

# Competitive Binding of $Mg^{2+}$ , $Ca^{2+}$ , $Na^+$ , and $K^+$ Ions to DNA in Oriented DNA Fibers: Experimental and Monte Carlo Simulation Results

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**ABSTRACT** Competitive binding of the most common cations of the cytoplasm ( $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$ ) with DNA was studied by equilibrating oriented DNA fibers with ethanol/water solutions (65 and 52% v/v EtOH) containing different combinations and concentrations of the counterions. The affinity of DNA for the cations decreases in the order  $Ca > Mg \gg Na \approx K$ . The degree of  $Ca^{2+}$  and/or  $Mg^{2+}$  binding to DNA displays maximum changes just at physiological concentrations of salts (60–200 mM) and does not depend significantly on the ethanol concentration or on the kind of univalent cation ( $Na^+$  or  $K^+$ ).  $Ca^{2+}$  is more tightly bound to DNA and is replaced by the monovalent cations to a lesser extent than is  $Mg^{2+}$ . Similarly,  $Ca^{2+}$  is a better competitor for binding to DNA than  $Mg^{2+}$ : the ion exchange equilibrium constant for a 1:1 mixture of  $Ca^{2+}$  and  $Mg^{2+}$  ions,  $K_c^{Ca_{Mg}}$ , changes from  $K_c^{Ca_{Mg}} \approx 2$  in 65% EtOH (in 3–30 mM NaCl and/or KCl) to  $K_c^{Ca_{Mg}} \approx 1.2$ –1.4 in 52% EtOH (in 300 mM NaCl and/or KCl). DNA does not exhibit selectivity for  $Na^+$  or  $K^+$  in ethanol/water solutions either in the absence or in the presence of  $Ca^{2+}$  and/or  $Mg^{2+}$ . The ion exchange experimental data are compared with results of grand canonical Monte Carlo (GCMC) simulations of systems of parallel and hexagonally ordered, uniformly and discretely charged polyions with the density and spatial distribution of the charged groups modeling B DNA. A quantitative agreement with experimental data on divalent-monovalent competition has been obtained for discretely charged models of the DNA polyion (for the uniformly charged cylinder model, coincidence with experiment is qualitative). The GCMC method gives also a qualitative description of experimental results for DNA binding competitions of counterions of the same charge ( $Ca^{2+}$  with  $Mg^{2+}$  or  $K^+$  with  $Na^+$ ).

## INTRODUCTION

In the living cell, RNA and DNA phosphate groups and negative charges on proteins (Asp<sup>−</sup> and Glu<sup>−</sup> amino acid residues) are in excess of the positive charges of proteins (Arg<sup>+</sup> and Lys<sup>+</sup>) and other organic cations. As a result, inorganic metal ions ( $K^+$ ,  $Na^+$ ,  $Mg^{2+}$ , and  $Ca^{2+}$ ) exceed the amount of small mobile anions in the plasma and (especially) in the nucleus of the cell. In the simplest organisms (i.e., *Escherichia coli*), where negative and positive charges on the proteins approximately balance each other, the nucleic acids are the dominant polyelectrolytes (Record et al., 1998a,b). In an elementary building block of eukaryotic chromatin, the nucleosome, only half of the DNA charge is neutralized by the positive charges on histone proteins (Mirzabekov and Rich, 1979). Little is known about the composition of species neutralizing the rest of the DNA charge. It is clear, however, that  $Mg^{2+}$ ,  $K^+$ ,  $Ca^{2+}$ , and  $Na^+$  should be the major part of these cations.

