et Cellulaire,
UMR 8126 CNRS-IGR-UPS, Institut Gustave-Roussy,
39 rue Camille Desmoulins, 94805 Villejuif Cedex, France

Introduction

DNA is a highly negatively charged polyelectrolyte and its chain is partly neutralized in physiological conditions by condensed cations, which form a thin counterion layer (Manning 1969; Record et al. 1978). The competition between counterions of different valences is important since the composition of the counterion layer plays a critical role in DNA condensation (Bloomfield 1997). Monovalent counterions just partly neutralize the DNA phosphates but multivalent counterions can, in addition, generate an attraction between two DNA segments. This attraction arises from the correlations of the multivalent counterions between two DNA segments. Divalent counterions only generate 2D condensation (Koltover et al. 2000) or DNA adsorption on highly charged surface (Pastré et al. 2003). For trivalent and higher charge cations, counterion correlations lead to the condensation of DNA in solution (Kjellander and Marcelja 1985; Lau and Pincus 1998; Levin et al. 1999; Murayama and Yoshikawa 1999; Rouzina and Bloomfield 1996). The polyelectrolyte behaviour of the DNA chain has been highlighted in several studies (Bloomfield 1997; Raspaud et al. 1998; Wilson and Bloomfield 1979) and it emerged that approximately 90% of the DNA charge must be neutralized for condensation to occur. However, these theoretical approaches did not generally associate the condensation with local helix perturbations (Burak et al. 2003; Khan et al. 1999; Raspaud et al. 1998). Besides, it has been established that multivalent counterions can stabilize local DNA bending (Flink and

Pettijohn 1975). Polyamines are able to dock in both major and minor DNA grooves and repel monovalent cations from the neighbouring phosphates, which are then strongly attracted to the bound cations, leading to groove closure (Feuerstein et al. 1990; Rouzina and Bloomfield 1998; Ruiz-Chica et al. 2004; Sines et al. 2000; Thomas and Thomas 2001). Condensation and local helix distortions are likely to be interrelated (Bloomfield 1997) and may together control higher order aspects of DNA structure and function.

In order to qualitatively describe the local bending by polyamines, we present an extension of a previously developed model (Rouzina and Bloomfield 1998). A new mechanism of DNA dynamical bending initiated by thermal agitation and mediated by the entropy loss in polyamine localization is described. We then study the effect of the binding competition between monovalent and multivalent counterions on the bending probability. The point is to estimate the probability of dynamical bending at the boundary of the condensation transition.

The theoretical results are compared to experimental ones, obtained by analysing the effect of spermidine on the double-strand cleavage by bleomycin, which is a glycopeptide antibiotic routinely used to treat cancer (Burger 1998; Claussen and Long 1999). This drug oxidatively damages DNA by inducing both double- and single-strand breaks. The point is that the DNA cleavage is improved by distortion of the DNA helix (Bailly et al. 1997; Strekowski et al. 1989). Indeed, bleomycin is likely to be a minor groove binder and DNA bending towards the major groove facilitates the DNA cleavage by bleomycin (Strekowski et al. 1989). DNA cleavage can be used as a probe of the dynamical and local bending induced by polyamines since a higher bending probability results in a more efficient cleavage of DNA by bleomycin. The mean number of double-strand breaks per DNA molecule is measured by analysing images of DNA molecules with atomic force microscope (AFM) as described in Piétrement et al. (2005). In addition, the condensation threshold can also be detected since condensed DNA is quite well protected from DNA cleavage by bleomycin due to a lower accessibility (Lopez-Larraza and Bianchi 1993; Smith et al. 1994).

Theory

The influence of multivalent counterions on DNA structure is a complex question that requires elaborate models for an accurate description (Korolev et al. 2002). However, for the purpose of this work, a simplified model can be useful for studying qualitatively the influence of multivalent counterion binding on the local and dynamical helix distortion. We only consider the dynamical bending of the major groove for an isolated DNA molecule (i.e. in dilute solution) and the intermolecular interactions, which are important at high DNA concentrations (>10 μg/ml), are not considered.

The major groove closure is just one of the possible helix distortion mechanisms which can be induced by the binding of polyamines. This study can be extended to other structural changes, which require counterion localization. Furthermore we only consider the dynamical bending of the B-form of DNA due to a purely electrostatic mechanism. The assumption is valid for condensing agents such as spermidine or spermine since both interact with the DNA chain mainly via non-specific electrostatic interactions. Polyamines can also promote $B \rightarrow A$ or $B \rightarrow Z$ secondary structure transformations. However, these transformations are not considered since their probabilities are rather low and experiments showed that the B-form structure is maintained upon condensation for genomic DNA (Deng et al. 2000).

DNA is a flexible worm-like polyelectrolyte chain with elastic properties, characterized by its persistence length L_p, which results from bending and twisting deformations. L_p depends on the sequence and ionic environmental conditions (Hagerman 1988). Local DNA conformation depends on anisotropic flexibility due to local curvature and local flexibility. However, in this article, DNA is considered as a flexible rod with a constant bending rigidity.

In the following section, we summarize a previous model (Rouzina and Bloomfield 1998) which describes the free energy of dynamical bending. A bit of reminding is necessary to fully understand the new mechanism of DNA bending by polyamines presented in "How is local curvature promoted? Are DNA bending probability and DNA condensation related?".

Mechanisms involved in the DNA bending

Bending energy

As the DNA molecule can be described as a flexible rod, the energy required to bend it is

$$E_{\text{bending}}(\alpha) = \frac{1}{2} c \alpha^2, \tag{1}$$

where c, the bending rigidity, is about 12 kT for a 6 bp fragment, which indicates that thermal fluctuations mainly induce small bending angles. Therefore the probability for DNA to be bent up to an angle α by thermal agitation is

$$P_{\text{bending}}(\alpha) \propto \exp\left(\frac{-E_{\text{bending}}(\alpha)}{kT}\right). \tag{2}$$

Electrostatic energy

Without external influences, the counterions condensed on the DNA surface are uniformly distributed. A non-uniform distribution of counterions along DNA could induce a local curvature (Maher 1998; McFail-Isom et al. 1999; Strauss and Maher 1994). In particular, the

localization of multivalent counterions in the middle of the major groove could lead to the groove closure (Boutonnet et al. 1993; Chiu and Dickerson 2000; Feuerstein et al. 1990; Rouzina and Bloomfield 1998), but the localization of polyamine in the major groove is still under debate (Deng et al. 2000; Ouameur and Tajmir-Riahi 2004). However, in our approach, a prior localization of the counterion in the major groove is not required because of thermal agitation, as described in "How is local curvature promoted? Are DNA bending probability and DNA condensation related?".

