Dielectric Constant and Ionic Strength Effects on DNA Precipitation

Stéphane Flock, Rudi Labarbe, and Claude Houssier
Laboratoire de Chimie Macromoléculaire et Chimie Physique, Université de Liège, Sart-Tilman (B6), B-4000 Liège, Belgium

ABSTRACT We have investigated the effect of different zwitterionic compounds on DNA precipitation induced by spermine^{4+}. Glycine, β-alanine, 4-aminobutyric acid, and 6-aminocaproic acid have shown an increasing capacity to attenuate DNA precipitation. This protection effect has been correlated with the dielectric constant increase of their corresponding solutions. Calculations based on these experimental data and counter-ion condensation theory have confirmed the importance of this parameter for DNA-ion interactions and precipitation mechanisms. We have also observed a resolubilization of DNA in the presence of 6-aminocaproic acid at high spermine^{4+} concentration and in the presence of glycine at high spermidine^{3+} concentration. This could be explained by an increase of screening effect with polyamine concentration.

INTRODUCTION

An exact description of the interactions of DNA with various compounds (cations, histones, polyamines, drugs, water, etc.) is important to the explanation of many biological processes. For example, within eukaryotic cells, DNA is complexed with histone and nonhistone proteins to form a highly condensed structure, the chromosomes. In viruses, DNA is condensed by polyamines into a compact form that allows its packaging into the limited space afforded inside the capsid. Moreover, the condensation/decondensation of nucleic acids is probably implicated in the mechanism of gene expression and repression.

Condensation and precipitation of DNA in vitro have thus been extensively studied, and a wide range of conditions that cause DNA to collapse into compact structures have been discovered. Trivalent metals (Gersanovski et al., 1985; Tajmir-Riahi et al., 1993), metal complexes (Widom and Baldwin, 1980, 1983; Schellman and Parthasarathy, 1984), polyamines (Chattoraj et al., 1978; Gosule and Schellman, 1978; Wilson and Bloomfield, 1979), and basic proteins (Clark and Thomas, 1986; Garcia-Ramirez and Subirana, 1994) have been found to be very efficient. On the contrary, monovalent and divalent cations like Na^+ and Mg^{2+} are unable to condense DNA except in the presence of organic solvents like methanol, ethanol, etc. (Gosule and Schellman, 1978; Wilson and Bloomfield, 1979). However, these cations are able to induce the condensation and precipitation of chromatin (DNA-histone complex) (Ausio et al., 1984; Widom, 1986; Marquet et al., 1988; Fredericq et al., 1988, 1991). This explains the chromatin hypercondensation appearing in normal cells submitted to a hyperosmotic shock (Delpire et al., 1985). Such dramatic changes resulting in cell death do not appear for cells of euryhaline invertebrates and some others cells like renal papillary cells, where the presence in the intracellular medium of low-molecular-weight organic compounds (glycine, taurine, proline, mannitol, sorbitol, etc.) called organic osmolytes (Gilles, 1988) seems to prevent such structural modifications.

Our laboratory, already involved in the study of nucleic acid condensation mechanisms (Gersanovski et al., 1985; Marquet et al., 1985, 1986, 1988; Fredericq et al., 1988, 1991; Marquet and Houssier, 1991), has included the investigation of this protection effect in its current research. The study of the influence of these organic osmolytes on the condensation and precipitation of nucleic acids in vitro has a double interest. From the biological point of view, this would yield information about the in vivo condensation/decondensation processes and could help to explain the mechanisms of resistance of cells to osmotic stress. From the physical point of view, this would lead to a better understanding of the behavior of charged polymers in solution. Indeed, DNA is a highly charged polyanion and is a good probe for studying the influence of cationic and organic substances on polyelectrolyte behaviors in solution. Moreover, the results obtained would probably make it possible to improve the models used to describe polyelectrolyte behavior.

Condensation and precipitation experiments in vitro have thus been conducted in the absence and presence of these osmotic effectors. Buche et al. (1989, 1990, 1993) have shown that these organic osmolytes were able to hinder chromatin precipitation induced by NaCl, KCl, $CaCl_2$, or $MgCl_2$. We have obtained a similar protection effect in the study of glycine addition on the DNA precipitation induced by spermine^{4+}, spermidine^{3+}, and Tb^{3+} (Flock et al., 1995). To explain this glycine protection effect, we have considered the increase of the medium dielectric constant resulting from the addition of this compound to a DNA solution. As a consequence, some counter-ions would be ejected from the DNA condensation layer, resulting in a decrease in DNA precipitation.

In this paper we have studied the DNA precipitation induced by spermine^{4+} in the presence of different aminocarboxylic acids (glycine, β-alanine, 4-aminobutyric acid, and 6-aminocaproic acid) that display increasing dielectric increment in aqueous solution (Cohn and Edsall, 1943).

Received for publication 19 October 1995 and in final form 11 December 1995.

Address reprint requests to Dr. Claude Houssier, University of Liège, Chimaphy, Sart-Tilman (B6), B-4000 Liège, Belgium. Tel.: 41-663406; Fax: 41-662934;, E-mail: houssie@gw.unipc.ulg.ac.be.

0006-3495/96/03/1456/10 $2.00

MATERIALS AND METHODS

Calf thymus DNA (Sigma Type I) was purified to reach a residual protein content smaller than 1%. Stock DNA solutions at high concentration (about 2 g/liter) were prepared and dialyzed against cacodylate buffer (1 mM, pH = 6.5). An extinction coefficient $\epsilon(260\ \text{nm}) = 6600\ M^{-1}\ cm^{-1}$ was used to determine their concentration.

Stock solutions of 2 M glycine (USB), β-alanine, 4-aminobutyric acid, and 6-aminocaproic acid (Fluka) were prepared in 1 mM cacodylate buffer (pH = 6.5) and were stored at 4°C.

Different stock solutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} M) of spermine^{4+} (spermine tetrahydrochloride; Sigma) and spermidine^{3+} (spermidine trihydrochloride; Sigma) in 1 mM cacodylate buffer (pH = 6.5) were prepared just before use and were stored at 4°C.

All samples were prepared by dilution of stock solutions in 1 mM cacodylate (pH = 6.5) to attain a DNA concentration of about 130 μM in mononucleotide residues (absorbance ≈ 1 at 260 nm for 1 cm path length).

For the precipitation experiments, the samples prepared by dilution with gentle stirring were left to equilibrate for about 5 min at room temperature before centrifugation at 5000 × *g* for 5 min. The amount of soluble material was then obtained from the supernatant absorbance at 260 nm, and at 400 nm to correct for turbidity. The percentage solubility is determined as $S^{\%} = (A^{cor}_{260}/A^{0}_{260}) \cdot 100$, where A^{cor}_{260} is the supernatant absorbance corrected for turbidity and absorbance of aminocarboxylic acids and polyamines, and A^{0}_{260} is the blank absorbance of DNA alone in the same conditions.