Most experimental and theoretical studies on the interactions between DNA and charged ligands (metal ions and complexes, DNA-binding proteins, and other species) are made in dilute water solutions. Under these conditions, the

DNA interaction with charged ligands, the helix-coil transition temperature, and other DNA properties are strongly dependent on the low-molecular-weight salt concentration (see recent reviews (Jayaram and Beveridge, 1996; Record et al., 1998c). However, for condensed DNA states (fibers, gels) or in vivo, similar characteristics are often independent of or only slightly dependent on the ionic composition of the solvent (Ha et al., 1992; Rupprecht et al., 1994; Schultz et al., 1994). This discrepancy is usually explained by the direct and indirect consequences of the so-called macromolecular crowding effect (Record et al., 1998a,b, and references cited therein). In the condensed state of DNA (fibers, gels, liquid crystals), the mean concentration of counterions is very high (1–2 M for distances between the DNA helix axes of  $\sim 20$ –40 Å), and this leads to loss of sensitivity of the binding and structural parameters of DNA with respect to the concentrations of ions in the bulk solution. Thus oriented fibers, gels, films, and liquid crystalline structures of DNA can serve as a basic model for describing the interactions between DNA and other ions in natural DNA states.

The competition between monovalent and multivalent counterions for binding to polyelectrolytes is usually studied by theoretical modeling of polyion-mobile ion interactions or in studies of the DNA-oligocation binding in dilute solutions (for references see reviews in Anderson and Record, 1982; Record et al., 1998c). There are relatively few experimental works devoted to the peculiarities of M1/M2, Ca/Mg, and Na/K competitions in binding to polyelectrolytes including DNA (Bleam et al., 1980; Gregor et al., 1956; Kuznetsov et al., 1984; Soldatov and Bichkova,

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1988) (the abbreviations M1 and M2 are used for unspecified mono- and divalent cations, respectively). Data obtained on synthetic ion exchangers with sulfonic, carboxylic, and phosphate groups are useful for understanding the effects observed in biologically relevant condensed polyelectrolyte systems. Data for sulfonic anions are useful because the behavior of these anions in ion exchange equilibria is considered to be “purely electrostatic” and usually gives the best agreement with predictions of polyelectrolyte theories. Results for  $COO^-$  groups are mentioned because they are important biological “ion exchangers.”

For the divalent-monovalent cation competition, experimental studies of the sulfonic ion exchange resins have shown that  $K^+$  is a more effective competitor than  $Na^+$  in substituting for  $Ca^{2+}$  and/or  $Mg^{2+}$  on  $SO_3^-$  groups (Soldatov and Bichkova, 1988). According to the interpretation of  $^{23}Na$  NMR relaxation data obtained in DNA solutions,  $Mg^{2+}$  is better in substituting for  $Na^+$  than is  $Ca^{2+}$ , although the difference between magnesium and calcium is close to the uncertainty of the NMR relaxation technique (Braunlin et al., 1986, 1991).

It is well established that specialized proteins (e.g., calmodulin; Gilli et al., 1998) bind  $Ca^{2+}$  with much higher affinity than  $Mg^{2+}$ : the apparent equilibrium constant of Ca-Mg exchange on the binding sites of proteins,  $K_c^{Ca_{Mg}}$ , is on the order of  $10^3$  or higher. Nonspecific binding (i.e., charge neutralizing) of these ions with carboxylic or sulfonic groups in the ion exchange resins shows much lower selectivity for  $Ca^{2+}$ :  $K_c^{Ca_{Mg}} \approx 3$  for carboxylic (Muraviev et al., 1996) and  $K_c^{Ca_{Mg}} \approx 2-3$  for sulfonic (Soldatov and Bichkova, 1988) ion exchangers. Measurements of Ca/Mg-polyelectrolyte binding in water solutions demonstrate that  $Mg^{2+}$  binds to the  $COO^-$  groups in a nonspecific electrostatic manner, while  $Ca^{2+}$  has a tendency toward site binding and coordination with carboxylic groups, resulting in cross-linking of the polymer chains (Malovikova et al., 1994). There is much less understanding of Ca/Mg binding to DNA. Some authors (Manzini et al., 1990; Rose et al., 1982) report a higher affinity of DNA for  $Mg^{2+}$ ; others (Braunlin et al., 1992; Nordmeier, 1995) show slight DNA selectivity for  $Ca^{2+}$  ( $K_c^{Ca_{Mg}} = 1.1-1.2$ ; Kuznetsov et al., 1984). From the results obtained in water solutions, it is clear that  $K_c^{Ca_{Mg}}$  is quite close to unity.