The gain in electrostatic energy due to the localization of a multivalent counterion in the middle of the major groove of a bent fragment is (Rouzina and Bloomfield 1998)

$$\Delta E_{\text{groove}}(\alpha,\Delta x) = -f(z)e^2\left[\frac{1}{\varepsilon\left(\frac{w_0-R\alpha}{2}\right)\frac{w_0-R\alpha}{2}}-\frac{1}{\varepsilon\left(\frac{w_0}{2}\right)\frac{w_0}{2}}\right]\times\left(1-\left(\frac{\Delta x}{w_0}\right)^2\right), \quad (3)$$

where e is the electron charge, R the DNA radius, w_0 the groove width (1 nm for the major groove), z the cation valence and $\varepsilon(r)$ the sigmoidal dielectric function. Δx represents the cation localization in the space which is roughly the width of the counterion density function. $f(z)$ is a function of the counterion valence: $f(z)=z^2$ for a point-like charge and $f(z)=z$ for linear cations (such as polyamines).

Entropy loss in counterion localization

Several studies have revealed that the counterions condensed on the DNA surface are highly mobile (Boutonnet et al. 1993; Braunlin et al. 1991; Xu et al. 1993). However, the bending of DNA molecules by polyamine ions requires their localization. It results in an energetic cost because of the entropy loss due to polyamine localization. On DNA, if a polyamine ion is situated between two multivalent counterions, its mobility is restrained since it should overcome an unfavourable electrostatic potential to move over a polyamine counterion. This phenomenon leads to the counterion correlations and the formation of a Wigner crystal structure composed of multivalent counterions (Grosberg et al. 2002). If we consider that polyamine ions are able to move between two other polyamines, the counterion entropy loss ΔS is given by

$$\Delta S \cong k\ln\left(\frac{r}{b}\Delta x\right), \quad (4)$$

where b is the distance between two base pairs. The distance between two nearest polyamines is b/r, where r is the number of counterions per base pair (binding ratio). In addition, here the collective translations of the counterions are neglected so that the entropy may be underestimated.

Free energy of the bending mechanism

The free energy change ΔF_0 due to the groove bending is the sum of the three major contributions described above (in kT units):

$$\Delta F_0(\alpha,\Delta x,r) = \frac{1}{2}c\alpha^2 + \Delta E_{\text{groove}}(\alpha,\Delta x) - \ln\left(\frac{r}{b}\Delta x\right). \quad (5)$$

Minimization of F_0 with respect to Δx gives

$$\Delta^2 x = -w_0^2\frac{1}{2E_{\text{groove}}(\alpha,0)}. \quad (6)$$

When we introduce this value in Eq. 5, we find (Rouzina and Bloomfield 1998):

$$F_0(\alpha,r) = \frac{1}{2}c\alpha^2 + E_{\text{groove}}(\alpha,0) + \frac{1}{2}\left(\ln(-2E_{\text{groove}}(\alpha,0))+1\right) - \ln\left(\frac{w_0}{b}r\right), \quad (7)$$

where r is the number of multivalent counterions per base pair. If it is assumed that $w_0 \approx 6b$, Eq. 7 is similar to that found previously (Rouzina and Bloomfield 1998).

Figure 1 represents the free energy versus the bending angle for two different binding ratios. At small angles ($\alpha < 20°$), bending is not favourable, which indicates that DNA should be bent by thermal agitation to initiate the reaction and to increase the occurrence of this mechanism. It can also be remarked that the free energy F_0 is reduced at higher binding ratio. This effect is quite obvious because the distance between two nearest multivalent counterions on DNA is shorter for a larger binding ratio. Therefore the entropy penalty due to counterions' localization is reduced, which favours the DNA bending. If a bending is initiated ($\alpha > 30°$ for $r=0.2$), the major groove is bent up to its largest possible angle. The most favourable angle is the largest angle allowed, as indicated by the free energy drop with the bending amplitude. However, as the bending is dynamical, unbending can also take place due to thermal

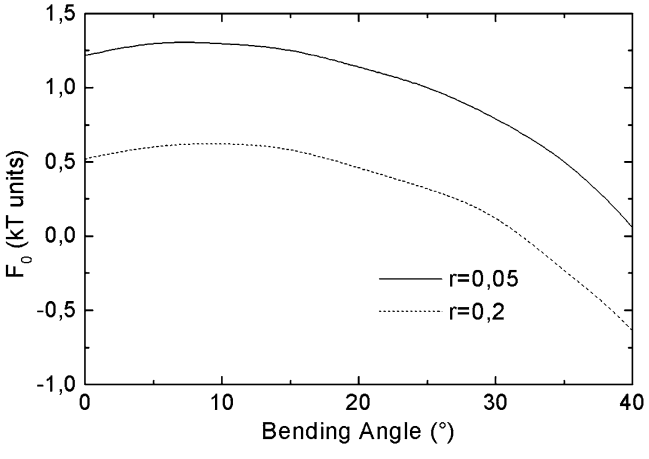

Fig. 1 Plot of the free energy of bending versus bending angle ($z=4$, linear molecule)

agitation. Since the maximum bending amplitude is limited by sterical hindrance, small cations could provoke a large bending ($>30°$ for polyamines; Rouzina and Bloomfield 1998). The energy benefit is then more important and the bending is more stable.

The point is that it is now possible to study the influence of the buffer composition on the bending probability since the free energy depends on r, the binding ratio (Rouzina and Bloomfield 1998):

$$r = \frac{2}{z}\frac{[L^{z+}]}{[L_1]^z} n_s^{z-1}\left(1-\frac{zr}{2}\right)^z, \quad (8)$$

where $[L^{z+}]$ is the polyamine concentration, z the charge of the polyamine, $[L_1]$ the monovalent counterion concentration and n_s the concentration of the counterions on DNA surface.