Counter-ion condensation theory

Several experimental techniques have been developed to probe the interactions of small cations with macromolecules (Kwak, 1973; Bleam et al., 1980; Mattai and Kwak, 1981; de Jong et al., 1987; Braunlin and Xu, 1992; Andreasson et al., 1993; Ma and Bloomfield, 1995). Simultaneously, numerous papers have been published in the field of the theoretical description of polyelectrolyte solutions (Manning, 1978; Anderson and Record, 1990; Jayaram et al., 1994). Among these theories, counter-ion condensation (Manning, 1978) has been widely used (Wilson and Bloomfield, 1979; Clark and Kimura, 1990), presumably because of its simplicity and its ability to predict with reasonable accuracy the various properties of DNA in solution (colligative properties, transport properties, binding equilibria, melting temperature, etc.).

According to this theory, DNA is considered as an infinite line of charge and is characterized by an axial charge density parameter: $\xi = q^2/(4\pi\epsilon_0\epsilon_r kTb)$, where q is the protonic charge, b the distance between two charge groups (1.7 Å for native DNA), ϵ_0 is the vacuum permittivity, ϵ_r the medium dielectric constant ($\epsilon_r = 80$ for water at 20°C), k the Boltzmann constant, and T the absolute temperature. For a DNA in water at 20°C, ξ amounts to 4.2. The counter-ions are treated as point charges, and their binding to the polyelectrolyte chain is nonspecific and delocalized. Under these conditions, the extent of binding of cations to DNA is determined by the balance between two opposing tendencies: i) the minimization of electrostatic free energy through charge neutralization on cation binding; and ii) the maximization of entropy through cation dissociation. At equilibrium, only a fraction ($F = \theta_1 + n \cdot \theta_n$) of the DNA phosphate charges are neutralized by counter-ion given by the following relationships:

$$\ln\left(\frac{\theta_1}{V(C_1 - \theta_1 C_p)\gamma_1}\right) + \phi = -2(1 - \theta_1 - n\theta_n)\xi\ln(1 - e^{-kb}) \quad (1)$$

$$\ln\left(\frac{\theta n}{V(C_n - \theta_n C_p)\gamma_n}\right) + \phi = -2n(1 - \theta_1 - n\theta_n)\xi\ln(1 - e^{-kb}), \quad (2)$$

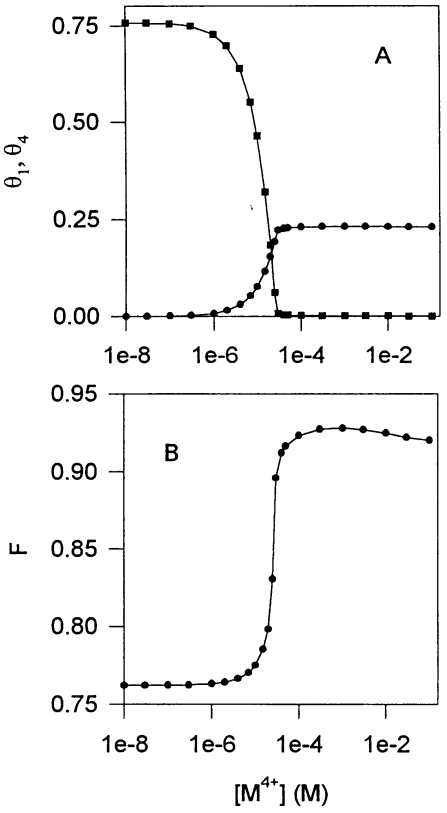

FIGURE 1 (*A*) Changes in monovalent and tetravalent binding fractions (θ_1 (■) and θ_4 (●)) during DNA titration by a tetravalent cation. (*B*) Total fraction F of DNA phosphate charges neutralized by counter-ions as a function of the tetravalent cations concentration (Eqs. 1 and 2, with C_p = 130 μM, C_1 = 1 mM, and $\phi = \gamma_1 = \gamma_4 = 1$).

where θ_1 and θ_n are the number of M^+ and M^{n+} ions, respectively, bound per DNA phosphate charge; C_1, C_n, and C_p are, respectively, the total concentrations of M^+, M^{n+}, and DNA; γ_1 and γ_n are the activity coefficients of unassociated counter-ions, and ϕ is the osmotic coefficient. V, the volume per mole of mononucleotide of the region surrounding the polynucleotide within which M^+ and M^{n+} cations are said to be "bound," is given by $V = 8 \cdot 10^3 \cdot \pi \cdot e \cdot N_{av}(\xi - 1)b^3$ (V has the units dm^3/mol mononucleotide if b is expressed in meters) and κ, the Debye screening parameter, is given by $\kappa^2 = 2 \cdot 10^3 \cdot N_{av} \cdot (q^2/\epsilon_0\epsilon_r kT) \cdot I$, where N_{av} is Avogadro's number and I the ionic strength computed from the salts concentrations.

RESULTS

DNA precipitation and counter-ion condensation theory

In spite of the large number of studies that have appeared in recent years on DNA condensation and precipitation (Riemer and Bloomfield, 1978; Manning, 1989; Bloomfield, 1991; Marquet and Houssier, 1991), the mechanism of this precipitation remains somewhat unknown. However, these studies have shown that these transitions are largely governed by the ability of counter-ions to neutralize the DNA phosphate charges.

On the basis of Eqs. 1 and 2, we have reported in Fig. 1 the fraction of DNA phosphate charges neutralized by monovalent (θ_1) and tetravalent (θ_4) cations during the titration of a DNA solution (130 μM) in a sodium buffer (1 mM). Starting at a concentration of about 1 μM, the tetravalent cations progressively replace the sodium ions condensed to DNA. This is illustrated by the sharp decrease of θ_1 and the concomitant increase of θ_4. However, the ejection of four M^+ from the condensation layer by one M^{4+} is entropically favorable. As a consequence, an increase of the phosphate charge neutralization from 76% at 1 μM M^{4+} to 93% at 1 mM M^{4+} will appear during the titration (Fig. 1 *B*).

Experimentally, Fig. 2 shows that 50% of DNA is precipitated in presence of 37 μM spermine^{4+}. According to the counter-ion condensation theory, this corresponds to 91% of DNA phosphate charge neutralization. This is in good agreement with the observations of Wilson and Bloomfield (1979), Yen et al. (1983), and Marquet et al. (1985) based on DNA condensation/precipitation induced by spermine^{4+} and by spermidine^{3+}. This value can thus be considered the percentage of phosphate charge neutralization necessary to decrease sufficiently the repulsive electrostatic forces between macromolecules and thus allow their precipitation due to attractive forces coming from the fluctuating counter-ion atmosphere surrounding DNA (Guldbrand et al., 1986) or from a hydration mechanism (Leikin et al., 1993).

We must notice here that the DNA concentration does not have a great influence on this threshold value: Wilson and Bloomfield (1979) have obtained the same result with 3 μM DNA solution, Yen et al. (1983) with 10 to 60 μM, and Marquet et al. (1985) with 75 μM. However, the intramolecular condensation is preferentially obtained at very low DNA concentration (<15 μM) (Gosule and Schellman, 1978; Post and Zimm, 1982). At the concentration used in this work (130 μM), the condensation is largely replaced by an intermolecular aggregation.