Available experimental data show that sulfonic groups of ion exchange resins bind  $K^+$  from  $K^+/Na^+$  mixtures preferentially, with the ion exchange equilibrium constant,  $K_c^{K_{Na}}$ , equal to  $\sim 2$  in water (Nordmeier, 1995; Soldatov and Bichkova, 1988). In methanol/water and ethanol/water mixtures the values of  $K_c^{K_{Na}}$  are much higher than in water (Fessler and Strobel, 1963).  $^{23}Na$  NMR relaxation studies of DNA solutions show small selectivity of DNA for  $K^+$  in comparison with  $Na^+$  (Bleam et al., 1980; Paulsen et al., 1988). On the contrary, equilibrium dialysis studies show that the DNA affinity for  $Na^+$  is slightly higher than for  $K^+$  (Nordmeier, 1995; Strauss et al., 1967). In our previous paper, we have found that DNA in oriented fibers equilibrated with NaCl/KCl ethanol/water mixtures does not sep-

arate  $K^+$  and  $Na^+$ . Values of  $K_c^{K_{Na}}$  are close to 1.0 under all conditions we have studied (Korolev et al., 1999).

In this paper we report the results of experimental studies of oriented DNA fibers equilibrated in ethanol/water solutions with different combinations of KCl, NaCl,  $MgCl_2$ , and  $CaCl_2$  at varying concentrations of ethanol and salts. We have obtained the relative thermodynamic affinities of these alkali and alkali earth metal ions for fibrous DNA by means of direct experimental determination of the concentrations of the ions in the DNA fibers and in the solvent. Within this approach, even small differences between  $Ca^{2+}$  and  $Mg^{2+}$  or  $K^+$  and  $Na^+$ , as well as details of divalent-monovalent ion competition, are clearly seen under conditions of reduced water activity, low dielectric constant, and small volume available for the competing ions between the DNA polyions separated from each other by only a few Ångströms. We compare our experimental data with the results of grand canonical Monte Carlo (GCMC) simulations of the system of parallel, hexagonally packed polyanions of different structures, namely uniformly and discretely charged polyions with density and spatial distribution of charged groups modeling B DNA (Fig. 1). Our data reveal that the Monte Carlo approach within the approximation of a dielectric continuum model can qualitatively and to some extent quantitatively explain the selectivity of DNA for the counterions studied. Quantitative agreement between the experimental and simulation data has been observed for the M2/M1 competition if the DNA polyion is modeled as a system of discrete charges arranged around an impenetrable cylinder. The competition of counterions of similar charge ( $Na^+$  with  $K^+$  or  $Ca^{2+}$  with  $Mg^{2+}$ ) gave qualitative agreement between the GCMC and experimental data. Based on a comparison of the theoretical and experimental results, we draw some general conclusions concerning the ion binding properties of nucleic acids in vivo and in vitro.

The manuscript is organized as follows:

In the Material and Methods section, details of the experimental ion exchange procedures and the theoretical grand canonical Monte Carlo simulation method are reported.

In the first part of the Results section, we present the data of the idealized GCMC calculations for the system of hexagonally packed polyions in mixtures of divalent cations with univalent counterions and coions.

In the second part of the Results, we present experimental ion exchange data and compare these with the estimations of the GCMC method.

In the Discussion, the possibilities and limitations of the Monte Carlo simulation approach as well as some implications of our data for the biologically relevant systems are discussed.

## MATERIALS AND METHODS

High-molecular-weight salmon testes NaDNA (Fluka Chemie AG, Buchs, Switzerland) was used without further purification. Ethanol (99.5% v/v) was purchased from Kemetyl AB (Stockholm, Sweden). All ethanol concentrations quoted in this paper are given as a percentage by volume.