Equation 8 needs to be numerically solved. The plot of F_0 for $\alpha=\alpha_{max}$ versus $[L^{z+}]$ is represented in Fig. 2. It can easily be observed that the free energy sharply decreases and reaches a saturation value at high polyamine concentration due to the nearly full occupancy of the DNA chain by the multivalent counterions. At low multivalent counterion concentrations, polyamines are free to move along the DNA chain and the entropy penalty for the counterion localization is very high, which explains the rather high energy needed for bending.

How is local curvature promoted? Are DNA bending probability and DNA condensation related?

When a polyamine ion is immobilized in the middle of the major groove, the DNA chain could be bent up to the largest angle sterically allowed. However, this mechanism could not take place without the help of thermal energy since $F_0>0$ for the small bending angle. The dynamical bending can be described by a three-step mechanism (see Fig. 3). First, the DNA molecule is bent by thermal agitation at least up to α_0, where α_0 is the angle limit for which $F_0=0$. Second, a polyamine ion could then be trapped in the middle of the major groove since this position is more favourable thanks to the bending initiated by thermal agitation. Third, the bending angle increases up to its maximum value for which the DNA bending is stabilized. As unbending can also occur via thermal agitation, a large bending amplitude induces a better stabilization of the DNA/ligand complexes.

The probability P of dynamical bending by polyamines is then directly proportional to the thermal bending which triggers it:

$$P([L^{z+}],[L_1]) \propto \exp\left(\frac{-E_{bending}(\alpha_0)}{kT}\right), \quad (9)$$

so the value of α_0 is of particular interest. It represents the minimum amplitude of thermal bending required for triggering the dynamical bending by a multivalent counterion. We should note that the bending probability is then not simply associated with the number of bound multivalent counterions (see "Experimental evidence of the increase of the monomolecular DNA bending by multivalent counterions"). Let us study the effect of the multivalent and monovalent counterion concentrations. Figure 4 represents the graphs of α_0 versus the multivalent counterion concentration for spermine and spermidine. It appears that α_0 is large for lower

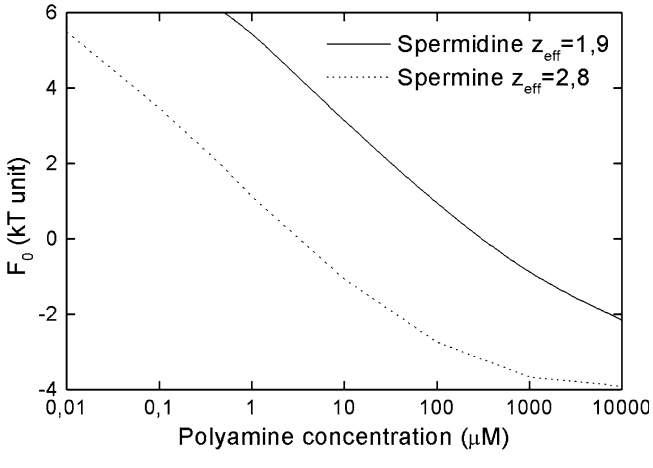

Fig. 2 Plot of the free energy of bending versus the concentration of polyamines for $\alpha=30°$ and 50 mM monovalent salt (see Fig. 7 for the estimation of z_{eff})

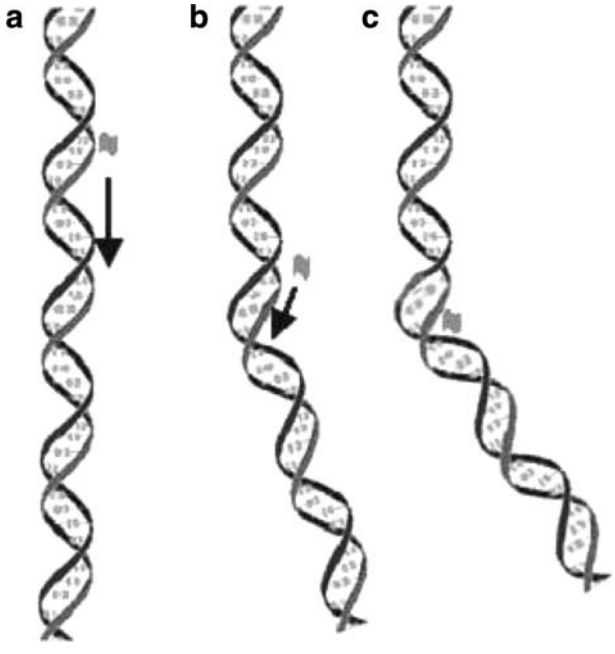

Fig. 3 Schematic representation of the dynamical bending mechanism by polyamine. **a** A free polyamine ion diffuses along the DNA chain. **b** A small bending is first initiated by thermal agitation, which then allows the polyamine localization in the major groove. **c** The bending angle is increased and stabilized by the polyamine ion

concentrations of multivalent counterions, so that the probability of bending is rather low. α_0 can even be larger than the maximum angle sterically allowed at very low polyamine concentrations (Fig. 4) and therefore the dynamical bending is theoretically not possible. The entropy loss in counterion localization can be reduced at higher binding ratio, which leads to a decrease of α_0 with polyamine concentration.

Figure 5 is the plot of the dynamical bending probability versus the multivalent ion concentration for spermine and spermidine. The point of this figure is to observe the slope of the curve, which increases continuously with the multivalent counterion concentration. More precisely, the slope of the curve becomes steep for $[L^{4+}] > 100$ μM for spermine ([NaCl] = 50 mM, see Fig. 5a). This means that small amounts of polyamines added to the solution can considerably enhance the bending probability. Thus we can expect a sharp transition from the extended state to the condensed state: the thermal fluctuations can easily induce a bending angle sufficient to trigger the groove closure. Figure 6 represents the bending probability versus monovalent salt concentration for spermine and spermidine. The local bending probability is considerably reduced at high monovalent counterion concentrations. The binding competition between monovalent and multivalent counterions limits the number of bound multivalent counterions, which leads to a larger entropy loss in counterion localization.