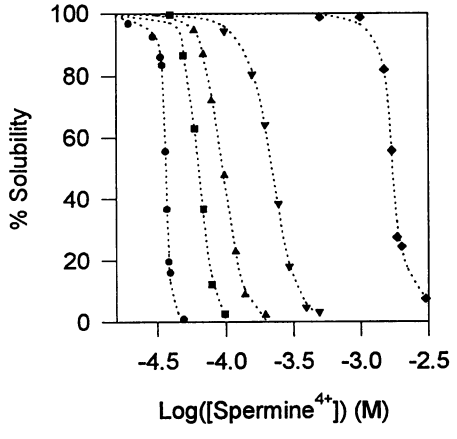

FIGURE 2 Percentage solubility of DNA (130 μM) as a function of spermine^{4+} concentration. ●, DNA alone; ■, in 1 M glycine; ▲, in 1 M β-alanine; ▼, in 1 M 4-aminobutyric acid; ♦, in 1 M 6-aminocaproic acid.

Dielectric constant effect

The fact that, in the presence of glycine, the concentration of spermine^{4+} required to produce DNA precipitation increases (Flock et al., 1995) is interesting in relation to the resistance of euryhaline cells to osmotic stress and to the role played by the solvent in the polyelectrolyte behavior. In the counter-ion condensation theory, the solvent is considered as a dielectric continuum. The only way to take into account a solvent change is thus to modify the dielectric constant. Because glycine is a zwitterionic compound at pH 6–7, its addition to a DNA solution increases the medium dielectric constant. As the axial charge density parameter ξ and the Debye screening parameter κ are dependent of this dielectric constant, such modification of the dielectric properties of the solvent changes the fraction of DNA phosphate charge neutralized by counter-ions. We have compared in Fig. 3 *A* the fraction of DNA phosphate charge neutralized during a titration by a tetravalent cation for dielectric constant values of ϵ_r = 80, 100, and 120. The phosphate charge neutralization decreases when the dielectric constant increases, so that more tetravalent cations have to be added in a solution of higher dielectric constant to reach the required level of phosphate charge neutralization producing DNA

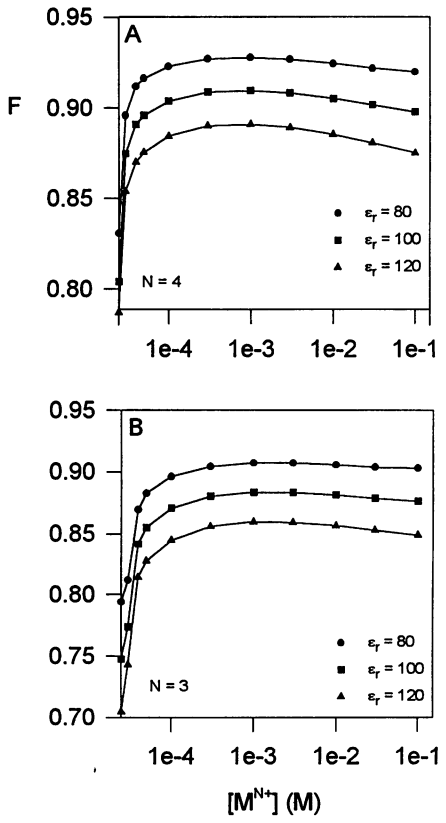

FIGURE 3 (*A*) Total fraction *F* of DNA phosphate charges neutralized by counter-ions as a function of the tetravalent cation concentration for different dielectric constants ϵ_r (Eqs. 1 and 2, with C_p = 130 μM, C_1 = 1 mM, and $\phi = \gamma_1 = \gamma_4 = 1$). (*B*) As in *A*, but for a titration by a trivalent cation.

aggregation. Moreover, for a sufficiently high dielectric constant we can suppose that the threshold value would not be attained, however high the tetravalent cation concentration is, and so DNA precipitation would thus become impossible (see also the change in the threshold value with dielectric constant later in this section).

To verify this theoretical prediction, we have studied DNA precipitation by spermine^{4+} in the presence of different aminocarboxylic acids: glycine, β-alanine, 4-aminobutyric acid, and 6-aminocaproic acid. The dielectric constant of their solutions increases linearly with the number of methyl groups between the amino and carboxyl groups (Cohn and Edsall, 1943). Fig. 2 shows that these compounds have a marked effect on DNA precipitation: the solubility curve of the macromolecule is moved to greater polyamine concentrations in the presence of these zwitterionic compounds. In Fig. 4 we have displayed the spermine^{4+} concentration necessary to reach 50% DNA precipitation as a function of aminocarboxylic acid concentration. It is clear that the efficiency of these compounds increases with their concentration and their charge separation. The largest effect is thus found with 1.5 M 6-aminocaproic acid, which prevents DNA precipitation by spermine^{4+} up to 0.02 M (the highest concentration tested).

The order of efficiency of these compounds can be correlated with their dielectric constant increments, namely: 22.6 for glycine, 34.6 for β-alanine, 51 for 4-aminobutyric acid, and 77.5 for 6-aminocaproic acid (Cohn and Edsall, 1943). On the basis of these data we can see that 50% precipitation is obtained with approximately the same spermine^{4+} concentrations when the solution displays the same dielectric constant: 64 and 53 μM in the presence of 1 M glycine (ϵ_r = 102.6) and 0.5 M β-alanine (ϵ_r = 97.3), respectively; 104 and 97 μM in the presence of 1.5 M glycine (ϵ_r = 113.9) and 1 M β-alanine (ϵ_r = 114.6); 203 and 223 μM in the presence of 1.5 M β-alanine (ϵ_r = 131.9) and 1 M 4-aminobutyric acid (ϵ_r = 131.0); 1160 and 1760 μM in the presence of 1.5 M 4-aminobutyric acid (ϵ_r = 156.5) and 1 M 6-aminocaproic acid (ϵ_r = 157.5). Fig. 5 shows the concentration of spermine^{4+} necessary to induce 50% DNA precipitation as a function of the calculated dielectric constant of the corresponding aminocarboxylic solutions; the correlation between these two parameters is manifest.

It has been shown above that in the absence of aminocarboxylic acid, the precipitation of DNA appears when 91% of phosphate charges are neutralized by spermine^{4+}. Thus, on the basis of the experimental data, we have used Eqs. 1 and 2 to calculate the fraction of phosphate charges neutralized at 50% DNA precipitation (Fig. 6). Variation of the resulting threshold value from 0.91 to 0.85 with the dielectric constant is not surprising. Indeed, until now, we have only discussed the effect of the increase in dielectric constant on DNA phosphate charge neutralization. However, the coulombic repulsive forces between phosphate charges of different DNA molecules are also dependent on the medium dielectric constant. As the increase of this parameter decreases the electrostatic forces, the repulsion between phosphate charges must decrease and, in consequence, the DNA precipitation appears for a lower phosphate charge neutralization.