Analytical-grade  $MgCl_2$  and  $CaCl_2$  were purchased from Riedel-de-Haen AG (Seelze, Germany), and NaCl and KCl were from Merck KGaA (Darmstadt, Germany). Ultrapure KCl and spectrapure NaCl from Alfa (Johnson Matthey GmbH, Karlsruhe, Germany) were used for preparations of ionization buffers in atom absorption spectroscopy (AAS) analyses.

Samples of highly oriented NaDNA fibers for ion exchange, x-ray diffraction, and mechanochemical studies were prepared by a wet spinning technique (Rupprecht, 1970). The conditions of wet spinning preparation of samples for ion exchange experiments were similar to those applied in the sample preparations for solid-state NMR experiments (Song et al., 1997). The resulting samples had accurate orientation of the DNA polymeric chains, as shown by rotor-synchronized 2D magic angle spinning (MAS)  $^{31}P$  NMR measurements and by x-ray diffraction (Song et al., 1997).

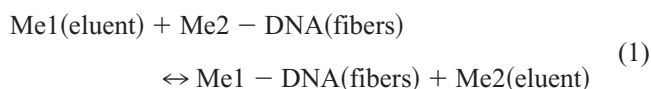
## Ion exchange measurements

Samples for ion exchange experiments were obtained by cutting pieces from a fiber bundle (length of the bundle  $\sim 200$  mm, width 6–8 mm, thickness 0.04–0.08 mm; these dimensions were for an air-dried sample). Each sample contained  $\sim 2$ –4 mg DNA. After cutting, the samples were transferred to a 65% ethanol/water mixture containing 10 mM NaCl and equilibrated in this mixture for at least 2 weeks. Then four to eight pieces of DNA fibers were transferred to the tubes with solvent (eluent) of the same ethanol concentration but containing a mixture of magnesium and/or calcium and potassium and/or sodium chlorides. The concentration of  $Mg^{2+}$ ,  $Ca^{2+}$ , or the sum of equal amounts of these ions was kept constant (4 mM), and the concentration of KCl, NaCl, or KCl + NaCl (the ratio K/Na was equal to 1:1) was varied from 3 to 300 mM. After 2 days of equilibrating, two to four samples of DNA fibers were separated and transferred to the eluents with lower ethanol concentration (52%) but with the same salt content and concentration. This precaution was due to the solubility of NaDNA in 52% ethanol/water mixtures. To avoid dissolution of the DNA fibers, these should be initially saturated with divalent cations at higher ethanol concentration. We have chosen 52% as the lower limit of the ethanol concentration because this concentration is reported to be the minimum for keeping MgDNA fibers resistant to the dissolution in the eluent (Schultz et al., 1994).

The volume of eluent above the DNA samples was  $\sim 40$  ml, and the amount of cation in the solvent was in great excess over the quantity of phosphate groups in the DNA fibers. Equilibrium concentrations of the ions in the DNA fibers and in the eluent were reached by changing solvent once a day for 7–10 days. Analysis of the last fraction of eluent in contact with DNA fibers was carried out, and the composition of this fraction never differed from that of the stock eluent.

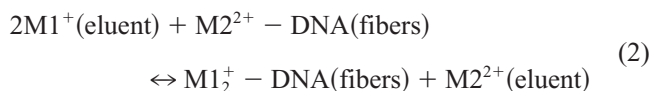
When NaDNA fibers come in contact with the eluent containing a mixture of cations, substitution of  $Na^+$  for a mixture of these cations begins. The composition of counterions neutralizing the phosphate groups of DNA depends on the thermodynamic affinity of DNA for these ions under given conditions (temperature, solvent composition, concentrations, and relative amounts of ions in eluent). Two types of competitive reactions can be distinguished:

1. Competition between the cations of same charge:



where Me1 and Me2 are, respectively,  $K^+$  and  $Na^+$  or  $Ca^{2+}$  and  $Mg^{2+}$ .