Could dynamical bending be involved in DNA condensation by polyamines? This hypothesis seems sound with regard to the sharp increase of the bending probability with the polyamine concentration. DNA bending favours the short-ranged attraction by counterion correlations between two DNA segments. Therefore condensation may be associated with localized helix distortion due to a condensing agent (Bloomfield 1997). In addition, the model of DNA condensation triggered by an accumulation of local bends by polyamine ions presents several analogies with the well-known characteristics of DNA condensation. First, the bending probability is affected by the addition of the monovalent salt. Second, there is a sharp transition between the condensed and extended states, as would be expected if DNA condensation was induced by an enhancement of the bending probability (Takahashi et al. 1997). Furthermore, the extended state changes into the condensed state by an increase in temperature (Murayama and

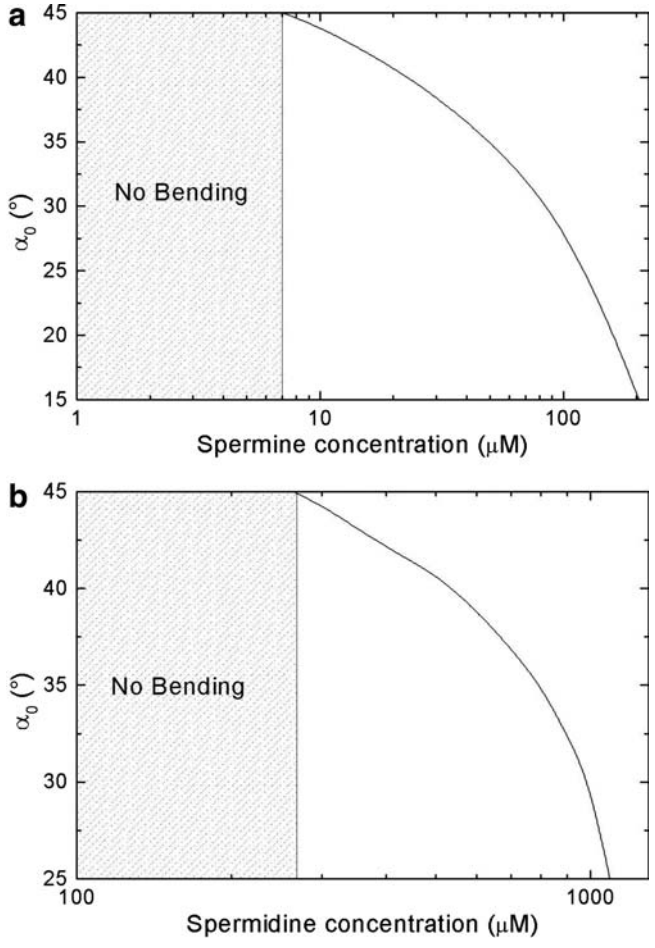

Fig. 4 Plot of α_0 versus polyamine concentration with 50 mM monovalent salt (the maximum angle sterically allowed is arbitrarily put to 45°) for **a** spermine and **b** spermidine

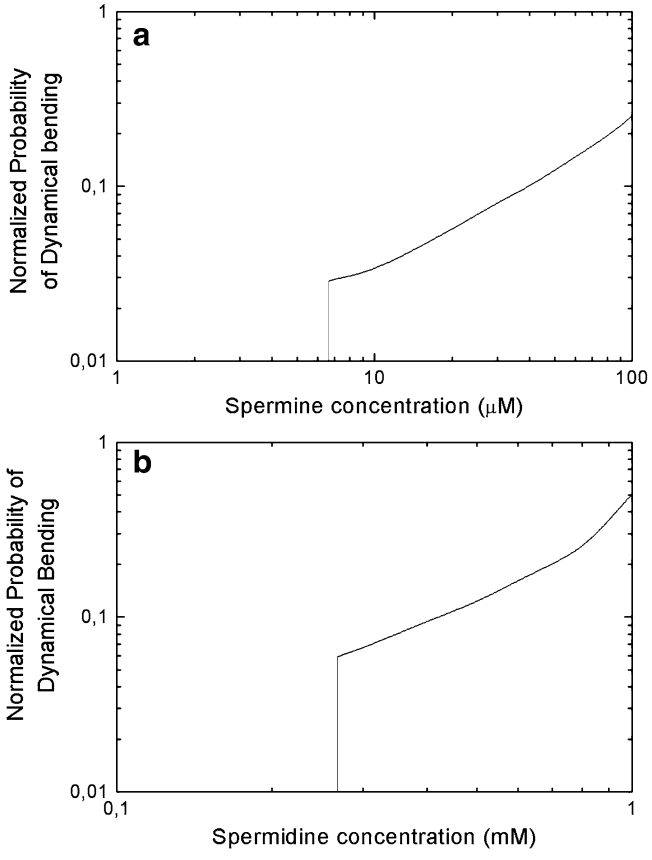

Fig. 5 Plot of the bending probability versus polyamine concentration with 50 mM monovalent salt for **a** spermine and **b** spermidine

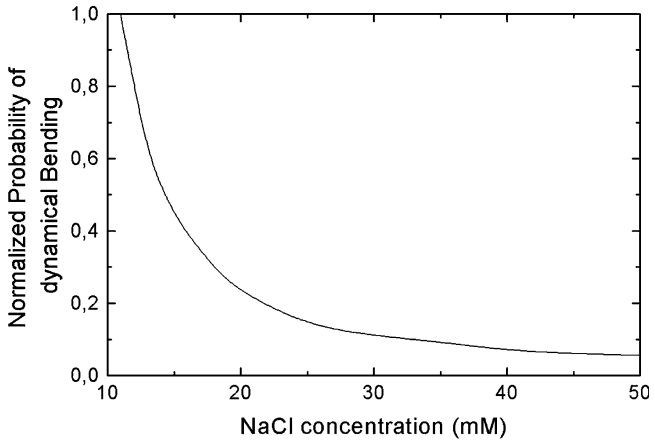

Fig. 6 Plot of the bending probability versus the monovalent salt concentration with 2 μM spermine

Yoshikawa 1999). This is in agreement with our model since, contrary to ion correlations, bending probability increases with the temperature. Third, condensing agents with a large radius of gyration are less efficient in condensing DNA. This is also valid for the dynamical bending since the bending amplitude is smaller for larger counterions due to sterical hindrance. It is worth noting that DNA curvature in toroidally condensed particles is gentle, with the radius of curvature comparable to the persistence length. In the condensed state, abrupt bending could be prevented by the interaction of DNA segment with its neighbours.