Resolubilization of DNA by addition of spermine^{4+} in the presence of 6-aminocaproic acid

It could have been supposed that an increase in spermine^{4+} concentration above the value responsible for 100% precipitation would not further change the behavior of DNA, which would remain totally aggregated. Fig. 7 shows that, in 1 M 6-aminocaproic acid, an increased solubility of DNA appears for a spermine^{4+} concentration greater than about 10 mM, and almost 100% of soluble DNA is found in the presence of 60 mM spermine^{4+} and above. It is also important to notice that, like the protection effect, this resolubilization is dependent on aminocarboxylic acid concentra-

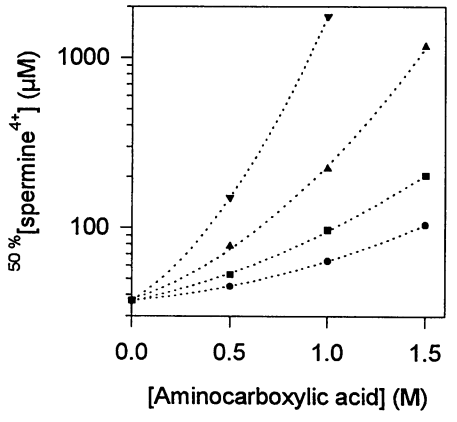

FIGURE 4 Spermine^{4+} concentrations required to reach 50% DNA precipitation as a function of aminocarboxylic acids concentration. ●, glycine; ■, β-alanine; ▲, 4-aminobutyric acid; ▼, 6-aminocaproic acid.

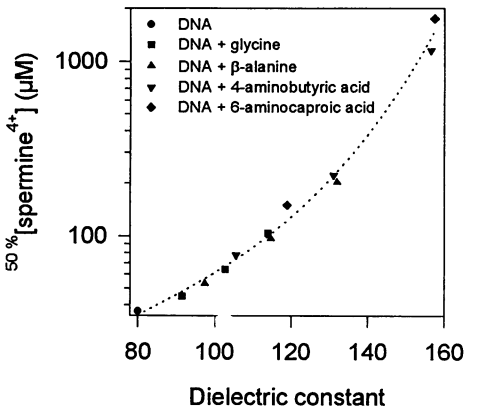

FIGURE 5 Spermine^{4+} concentrations required to reach 50% DNA precipitation as a function of dielectric constant of the aminocarboxylic acid solutions.

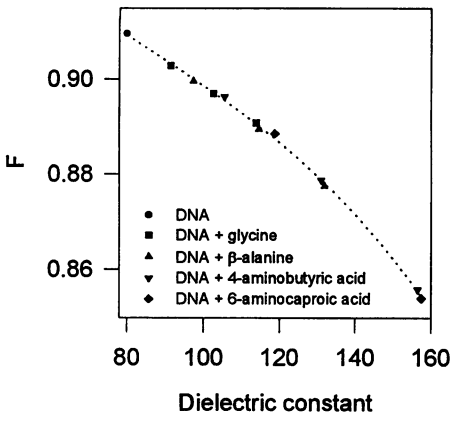

FIGURE 6 Variation of the total fraction of phosphate charge neutralized at 50% DNA precipitation with the dielectric constant of the aminocarboxylic solutions (Eqs. 1 and 2, with C_p = 130 μM, C_1 = 1 mM, ϕ = γ_1 = γ_4 = 1, and C_4 given by the precipitation experiments).

tion. Indeed, 100%, 70%, and 4% of DNA is found to be precipitated by the addition of 0.1 M spermine^{4+} in the absence and presence of 6-aminocaproic acid (0.5 M and 1 M, respectively) (Fig. 7, *right ordinate*).

The soluble DNA found in these conditions has the same UV and circular dichroism spectra as in the absence of spermine^{4+} (not shown). No denaturation or transition to another form than the B right-handed double helix can thus be found to explain this resolubilization (it would be preferabe to speak of increased solubility because we added the aminocarboxylic compound before polyamine addition to the DNA solution).

According to Eqs. 1 and 2, an increase in the tetravalent cation concentration over about 1 mM slightly decreases the fraction of phosphate charges neutralized (Fig. 1). Thus, for a sufficiently high spermine^{4+} concentration, a decrease in the phosphate charge neutralization under the threshold value for DNA precipitation could be obtained, resulting in a DNA resolubilization. However, as the decrease in the phosphate charge neutralization with the increase of the M^{4+} concentration is small, the DNA resolubilization would be possible only when the maximum of the neutralization curve is close to the threshold value for DNA precipitation. This is probably why no resolubilization is found in the absence of aminocarboxylic acid and why the effect is more important in the presence of a higher concentration of 6-aminocaproic acid.

If this explanation is correct, it should also be possible to observe a resolubilization effect with DNA precipitation induced by spermidine.$^{3+}$ However, trivalent cations are less efficient than tetravalent cations in neutralizing DNA phosphate charges (Fig. 3). As the resolubilization phenomenon is expected to occur when the maximum of the neutralization curve is close to the threshold value for a DNA precipitation, it should thus appear in a medium of lower dielectric constant with spermidine^{3+} (Fig. 3 *B*) rather than with spermine^{4+} (Fig. 3 *A*). We have shown in a preceding paper (Flock et al., 1995) that, in the presence of 1.5 M glycine (ϵ_r = 113.9), no DNA precipitation is detected for a concentration of spermidine^{3+} up to 20 mM. The maximum of the charge neutralization curve (Fig. 3 *B*) is under the threshold value at this glycine concentration. We could thus suppose that for a slightly lower dielectric constant, i.e., in the presence of 1 M glycine (ϵ_r = 102.6), a resolubilization effect would be visible. This is indeed what we observed, as shown in Fig. 8: in 0.06 M spermidine^{3+} almost all of the DNA remains in solution in 1 M glycine.

DISCUSSION

Although obtained from rather simple precipitation experiments, the above experimental data provide much information about the DNA solutions and their polyelectrolyte behaviors.

Dielectric constant and protection effect

The polyelectrolyte sensitivity to the medium dielectric constant is manifest. Fig. 5 clearly illustrates the effect of an

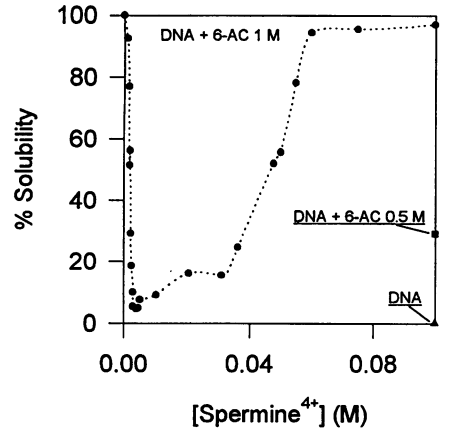

FIGURE 7 Solubility profile of DNA (130 μM) as a function of spermine^{4+} concentration in the presence of 1 M 6-aminocaproic acid (6-AC).

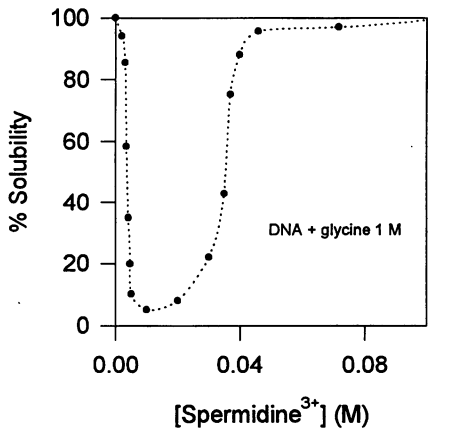

FIGURE 8 Solubility profile of DNA (130 μM) as a function of spermidine^{3+} concentration in the presence of 1 M glycine.

increase in this parameter: DNA becomes less sensitive to the action of precipitating agents like polyamines when the dielectric constant of the medium increases. Already in 1979, Wilson and Bloomfield had pointed out the importance of this parameter for the structural behavior of DNA in solution. Indeed, Gosule and Schellman (1978) had been unable to detect any evidence for DNA collapse in water by using the divalent cations Mg^{2+} and putrescine^{2+}. On the contrary, by decreasing the solution dielectric constant by adding methanol, Wilson and Bloomfield observed a DNA condensation by these cations. They have also observed that the spermidine^{3+} concentration needed to cause DNA condensation was reduced on going from water to methanol/water mixtures. Recently, Arscott et al. (1995) have shown that the critical concentration of cobalt-hexaammine^{3+} required to induce DNA condensation decreases from 21 μM to about 16 μM as the dielectric constant decreases from 80 to 70.