2. Competition between ionic species differing in charge:



Here  $M1^+$  is  $Na^+$  and/or  $K^+$ , and  $M2^{2+}$  is  $Ca^{2+}$  and/or  $Mg^{2+}$ .

Equilibrium constants for the first reaction (Eq. 1) are

$$K_c^{\text{Me1}}_{\text{Me2}} = ([C_{\text{Me1}}]/[C_{\text{Me2}}]) \cdot (C_{\text{Me2}}/C_{\text{Me1}}) \quad (3)$$

and

$$\begin{aligned} K_a^{\text{Me1}}_{\text{Me2}} &= [C_{\text{Me1}}][C_{\text{Me2}}] \cdot (a_{\text{Me1}}/a_{\text{Me2}}) \\ &= K_c^{\text{Me1}}_{\text{Me2}} \cdot (\gamma_{\text{Me2}}/\gamma_{\text{Me1}}) \end{aligned} \quad (4)$$

Here  $K_c^{\text{Me1}}_{\text{Me2}}$  is the apparent equilibrium or selectivity constant, and  $K_a^{\text{Me1}}_{\text{Me2}}$  is the so-called corrected selectivity constant, because it is obtained after correction of  $K_c^{\text{Me1}}_{\text{Me2}}$  for the preference of the solution phase;  $[C_{\text{Me1}}]$ ,  $[C_{\text{Me2}}]$ ,  $C_{\text{Me1}}$ , and  $C_{\text{Me2}}$  are, respectively, the concentrations of Me1 and Me2 in the DNA fibers and in the eluent;  $a_{\text{Me1}}$ ,  $a_{\text{Me2}}$  and  $\gamma_{\text{Me1}}$ ,  $\gamma_{\text{Me2}}$  are the activities and activity coefficients of Me1 and Me2 in the eluent.

The numerical value of the equilibrium constant for the second reaction (Eq. 2) depends on the choice of units used in determining the ion concentrations. To compare our data with those available in the literature, we express cation concentrations in molar parts with the apparent equilibrium constant ( $D_c^1$ ) of reaction 2:

$$D_c^1 = (p_{M1}^b/p_{M1}^f)^2 \cdot (p_{M2}^f/p_{M2}^b) \quad (5)$$

Here  $p_{M1}^b$ ,  $p_{M2}^b$  and  $p_{M1}^f$ ,  $p_{M2}^f$  are molar fractions of bound (found in analysis of DNA fibers) and free (determined in eluent) cations  $M1^+$  and  $M2^{2+}$ , respectively. We use the sum of the molar fractions of monovalent and divalent cations when, respectively, both  $Na^+$  and  $K^+$  or  $Ca^{2+}$  and  $Mg^{2+}$  are present in the system.

The ion exchange equilibrium constant calculated with Eq. 5 differs from the definition of the competitive parameter  $D$ , which was proposed to measure the selectivity of DNA-counterion interactions from  $^{23}Na$  NMR relaxation studies (Bleam et al., 1980). This difference is due to the fact that the  $D$  values are based on the calculated relative amounts of counterions located in the close vicinity of the polyion, whereas in the determination of  $K_c^{\text{K}_M}$  and  $K_a^{\text{K}_M}$  the total quantities of counterions in the DNA fiber phase are used. However, the separation between the DNA polyions in condensed ordered fibers is very small, and thus every ion within the "DNA phase" can be considered as bound to the nearest polyion.

The amounts of ions in the samples of DNA fibers after the ion exchange procedure have been determined as follows.

The DNA samples were quickly removed from the solvent, slightly pressed between sheets of weighing papers, weighed, and dried over phosphorus anhydride. The dried DNA samples were weighed again (to find the content of the volatile components) and dissolved in bidistilled water. The concentrations of  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Na^+$ , and  $K^+$  in these DNA solutions were determined with a PU9100 AAS Spectrometer (Phillips Scientific, Cambridge, England). To eliminate interference from the phosphate groups of DNA on the atomic absorption data, 0.2% (w/v) solutions of ultrapure potassium (as KCl) or sodium (as NaCl) solutions were used for the preparation of probes and standard solutions. The DNA concentrations,  $C_p$ , in the probes were determined by UV absorption measurements of acid-hydrolyzed DNA solutions. Other details of our ion exchange technique have been reported elsewhere (Korolev et al., 1999).