Let us set the bending probability fixed by α_0, at which the DNA condensation is initiated. This assumption allows one to study the effect of the monovalent counterion concentration on the polyamine concentration required to condense DNA. Figure 7 shows the polyamine concentration at the onset of DNA condensation versus the monovalent counterion concentration for spermine and spermidine, from previous reports (Raspaud et al. 1998; Saminathan et al. 1999; Wilson and Bloomfield 1979). The results are in pretty good agreement for high monovalent salt concentrations. However, at small monovalent ion concentration ([NaCl] < 20 mM), the condensation happens after the replacement of monovalent counterions by multivalent counterions (Wilson and Bloomfield 1979). The effect of the cloud of strongly correlated multivalent counterions needs to be incorporated in the model for a better accuracy.

Experiments

The aim of this section is to show whether there is a sharp increase in the local bending near the transition between the extended and condensed states. This could be an indication that helix distortion by multivalent counterions and DNA condensation are interrelated.

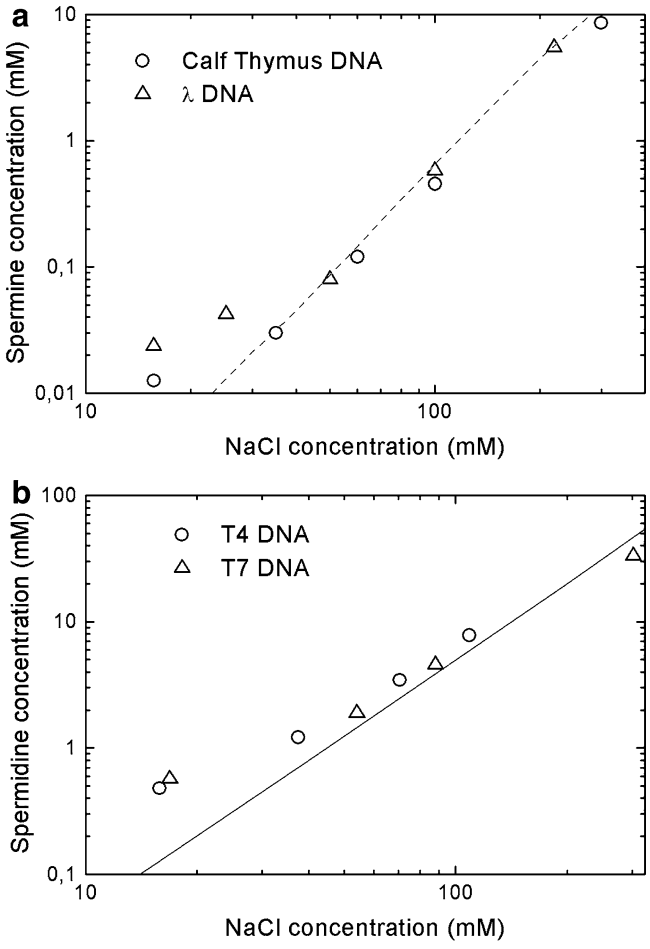

Fig. 7 Iso-probability curve of DNA bending for **a** spermine and **b** spermidine. Data points are the experimental measurements of the collapse transition versus monovalent salt concentration and were collected from several works. The *dotted line* is the theoretical curve representing the coil/globule transition by assuming that the trigger is an accumulation of local bending. The slope of the curve in a log/log plot is equal to z_{eff}. The parameter α_0 is calculated to best fit the experimental measurements: $\alpha_0 = 28°$ and 23° for spermine and spermidine, respectively. These values are in agreement with our model since the dynamical bending probability increases strongly for $\alpha < \alpha_0$ (see Fig. 4)

Materials and method

Reactives

Bleomycin sulphate (Blenoxane) was obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). Fe(III)-bleomycin was prepared by dissolving ferric ammonium sulphate dodecahydrate in water and immediately adding a 10% molar excess of iron to bleomycin as already described (Li et al. 2002). A 1 mM Fe(III)-bleomycin solution was then refrigerated (−20°C). pUC19 plasmid DNA was purchased from Sigma-Aldrich. Circular DNA molecules allow an easier determination of the DNA double-strand breakage.

AFM experiments

Atomic force microscope experiments were carried out with a Nanoscope IIIa AFM (Digital Instruments/Veeco, Santa Barbara, CA, USA) using tapping-mode in air. We used OTESPA cantilevers (Digital Instruments/Veeco), with resonant frequencies in air contained between 290 and 350 kHz. The scan frequency was typically 1 Hz per line and the modulation amplitude was about a few nanometres.

Bleomycin cleavage of DNA

A 2.5 μg/ml pUC19 DNA solution is reacted with spermidine for 15 min in 10 mM Hepes (pH 7.5) buffer with or without 5 mM $MgCl_2$. The cleavage of DNA by Fe(III)-bleomycin was performed by adding a 4 μl droplet of a buffer solution containing Fe(III)-bleomycin to 16 μl of the DNA solution. Bleomycin reacted with DNA in 10 min. The reaction was stopped by adding 5 μl of 100 mM EDTA solution. The samples were supplemented with NaCl to yield a final concentration of 100 mM so that DNA molecules cannot be in the condensed state anymore (condensation is a readily reversible process). This step is necessary for an accurate measurement of the DNA contour lengths. The reaction solution was deposited on a mica surface by adding $MgCl_2$ for AFM observation. A drying step is performed after using a 0.02% diluted uranyl acetate solution in order to fix DNA molecules in their conformations. The analysis of the DNA breaks was performed as previously described (Piétrement et al. 2005).

Experimental evidence of the increase in monomolecular DNA bending by multivalent counterions

Several experiments have shown evidence that DNA bending probability is enhanced by multivalent counterions. These measurements were performed by optical technique (Porschke 1991; Vijayanathan et al. 2001) or laser tweezers (Baumann et al. 2000). DNA bending was also imaged by electron microscopy (Conwell et al. 2003; Frontali et al. 1979; Laundon and Griffith 1987; Théveny et al. 1988) and atomic force microscopy (Fang and Hoh 1999; Lin et al. 1998). The radius of gyration of DNA molecules appears to be reduced by polyamines before condensation. Figure 8 shows DNA molecules adsorbed on mica for three spermidine concentrations. At low spermidine concentration (<1 μM), the molecules are extended on the surface. As spermidine concentration approaches the onset of DNA condensation (10 μM), the molecules are more and more entangled. The presence of a lot of crossovers could indicate a high bending probability. For spermidine concentrations larger than 100 μM, the DNA molecules are condensed: DNA toroids appear as bright spots in the image. However, the AFM image cannot allow a precise measurement of the condensation threshold or the bending probability since the DNA adsorption onto mica can alter the measurement (Pastré et al. 2003). Thus, it is not possible to obtain quantitative measurements with this method.