In conclusion, DNA precipitation is largely dependent on the dielectric constant of the solution, and the greater this parameter, the more difficult it is to aggregate the macromolecule. From the electrostatic point of view, we can assume that, in sodium salt solution, the strong repulsive forces between phosphate charges of DNA molecules prevent their precipitation. Thus, this transition appears only when the negative charges are sufficiently neutralized by the addition of multivalent cations. Therefore, the dielectric constant increase acts at two levels:

i) As the electrostatic force is inversely proportional to the dielectric constant, the repulsion between the remaining phosphate charges decreases when this parameter increases. The DNA precipitation thus occurs for a lower phosphate charge neutralization.

ii) However, such a variation of the dielectric constant decreases the neutralization of the DNA charges. In a first approximation, we can see (Fig. 3) that the charge remaining on a phosphate group at a given salt concentration increases proportionally with the dielectric constant. Thus the same amount of phosphate charge neutralization is obtained for a higher cation concentration.

As a result, the total repulsion force between two macromolecules would increase with the dielectric constant and more multivalent cations would be necessary to aggregate the DNA. Our experimental data are well explained on the basis of these hypotheses: although the neutralization of phosphate charges required to induce 50% DNA precipitation slightly decreases with the dielectric constant (Fig. 6), a larger concentration of spermine^{4+} is necessary to reach this threshold value (Fig. 5).

A modification of the threshold value with the dielectric constant has also been observed by Arscott and co-workers (1995), but the slight increase in this threshold value (from 0.89 to 0.91, with methanol) with the slight decrease in the dielectric constant (from 80 to 70) was not considered meaningful. On the contrary, in our work, the large increase in the dielectric constant (from 80 to 160) is clearer evidence of the variation in the threshold (Fig. 6).

Some controversy over the nature of the forces responsible for DNA condensation has appeared in the literature (Guldbrand et al., 1986; Marquet and Houssier, 1991; Bloomfield, 1991; Leikin et al., 1993). The fact that different zwitterionic compounds with different dipole moments give rise to the same effects at the same medium dielectric constant argues for an electrostatic explanation rather a hydration force mechanism based on polarization of water dipoles. This conclusion has also been reached by Arscott et al. (1995) from their experiments in different alcohol-water mixtures with $\epsilon_r = 80-70$. In addition, activity coefficients reported for glycine, β-alanine, and 6-aminocaproic acid (Edsall and Wyman, 1958) are not correlated with the observed protection effect. The possibility that the effects of zwitterionic compounds come from their osmotic coefficients, as suggested by Buche et al. (1993) for glycine, proline, and taurine, can thus be rejected. In conclusion, experimental results reported in this paper and a previous one (Flock et al., 1995) are well explained by considering only electrostatic repulsive forces, and we believe that it is not necessary to consider other forces, which have probably a minor importance in the protection effect of aminocarboxylic acids.

In a forthcoming paper, we analyze by ^{23}Na NMR the effect of aminocarboxylic acids on the sodium condensation layer of DNA. Results clearly demonstrate the decrease in the amount of sodium ions in the vicinity of DNA with an increase in the medium dielectric constant (in accord with counter-ion condensation theory). As interaction between DNA and spermine^{4+} has been described as a loose electrostatic interaction (Braunlin et al., 1982; Wemmer et al., 1985; Besley et al., 1990), we can expect the same behavior with this polyamine in the presence of zwitterionic compounds, and work is in progress to check this hypothesis.

Ionic strength and the resolubilization phenomenon

We turn our attention now to the variation of the fraction of DNA neutralization with the cation concentration. Although Eqs. 1 and 2 qualitatively explain the behavior of DNA in the conditions of our precipitation experiments, the slight decrease in the fraction of phosphate charge neutralization when the multivalent cation concentration becomes higher than about 1 mM is surprising (Fig. 1 and 3). Our experiments seem to verify this theoretical analysis.

An explanation for that decrease has been found by studying the role played by the ionic strength in Eqs. 1 and 2. For a titration by a tetravalent cation, we have seen (Fig. 1) that, after a sharp increase due to the displacement of monovalent counterions, the fraction of phosphate charge neutralized became invariant at moderate cation concentration and slightly decreased at high concentration. This profile contrasts with the theoretical titration curve obtained by taking a constant ionic strength in Eqs. 1 and 2 ($I = 10^{-3}$): after the titration rise, the fraction of phosphate charge

neutralized increases continuously with the tetravalent cation concentration (Fig. 9). We have also reported in this figure the total fraction of phosphate charge neutralized during a DNA titration by di- and trivalent cations. As for tetravalent cations, when a constant ionic strength is used for the calculation, the neutralization fraction increases continuously with the cation concentration, whereas an invariance of this fraction is found at moderate salt concentration when the cation concentration added is taken into account in the ionic strength expression. For the trivalent cations, a slight decrease in the fraction of phosphate charge neutralization is also obtained at high salt concentration (Fig. 9 *B*) and probably explains the resolubilization effect obtained with spermidine^{3+}. Such a neutralization decrease does not appear when the titration is done with a divalent cation (Fig. 9 *C*).

Similar results are obtained when we study the neutralization of phosphate charges in sodium salt solutions (Eq. 1 with $\theta_n = 0$). When the ionic strength due to NaCl concentration is used, the calculations predict a nearly constant phosphate charge neutralization (76%) up to 0.1 M NaCl. Above this concentration, an increase is observed. On the contrary, the calculations made by considering a constant ionic strength predict a continuous increase of the binding fraction (Fig. 10). The effect of the increase of ionic strength with salt concentration can be discussed in more detail by analyzing this rather simple equation. When NaCl is added to DNA, the free sodium concentration ($C_1 - \theta_1 C_p$) increases and the left member of the equation decreases. However, at the same time, we have an increase in ionic strength that decreases the right member. As a consequence,

if the left member decreases to a larger extent as compared to the right member, the system will react by increasing the sodium-bound fraction;

if the left and right members of the equation decrease in the same way, no change in the fraction of phosphate charge neutralization appears;

the left member could also become bigger than the right member of the equation. In that case, the system will restore the equality by decreasing the phosphate charge neutralization.

In summary, whatever the valence of the cation used (1, 2, 3, or 4), the ionic strength increase with salt concentration explains the invariance of the fraction of charges neutralized at moderate salt concentration. This well-known invariance has been experimentally verified by NMR and has been called "the condensation limit." The ionic strength increase also explains the slight decrease in the fraction of phosphate charge neutralization when the tri- or tetravalent cation concentration increases further.