## The grand canonical Monte Carlo simulation method

### The model

In our theoretical calculations of ion exchange properties, we have used two models that represent the DNA polyion in solution. In the simplest model (cylindrical polyion), DNA was considered as an infinitely long uniformly charged hard cylinder of radius  $a = 9.5$  Å and reduced linear charge density  $\xi = l_b/b$ , where  $b = 1.7$  Å is the length of the cylinder corresponding to the unit charge for B DNA and  $l_b = e^2/\epsilon kT$  is the Bjerrum length ( $l_b = 7.13$  Å for water at room temperature). In the simulation



program, we put negative unit charges on the polyion axis, so that they are separated by the distance  $b$  from each other (CB model in Fig. 1). Because  $b \ll a$  and because of the Gauss theorem, such a charge distribution is equivalent to an equilibrium distribution of charge over the polyion surface. The radius  $a$  limits the area that is impenetrable for the centers of the smallest ion species. The larger ions of  $i$ th type cannot penetrate closer to the polyion axis than  $a_i = a + (\sigma_i - \sigma_{\min})$ , where  $\sigma_i$  and  $\sigma_{\min}$  are radii of the  $i$ th and smallest ion species, respectively.

The other model (helical polyion) was devised to incorporate effects of the helical DNA grooves and the discrete charge localization on the DNA surface. This model has been used in previous papers (Lyubartsev and Nordenskiöld, 1995, 1997). The model has a hard cylindrical core of radius  $a = 8 \text{ \AA}$  for B-DNA (HB model in Fig. 1) and charged “phosphate groups” situated at the sites corresponding to the B form of DNA. The value  $a = 8 \text{ \AA}$  determines the distance of closest approach for the centers of all of the small ions in the simulation cell. Each phosphate group has a charge  $-e$  and a soft repulsive  $r^{-12}$  potential of effective radius  $\sigma_- = 2 \text{ \AA}$ . This set of phosphate groups forms an idealized model of double-stranded DNA with two grooves (model HB in Fig. 1). Because of the repulsive short-range interactions, the average effective radius of DNA is  $\sim 10 \text{ \AA}$  for the B form. Unlike in our previous study (Korolev et al., 1999), where we also tested DNA polyion models representing the A form, we restricted ourselves in this study to modeling only B-DNA. The reason is that according to experimental data (Rupprecht et al., 1991, 1994), Na-, K-, Mg-, and CaDNA are in the B form in fibers equilibrated with 65% and 52% EtOH.

In all of the cases the ions are modeled as soft charged spheres of effective radius  $\sigma_i$ . The ions interact with each other and with the phosphate groups of DNA by the potential

$$V_{ij} = \frac{z_i z_j e^2}{4\pi\epsilon_0 \epsilon r_{ij}} + kT \left( \frac{\sigma_i + \sigma_j}{r_{ij}} \right)^{12} \quad (6)$$

(The first term is the Coulomb interaction of charges, and the second is the “soft spheres” repulsion.) The specific choice of ion radius  $\sigma_i$  for each ion type is discussed below.

### Simulation details

The standard grand canonical Monte Carlo (GCMC) method (Valleau and Cohen, 1980) was employed for the simulation and performed as in our previous work (Lyubartsev and Nordenskiöld, 1997) for the situation of an ordered DNA phase in equilibrium with a bulk salt solution. In the present study, we have calculated the activity coefficients for water solutions of  $CaCl_2 + NaCl$  and received a good agreement with experimental values (Butler, 1968).