A possible way to overcome the problem is to use an indirect measurement method. A ligand, for which the interaction with DNA depends strongly on conformational changes (i.e. the major groove width), could act on the DNA molecule at various polyamine concentrations. Thus, the action of a ligand, such as bleomycin, on DNA can be used as a probe of the DNA dynamical bending. Bleomycin induced both double- and single-strand breaks but its activity is not yet understood since its mode of binding is complex. It involves minor groove binding and partly intercalation or some other conformational changes (Steighner and Povirk 1990). However, there are several evidences suggesting that major groove closure results in a better fit for cleavage by bleomycin (Strekowski et al. 1989). So the amplification of the DNA cleavage can be used as a probe of the bending probability. The point is to measure the cleavage efficiency of bleomycin on isolated DNA for various buffer compositions.

We have recently presented a new technique that allows a quantitative measurement of the number of DNA breaks per molecule by AFM imaging (Piétrement et al. 2005). This technique is based on the measurement of the number of double-strand breaks per circular DNA molecule. According to Poisson distribution, the probability $f_{n,a}$ that a DNA molecule has exactly a breaks is equal to

$$f_{n,a} = \exp(-n)\frac{n^a}{a!}, \tag{10}$$

where n is the mean number of DNA cuts per molecule.

By measuring the DNA contour length of the molecules after interaction with Fe(III)-bleomycin, we are able to determine quantitatively the mean number of cuts per molecule. The experiments were performed in diluted solution, which reduces the intermolecular interactions. The reader should refer to our previous work for further details about this experiment (Piétrement et al. 2005). The only additional step in the experimental procedure is that it is necessary to uncondense DNA before deposition by adding monovalent salt (see "Material and methods").

Figure 9a represents the number of DNA cuts versus spermidine concentration. It indicates that the number of DNA cuts per circular DNA increases sharply until the condensation takes place. The extended/condensed transition is represented by grey areas in Fig. 9a and b. In this region, uncut DNA molecules (condensed state) and highly damaged DNA molecules (extended state) coexist (Murayama and Yoshikawa 1999; Takahashi et al. 1997), so it is difficult to perform reliable measurements. For larger spermidine concentrations, almost all the DNA molecules are condensed and the bleomycin cleavage is inhibited due to the lower accessibility. The main result is that the DNA bending sharply increases up to the condensation transition, as predicted by the

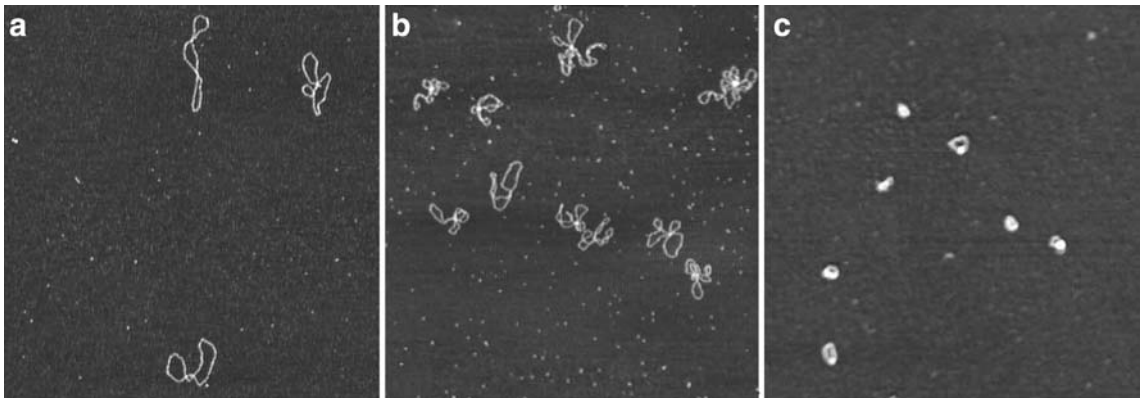

Fig. 8 AFM image of pUC19 DNA molecules (2.5 μg/ml DNA in Hepes 10 mM, pH 7.5, $MgCl_2$ 5 mM buffer) after interaction with three different concentrations of spermidine: **a** 1 μM, **b** 10 μM and **c** 50 μM. Image area 2×2 μm^2 and *z*-scale 3 nm

model. This observation is also in agreement with AFM observations (Fig. 8). The high level of DNA deformation just before the extended/collapsed transition was not clearly observed by other techniques. In addition, this experiment points out that a slight variation of the concentration of polyamine can lead to a high variation in the bleomycin activity.

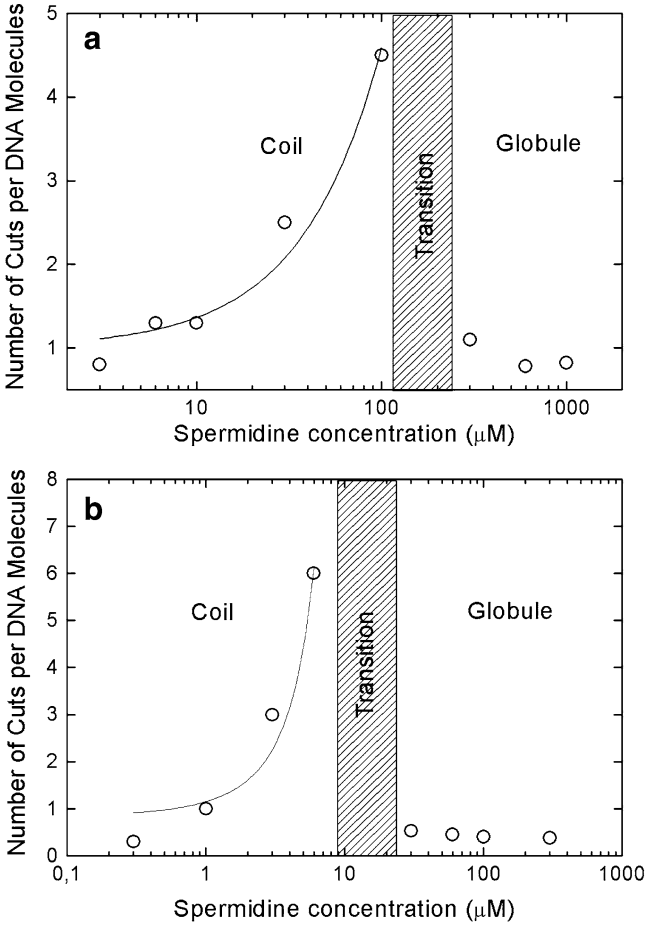

Fig. 9 Plot of the number of DNA cuts per pUC19 circular DNA molecules (2.5 μg/ml DNA in Hepes 10 mM, pH 7.5 buffer) versus spermidine concentration. The transition area indicates the beginning of the DNA condensation: **a** without $[Mg^{2+}]$, [Fe-blm] = 10 nM; **b** $[Mg^{2+}]$ = 5 mM, [Fe-blm] = 200 nM