A few years ago, Fenley et al. (1990) modified the line model of Manning's theory to take into account the double-helical array of B-DNA. They were surprised by the fact that this model predicted a decrease in the binding fraction as salt concentration increases. Considering that no experimental data had evidenced such a behavior, they restored

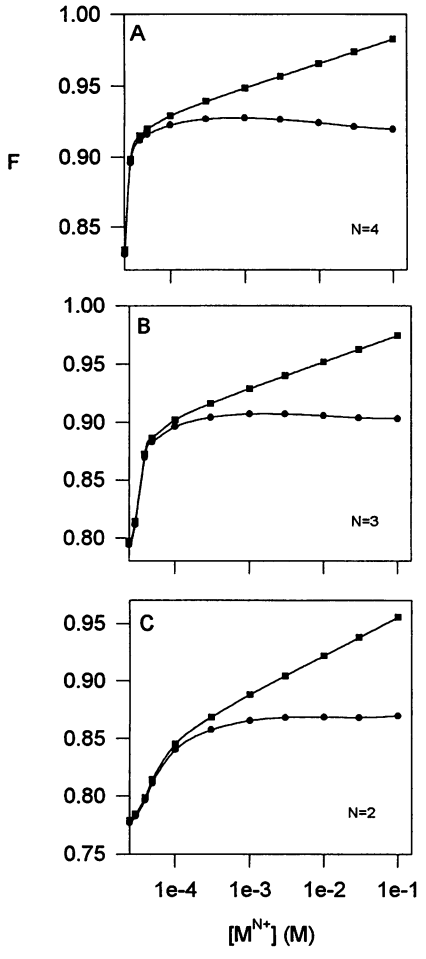

FIGURE 9 Total fraction F of DNA phosphate charges neutralized by counterions as a function of the N-valent cation concentration (N = 2, 3 and 4) for a concentration-dependent ionic strength $I = C_1 + C_N.(N^2 + N)/2$ (●) and a constant ionic strength $I = 10^{-3}$ (■) (Eqs. 1 and 2, with C_p = 130 μM, C_1 = 1 mM, and $\phi = \gamma_1 = \gamma_4 = 1$).

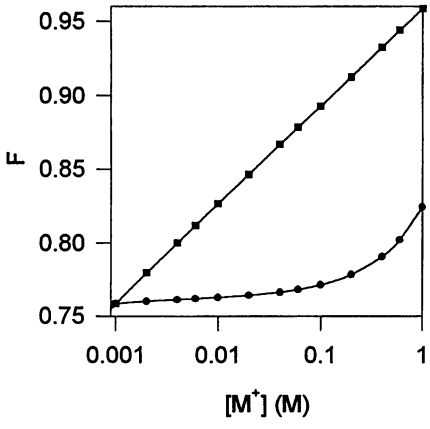

FIGURE 10 Variation of the DNA phosphate charges neutralized with the sodium salt concentration for a concentration-dependent ionic strength $I = C_1$ (●) and a constant ionic strength $I = 10^{-3}$ (■) (Eq. 1, with θ_n = 0, C_p = 130 μM, and $\phi = \gamma_1 = \gamma_4 = 1$).

the invariance of θ_n in their theory by means of a distance-dependent dielectric function. However, considering the above discussion, we cannot categorically reject the idea of a decrease in phosphate charge neutralization at high salt concentration.

Similar "resolubilization" of short DNA fragments (130 to 600 base pairs) at high spermidine^{3+}, spermine^{4+}, and cobalt-hexaammine^{3+} concentration (± 0.1 M) have recently been observed by Pelta and co-workers (Pelta et al., manuscript submitted for publication). Two explanations have been envisaged by the authors: i) a screening of the short-range electrostatic attraction between multivalent ions and DNA and ii) a charge reversal of the macromolecule (a well-known phenomenon in colloid chemistry). Because electrophoresis experiments have shown that the net charge of DNA remains negative, they have rejected the last hypothesis. Our results agree also, as discussed above, with a screening effect. According to our views, "resolubilization" occurs if the dielectric constant increment $\Delta\epsilon_r$ is sufficient for the total fraction F of phosphate charges neutralized to decrease just below the threshold value for precipitation. If $\Delta\epsilon_r$ is too small, F will remain above the threshold and no "resolubilization" will be possible. If it is too large, F will remain below the threshold whatever the spermine^{4+} concentration, and precipitation of DNA will be impossible. Thus, for $\Delta\epsilon_r = 116.3$ (1.5 M 6-aminocaproic acid) no precipitation of DNA is detected up to 0.1 M spermine^{4+}, and for $\Delta\epsilon_r = 77.5$ (1 M 6-aminocaproic acid), the precipitation and resolubilization of DNA when spermine^{4+} concentration increases from 1 mM to 0.1 M are clearly apparent in Fig. 7. Because of its lower charge, dielectric constant increments required to observed the same effect with spermidine^{3+} are lower than for spermine^{4+}: for $\Delta\epsilon_r = 33.9$ (1.5 M glycine) no precipitation of DNA is detected, and for $\Delta\epsilon_r = 22.6$ (1 M glycine) the precipitation and resolubilization of DNA are observed (Fig. 8). We have not observed a resolubilization phenomenon for DNA in the absence of zwitterionic compounds. This contrasts with the results of Pelta et al. (manuscript submitted for publication) but can probably be explained by the short length of the DNA fragments used in their work.

Limitations to the counter-ion condensation theory: arbitrary choice of *V*

It is important to notice here that the parameter V, the volume of the region surrounding the polynucleotide within which counter-ions are said to be "bound," is present in the left side of Eqs. 1 and 2. Its value will thus influence the equilibrium discussed above. We have reported in Fig. 11 the fraction of phosphate charge neutralized as a function of the salt concentration for DNA in sodium salt solution. Three different values of V has been used for these calculations: $V = 643.3$ cm^3/mol phosphate (the value used in this work), and half (321.6 cm^3/mol) and twice (1286.5 cm^3/mol) this value. The influence of this parameter is clearly evidenced.

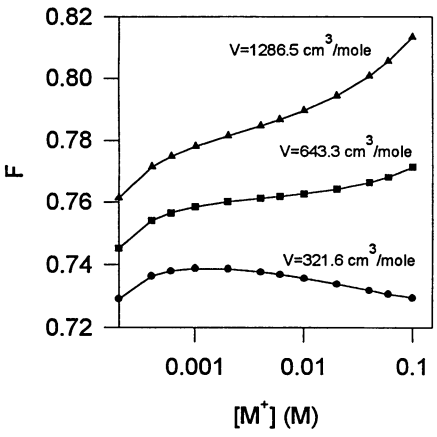

FIGURE 11 Variation of the DNA phosphate charges neutralized with the sodium salt concentration for different values of V (Eq. 1, with $\theta_n = 0$, $C_p = 130$ μM, and $\phi = \gamma_1 = \gamma_4 = 1$).

This figure summarises the main drawbacks of Manning's theory: if we want to estimate the fraction of charge neutralization at finite ionic strength, a priori knowledge of the condensed phase volume V is necessary. However, we have no way of determining this value. In the literature, the bound volume is generally assumed to be constant and equal to its limiting value for $C_n \rightarrow 0$. However, V given by this method is a function of the valence and number of counter-ions and co-ions in the salt formula (equation 13 of Manning, 1978). It is thus difficult to choose a value of V when different salts are present in solution.