With 5 mM magnesium, the condensation takes place at lower spermidine concentration and a significant amplification of the DNA cleavage arises at lower spermidine concentration just before condensation (Fig. 9b). Previous experiments show that the addition of a small concentration of magnesium to the buffer can promote DNA condensation in the presence of polyamines (Rowatt and Williams 1992). Thus the concentration of polyamines required to condense DNA can be significantly lowered. From the models previously developed, it is thought that divalent counterions are more efficient for DNA neutralization than monovalent counterions (Bloomfield 1997). In addition divalent counterions can also participate in the counterions correlations. We observe that the amplification of bleomycin activity starts at a lower concentration of spermidine than without magnesium. It can be due to a facilitated localization because of the presence of magnesium on the DNA chain. This discussion points out that the dynamical bending probability is not simply proportional to the polyamine binding ratio but is more likely related to the entropy loss in polyamine localization. It could also be remarked that magnesium alone cannot amplify the DNA cleavage by bleomycin (Piétrement et al. 2005). The hydrated radius of magnesium ions ((5.3 Å) is too large to permit a large bending angle, which means that DNA bending is not as stable as for smaller cations (the energy benefit in bending is dependent on the maximum angle allowed). For example, a divalent cation like putrescine, which is smaller ((3 Å) than magnesium, amplifies the bleomycin cleavage (Strekowski et al. 1989).

Conclusion

The local distortion of the DNA helix by polyamines strongly depends on the entropy penalty due to the counterion localization. Because of the self-repulsion

between multivalent counterions, a high binding ratio of polyamines (up to the polyamine concentration required for DNA condensation) favours DNA bending by polyamines. It results that the DNA bending depends on the binding competition between monovalent and multivalent counterions. In addition, we describe a new bending mechanism. First, the DNA molecule is bent by thermal agitation. Second, a polyamine ion could then be trapped in the middle of the major groove since this position is more favourable thanks to the bending initiated by thermal agitation. Third, the bending angle increases up to its maximum value.

Bleomycin was used as a new probe to study the dynamical bending of DNA molecules by polyamines. Experimental results indicate that the dynamical bending probability is sharply enhanced just at the boundary of DNA condensation, which is in agreement with the model. The local distortion mechanism may be closely related to DNA condensation by polyamines. This study could provide additional information for understanding the interplay between polyamines, DNA and DNA-binding proteins like transcription factors.

References

Bailly C, Kenani A, Waring MJ (1997) Altered cleavage of DNA sequences by bleomycin and its deglycosylated derivative in the presence of actinomycin. Nucleic Acids Res 25:1516–1522

Baumann CG, Bloomfield VA, Smith SB, Bustamante C, Wang MD, Block SM (2000) Stretching of single collapsed DNA molecules. Biophys J 78:1965–1978

Bloomfield VA (1997) DNA condensation by multivalent cations. Biopolymers 44:269–282

Boutonnet N, Hui X, Zakrzewska K (1993) Looking into the grooves of DNA. Biopolymers 33:479–490

Braunlin WH, Nordenskiold L, Drakenberg T (1991) A reexamination of 25Mg2+ NMR in DNA solution: site heterogeneity and cation competition effects. Biopolymers 31:1343–1346

Burak Y, Ariel G, Andelman D (2003) Onset of DNA aggregation in presence of monovalent and multivalent counterions. Biophys J 85:2100–2110

Burger RM (1998) Cleavage of nucleic acids by bleomycin. Chem Rev 98:1153–1170

Chiu TK, Dickerson RE (2000) 1 A crystal structures of B-DNA reveal sequence-specific binding and groove-specific bending of DNA by magnesium and calcium. J Mol Biol 301:915–945

Claussen CA, Long EC (1999) Nucleic acid recognition by metal complexes of bleomycin. Chem Rev 99:2797–2816

Conwell CC, Vilfan ID, Hud NV (2003) Controlling the size of nanoscale toroidal DNA condensates with static curvature and ionic strength. Proc Natl Acad Sci USA 100:9296–9301

Deng H, Bloomfield VA, Benevides JM, Thomas GJ Jr (2000) Structural basis of polyamine-DNA recognition: spermidine and spermine interactions with genomic B-DNAs of different GC content probed by Raman spectroscopy. Nucleic Acids Res 28:3379–3385

Fang Y, Hoh JH (1999) Early intermediates in spermidine-induced DNA condensation on the surface of mica. J Am Chem Soc 120:8903–8909

Feuerstein BG, Pattabiraman N, Marton LJ (1990) Molecular mechanics of the interactions of spermine with DNA: DNA bending as a result of ligand binding. Nucleic Acids Res 18:1271–1282

Flink I, Pettijohn DE (1975) Polyamines stabilise DNA folds. Nature 253:62–63

Frontali C, Dore E, Ferrauto A, Gratton E, Bettini A, Pozzan MR, Valdevit E (1979) An absolute method for the determination of the persistence length of native DNA from electron micrographs. Biopolymers 18:1353–1373

Grosberg AY, Nguyen TT, Shklovskii BI (2002) Colloquium: the physics of charge inversion in chemical and biological systems. Rev Mod Phys 74:329–337

Hagerman PJ (1988) Flexibility of DNA. Annu Rev Biophys Biophys Chem 17:265–286

Khan MO, Mel'nikov SM, Jönsson B (1999) Anomalous salt effects on DNA conformation: experiment and theory. Macromolecules 32:8836–8840

Kjellander R, Marcelja S (1985) Correlation and image charge effects in electrical double layers. Chem Phys Lett 114:124

Koltover I, Wagner K, Safinya CR (2000) DNA condensation in two dimensions. Proc Natl Acad Sci USA 97:14046–14051

Korolev N, Lyubartsev AP, Laaksonen A, Nordenskiold L (2002) On the competition between water, sodium ions, and spermine in binding to DNA: a molecular dynamics computer simulation study. Biophys J 82:2860–2875

Lau AWC, Pincus P (1998) Charge-fluctuation-induced nonanalytic bending rigidity. Phys Rev Lett 81:1338–1341

Laundon CH, Griffith JD (1987) Cationic metals promote sequence-directed DNA bending. Biochemistry 26:3759–3762

Levin Y, Arenzon JJ, Stilck JF (1999) The nature of attraction between like-charged rods. Phys Rev Lett 83:2680

Li W, Antholine WE, Petering DH (2002) Kinetics of reaction of DNA-bound Fe(III)bleomycin with ascorbate: interplay of specific and non-specific binding. J Inorg Biochem 90:8–17

Lin Z, Wang C, Feng X, Liu M, Li J, Bai C (1998) The observation of the local ordering characteristics of spermidine-condensed DNA: atomic force microscopy and polarizing microscopy studies. Nucleic Acids Res 26:3228–3234

Lopez-Larraza DM, Bianchi NO (1993) DNA response to bleomycin in mammalian cells with variable degrees of chromatin condensation. Environ Mol Mutagen 21:258–264

Maher LJ 3rd (1998) Mechanisms of DNA bending. Curr Opin Chem Biol 2:688–694

Manning GS (1969) Limiting laws and counterion condensation in polyelectrolyte solutions I. Colligative properties. J Chem Phys 51:924–933

McFail-Isom L, Sines CC, Williams LD (1999) DNA structure: cations in charge? Curr Opin Struct Biol 9:298–304

Murayama H, Yoshikawa K (1999) Thermodynamics of the collapsing phase transition in a single duplex DNA molecule. J Phys Chem B 103:10517–10523

Ouameur AA, Tajmir-Riahi HA (2004) Structural analysis of DNA interactions with biogenic polyamines and cobalt(III)hexamine studied by Fourier transform infrared and capillary electrophoresis. J Biol Chem 279:42041–42054

Pastré D, Piétrement O, Fusil S, Landousy F, Jeusset J, David MO, Hamon L, Le Cam E, Zozime A (2003) Adsorption of DNA to mica mediated by divalent counterions: a theoretical and experimental study. Biophys J 85:2507–2518

Piétrement O, Pastré D, Landousy F, David MO, Fusil S, Hamon L, Zozime A, Le Cam E (2005) Studying the effect of a charged surface on the interaction of bleomycin with DNA using an atomic force microscope. Eur Biophys J 34:200–207

Porschke D (1991) Persistence length and bending dynamics of DNA from electrooptical measurements at high salt concentrations. Biophys Chem 40:169–179

Raspaud E, Olvera de la Cruz M, Sikorav JL, Livolant F (1998) Precipitation of DNA by polyamines: a polyelectrolyte behavior. Biophys J 74:381–393

Record MT Jr, Anderson CF, Lohman TM (1978) Thermodynamic analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the roles of ion association or release, screening, and ion effects on water activity. Q Rev Biophys 11:103–178

Rouzina I, Bloomfield VA (1996) Macroion attraction due to electrostatic correlation between screening counterions. J Phys Chem 100:9977–9989

Rouzina I, Bloomfield VA (1998) DNA bending by small, mobile multivalent cations. Biophys J 74:3152–3164

Rowatt E, Williams RJ (1992) The binding of polyamines and magnesium to DNA. J Inorg Biochem 46:87–97

Ruiz-Chica AJ, Medina MA, Sanchez-Jimenez F, Ramirez FJ (2004) On the interpretation of Raman spectra of 1-aminooxyspermine/DNA complexes. Nucleic Acids Res 32:579–589

Saminathan M, Antony T, Shirahata A, Sigal LH, Thomas T, Thomas TJ (1999) Ionic and structural specificity effects of natural and synthetic polyamines on the aggregation and resolubilization of single-, double-, and triple-stranded DNA. Biochemistry 38:3821–3830

Sines CC, McFail-Isom L, Howerton SB, VanDerveer D, Williams LD (2000) Cations mediate B-DNA conformational heterogeneity. J Am Chem Soc 122:11048–11056

Smith B, Bauer G, Povirk L (1994) DNA damage induced by bleomycin, neocarzinostatin, and melphalan in a precisely positioned nucleosome. Asymmetry in protection at the periphery of nucleosome-bound DNA. J Biol Chem 269:30587–30594

Steighner RJ, Povirk LF (1990) Bleomycin-induced DNA lesions at mutational hot spots: implications for the mechanism of double-strand cleavage. Proc Natl Acad Sci USA 87:8350–8354

Strauss JK, Maher LJ 3rd (1994) DNA bending by asymmetric phosphate neutralization. Science 266:1829–1834

Strekowski L, Harden DB, Wydra RL, Stewart KD, Wilson WD (1989) Molecular basis for potentiation of bleomycin-mediated degradation of DNA by polyamines. Experimental and molecular mechanical studies. J Mol Recognit 2:158–166

Takahashi M, Yoshikawa K, Vasilevskaya VV, Khokhlov AR (1997) Discrete coil-globule transition of single duplex DNAs induced by polyamines. J Phys Chem B 101:9396–9401

Théveny B, Coulaud D, Le Bret M, Revet B (1988) Local variations of curvature and flexibility along DNA molecules analyzed from electron micrographs. In: Sundaralingam M (ed) Structure and expression. DNA bending and curvature, vol 3. Adenine Press, New York

Thomas T, Thomas TJ (2001) Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. Cell Mol Life Sci 58:244–258

Vijayanathan V, Thomas T, Shirahata A, Thomas TJ (2001) DNA condensation by polyamines: a laser light scattering study of structural effects. Biochemistry 40:13644–13651

Wilson RW, Bloomfield VA (1979) Counterion-induced condensation of deoxyribonucleic acid. A light-scattering study. Biochemistry 18:2192–2196

Xu Q, Deng H, Braunlin WH (1993) Selective localization and rotational immobilization of univalent cations on quadruplex DNA. Biochemistry 32:13130–13137