In their equations, Wilson and Bloomfield (1979) have considered different values of V according to the valence of the cation considered. Using this method in our calculation yielded results similar to those reported here and obtained with V corresponding to NaCl solution. It does not affect the discussion about the dielectric constant and ionic strength effects at low and moderate salt concentration. However, at high salt concentration, the slight decrease in the fraction of phosphate charge neutralization with the increase in the tri- and tetravalent cation concentration (evidenced experimentally by DNA resolubilization) is replaced by an increase.

Possibility of control of the osmotic stress by a variation of the intracellular medium dielectric constant

The effect of dielectric constant change on the DNA precipitation is also found in the case of chromatin precipitation. Indeed, Atchley and Bhagavan (1964), who studied the aminocarboxylic acids effects on the solubility of crude deoxyribonucleoprotein (DNP) extruded from malignant mammalian cells, have observed that the addition of glycine, β-alanine, 4-aminobutyric acid, and 6-aminocaproic acid significantly increased the solubility of DNP in 0.01 $MgCl_2$. By a careful analysis of their results, we can see that the same percentage inhibition of precipitation was obtained for the same dielectric constant in solution: 15% and 12% in

the presence of 1.2 M glycine (ϵ_r = 107) and 0.8 M β-alanine (ϵ_r = 108), respectively; 23% and 25% in the presence of 1.6 M glycine (ϵ_r = 116) and 1.2 M β-alanine (ϵ_r = 122); 82% and 80% in the presence of 1.2 M γ-aminobutyric acid (ϵ_r = 141) and 0.8 M ε-aminocaproic acid (ϵ_r = 142). These results suggest that the resistance in vivo of euryhaline cells to osmotic stress is partially controlled by a medium dielectric constant modification due to variation in the concentrations of intracellular zwitterionic compounds (glycine, taurine, proline, etc.). Such variation of osmotic effector concentrations with the change in the osmolality of the external medium of these cells is a well-known phenomenon (Gilles, 1988).

CONCLUSION

Experiments conducted in this work with zwitterionic compounds of different chain lengths make it possible to understand the protection effect of glycine met in DNA and chromatin precipitation experiments. Because of its zwitterionic character, this compound increases the medium dielectric constant of solution and produces a decrease of the polymer charge interaction with counter-ions. In the formalism of Manning's counter-ion condensation theory, this corresponds to a reduction in the fraction of phosphate charge neutralization, which explains why the polyanion precipitation is hindered.

We have also shown that in the presence of glycine and 6-aminocaproic acid a complete resolubilization of DNA appears at high spermidine^{3+} or spermine^{4+} concentration (± 0.1 M). According to counter-ion condensation theory, the screening effect resulting from the ionic strength increase with the polyamine concentration would be responsible for this surprising behavior. Indeed, by increasing the multivalent cation concentration we should increase the phosphate charge neutralization. However, the ionic strength concomitantly increases and the electrostatic forces between phosphate charges and counter-ions decrease. For a moderate salt concentration, the two effects would cancel, resulting in an invariance of the counter-ion binding fraction with cation concentration, as predicted by Manning's limiting equation over a wide range of salt concentration and verified experimentally. At high salt concentration, the ionic strength effect would become more important and would then explain the resolubilization phenomenon.

However, we have also shown that the competition between the cation concentration increase and the concomitant ionic strength increase is dependent on the value of the volume of condensation layer used in the counter-ion condensation equations. This volume, introduced in the counter-ion condensation model, is an ill-defined concept. It is pictured as the volume surrounding the macromolecule and containing the condensed counter-ions. Its value is assumed to be constant and equal to its limiting value when the salt concentration tends to 0. In this study, we have used the binding volume corresponding to DNA in sodium salt at infinite dilution. Using another value does not change our conclusions, except in the case of the resolubilization experiments: the decrease in the charge neutralization is not predicted for a higher V.

We are grateful to Dr. J. Pelta and J-L. Sikorav for giving us access to his submitted paper.

This work was supported by the Ministère de l'Education, de la Recherche et de la Formation, Communauté Française de Belgique (ARC contract 91/95-152), the Fonds National de la Recherche Scientifique (FNRS Aspirant status to RL), and a research fellowship from IRSIA to SF.

REFERENCES

Anderson, C. F., and M. T. Record, Jr. 1990. Ion distributions around DNA and other cylindrical polyions: theoretical descriptions and physical implications. *Annu. Rev. Biophys. Biophys. Chem.* 19:423–465.

Andreasson, B., L. Nordenskiöld, W. H. Braunlin, J. Schultz, and P. Stilbs. 1993. Localized interaction of the polyamine methylspermidine with double-helical DNA as monitored by ^{1}H-NMR self-diffusion measurements. *Biochemistry.* 32:961–967.

Arscott, P. G., C. Ma, J. R. Wenner, and V. A. Bloomfield. 1995. DNA condensation by cobalt hexaammine (III) in alcohol-water mixtures: dielectric constant and other solvent effects. *Biopolymers.* 36:345–364.

Atchley, W. A., and N. V. Bhagavan. 1964. Saline-soluble preparations of deoxyribonucleoproteins. *Arch. Biochem. Biophys.* 106:505–515.

Ausio, J., N. Borochov, D. Seger, and H. Eisenberg. 1984. Interaction of chromatin with NaCl and $MgCl_2$. *J. Mol. Biol.* 177:373–398.

Besley, S., P. M. Cullis, R. Partridge, M. C. R. Symons, and R. T. Wheelhouse. 1990. Motion of polyammonium ions on aqueous solutions of DNA. *Chem. Phys. Lett.* 165:120–123.

Bleam, M. L., C. F. Anderson, and M. T. Record, Jr. 1980. Relative binding affinities of monovalent cations for double-stranded DNA. *Proc. Natl. Acad. Sci. USA.* 77:3085–3089.

Bloomfield, V. A. 1991. Condensation of DNA by multivalent cations: considerations on mechanism. *Biopolymers.* 31:1471–1481.

Braunlin, W. H., T. J. Strick, and M. T. Record, Jr. 1982. Equilibrium dialysis studies of polyamine binding to DNA. *Biopolymers.* 21: 1301–1314.

Braunlin, W. H., and Q. Xu. 1992. Hexaamminecobalt (III) binding environments on double-helical DNA. *Biopolymers.* 32:1703–1711.

Buche, A., P. Colson, and C. Houssier. 1990. Organic osmotic effectors and chromatin structure. *J. Biomol. Struct. Dyn.* 8:601–618.

Buche, A., P. Colson, and C. Houssier. 1993. Effect of organic effectors on chromatin solubility, DNA-histone H1 interaction, DNA and histone H1 structures. *J. Biomol. Struct. Dyn.* 11:95–119.

Buche, A., A. Ouassaidi, R. Hacha, E. Delpire, R. Gilles, and C. Houssier. 1989. Glycine and other amino compounds prevent chromatin precipitation at physiological ionic strength. *FEBS Lett.* 247:367–370.

Chattoraj, D. K., L. C. Gosule, and J. A. Schellman. 1978. DNA condensation with polyamines. II. Electron microscopic studies. *J. Mol. Biol.* 121:327–337.

Clark, D. J., and T. Kimura. 1990. Electrostatic mechanism of chromatin folding. *J. Mol. Biol.* 211:883–896.

Clark, D. J., and J. O. Thomas. 1986. Salt-dependent co-operative interaction of histone H1 with linear DNA. *J. Mol. Biol.* 187:569–580.

Cohn, E. J., and J. T. Edsall. 1943. Dielectric constants and dipole movements of dipolar ions. *In* Proteins, Amino Acids and Peptides as Ions and Dipolar Ions. Reinhold Publishing, New York. 145–148.

de Jong, H. G., J. Lyklema, and H. van Leeuwen. 1987. Conductometric analysis of the competition between monovalent and divalent counterions in their interaction with polyelectrolytes. *Biophys. Chem.* 27: 173–182.

Delpire, E., C. Duchêne, G. Goessens, and R. Gilles. 1985. Effects of osmotic shocks on the ultrastructure of different tissues and cell types. *Exp. Cell. Res.* 160:106–116.

Delpire, E., R. Gilles, C. Duchêne, and G. Goessens. 1985. Effects of osmotic shocks on the ion content and ultrastructure of rat pheochromocytoma cells of line PC12. *Mol. Physiol.* 8:293–306.

Edsall, J. T., and J. Wyman. 1958. *In* Biophysical Chemistry, Vol. 1. Academic Press, New York. 195–198.

Fenley, M. O., G. S. Manning, and W. K. Olson. 1990. Approach to the limit of counterion condensation. *Biopolymers.* 30:1191–1203.

Flock, S., R. Labarbe, and C. Houssier. 1995. Osmotic effectors and DNA structure: effect of glycine on precipitation of DNA by multivalent cations. *J. Biomol. Struct. Dyn.* 13:87–102.

Fredericq, E., R. Hacha, P. Colson, and C. Houssier. 1991. Condensation and precipitation of chromatin by multivalent cations. *J. Biomol. Struct. Dyn.* 8:847–865.

Fredericq, E., R. Hacha, and C. Houssier. 1988. Condensation of chromatin films in contact with divalent cation solutions at physiological ionic strength. *Int. J. Biol. Macromol.* 10:51–54.

Garcia-Ramirez, M., and J. A. Subirana. 1994. Condensation of DNA by basic proteins does not depend on protein composition. *Biopolymers.* 34:285–292.

Gersanovski, D., P. Colson, C. Houssier, and E. Fredericq. 1985. Terbium(3+) as a probe of nucleic acids structure. Does it alter the DNA conformation in solution? *Biochim. Biophys. Acta.* 824:313–323.

Gilles, R. 1988. Comparative aspects of cell osmoregulation and volume control. *Renal Physiol. Biochem.* 11:277–288.

Gosule, L. C., and J. A. Schellman. 1978. DNA condensation with polyamines 1. Spectroscopic studies. *J. Mol. Biol.* 121:311–326.

Guldbrand, L., L. G. Nilsson, and L. Nordenskiöld. 1986. A Monte Carlo simulation study of electrostatic forces between hexagonally packed DNA double helices. *J. Chem. Phys.* 85:6686–6698.

Jayaram, B., N. Aneja, E. Rajasekaran, V. Arora, A. Das, V. Ranganathan, and V. Gupta. 1994. Modelling DNA in aqueous solutions. *J. Sci. Ind. Res.* 53:88–105.

Kwak, J. C. T. 1973. Mean activity coefficient for the simple electrolyte in aqueous mixtures of polyelectrolyte and simple electrolyte. The system sodium polystyrenesulfonate-sodium chloride. *J. Phys. Chem.* 77: 2790–2793.

Leikin, S., V. A. Parsegian, D. C. Rau, and R. P. Rand. 1993. Hydration forces. *Annu. Rev. Phys. Chem.* 44:369–395.

Ma, C., and V. A. Bloomfield. 1995. Gel electrophoresis measurements of counterion condensation on DNA. *Biopolymers.* 35:211–216.

Manning, G. S. 1978. The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Q. Rev. Biophys.* 11:179–246.

Manning, G. S. 1989. Self-attraction and natural curvature in null DNA. *J. Biomol. Struct. Dyn.* 7:41–61.

Marquet, R., P. Colson, and C. Houssier. 1986. The condensation of chromatin and histone H1-depleted chromatin by spermine. *J. Biomol. Struct. Dyn.* 4:205–217.

Marquet, R., P. Colson, A. M. Matton, C. Houssier, M. Thiry, and G. Goessens. 1988. Comparative study of the condensation of chicken erythrocyte and calf thymus chromatin by di- and multivalent cations. *J. Biomol. Struct. Dyn.* 5:839–857.

Marquet, R., and C. Houssier. 1991. Thermodynamics of cation-induced DNA condensation. *J. Biomol. Struct. Dyn.* 9:159–167.

Marquet, R., C. Houssier, and E. Fredericq. 1985. An electro-optical study of the mechanisms of DNA condensation induced by spermine. *Biochim. Biophys. Acta.* 825:365–374.

Mattai, J., and J. C. T. Kwak. 1981. A dye spectrophotometric method for binding studies of Zn^{2+} and Mn^{2+} by biopolyelectrolytes. *Biophys. Chem.* 14:55–64.

Post, C. B., and B. H. Zimm. 1982. Theory of DNA condensation: collapse versus aggregation. *Biopolymers.* 21:2123–2137.

Riemer, S. C., and V. A. Bloomfield. 1978. Packaging of DNA in bacteriophage heads: some considerations on energetics. *Biopolymers.* 17: 785–794.

Schellman, J. A., and N. Parthasarathy. 1984. X-ray diffraction studies on cation-collapsed DNA. *J. Mol. Biol.* 175:313–329.

Tajmir-Riahi, H. A., R. Ahmad, and M. Naoui. 1993. Interaction of calf-thymus DNA with trivalent La, Eu and Tb ions. Metal ion binding, DNA condensation and structural features. *J. Biomol. Struct. Dyn.* 10: 865–877.

Wemmer, D. E., K. S. Srivenugopal, B. R. Reid, and D. R. Morris. 1985. Nuclear magnetic resonance studies of polyamine binding to a defined DNA sequence. *J. Mol. Biol.* 185:457–459.

Widom, J. 1986. Physicochemical studies of the folding of the 100 Å nucleosome filament into the 300 Å filament. *J. Mol. Biol.* 190: 411–424.

Widom, J., and R. L. Baldwin. 1980. Cation-induced toroidal condensation of DNA. *J. Mol. Biol.* 144:431–453.

Widom, J., and R. L. Baldwin. 1983. Monomolecular condensation of λ-DNA induced by cobalt hexammine. *Biopolymers.* 22:1595–1620.

Wilson, R. W., and V. A. Bloomfield. 1979. Counterion-induced condensation of deoxyribonucleic acid. A light-scattering study. *Biochemistry.* 18:2192–2196.

Yen, W. S., K. W. Rhee, and B. R. Ware. 1983. Condensation of polyamines onto nucleic acids. *J. Phys. Chem.* 87:2148–2152.