Because during the simulations the system should be kept electroneutral, steps including insertion or deletion of ions must be performed with pairs of ions of opposite charge (Lyubartsev and Nordenskiöld, 1995; Valleau and Cohen, 1980). Normally, in a dense polyion system, the

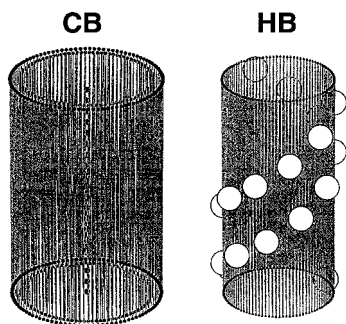


FIGURE 1 Models of the B-DNA polyion. CB is a model representing a uniformly charged cylinder; HB is a model of the simplified helical polyion.

number of counterions in the simulation cell greatly exceeds the number of coions; thus exchange between different counterion species is hindered by the fact that coions must also be involved in this process also. To facilitate the exchange between different species of counterions, we have introduced another kind of Monte Carlo step, in which one counterion is replaced by an ion of another type but of the same valency. This kind of step, which also follows the rules for the grand canonical ensemble, greatly accelerates the process of approaching thermodynamic equilibrium between different ions of the same charge and is important for modeling ion exchange processes.

The height of the simulation cell was taken as corresponding to three full turns of DNA (102  $\text{\AA}$  for B-DNA) in our calculations within the HB model, and the size of the cell in the perpendicular direction was taken as corresponding to the distance between DNA helices in fibers,  $R = 25 \text{ \AA}$  (Schultz et al., 1994). When performing calculations with the CB model, we have added 2  $\text{\AA}$  to this value to account for the extra space in the DNA grooves. The total number of simulated ions varied from 100 to 400, depending on the salt concentration and the number of polyions in the simulation cell. Additional simulation details are given in our previous work (Lyubartsev and Nordenskiöld, 1995).

### Dielectric constant

The most common assumption used in polyelectrolyte theories is that the solvent can be approximated as a dielectric continuum. An alternative molecular description of the solvent around a polyion demands a tremendous increase in computer resources, which has only recently become available for systems of reasonable size. Usually the dielectric constant,  $\epsilon$ , of the pure solvent is used and water is the solvent in the vast majority of calculations. We have used values of  $\epsilon$  determined in ethanol/water mixtures at 20°C:  $\epsilon = 53$  and 45 for 52% and 65% EtOH, respectively (Åkerlöf, 1932). In a previous paper (Korolev et al., 1999) we discussed the correctness of this approximation and showed that it can produce qualitative agreement between experimental and GCMC simulation results for ordered DNA fiber systems.

### Ionic radii

A natural consequence of the dielectric continuum approximation is the introduction of “effective” radii of small ions and charged groups of the polyion to account for solvation effects. Because the equilibrium concentrations of ions in the DNA fibers are defined by thermodynamic relationships, it is a natural choice to define the effective radii of ions used in our computations by their thermodynamic properties. Values of monovalent ion radii obtained by fitting osmotic and activity coefficients, calculated within the MSA (mean spherical approximation) theory, to the corresponding experimental values (Fawcett and Tikanen, 1996; Simonin et al., 1996) were used in our work. Actually, only the best-fitting values of cation-anion separation are reported by Simonin et al. (1996). To evaluate the radii ( $\sigma$ ) of individual ions, we have assumed equal hydration of  $K^+$  and  $Cl^-$  in KCl solutions and obtained the following values of  $\sigma$  for  $Na^+$ ,  $K^+$ , and  $Cl^-$ : 1.88, 1.62, and 2.00  $\text{\AA}$ , respectively. We did not find literature data for  $Ca^{2+}$  and  $Mg^{2+}$  of a degree of reliability similar to that of univalent cations. For this reason, we have used the value  $\sigma = 3.00 \text{ \AA}$  as an estimation of the radius of the hydrated magnesium ion. According to the data of experimental (Black and Cowan, 1994) and molecular dynamics (MD) studies (MacKerell, 1997; York et al., 1992),  $Mg^{2+}$  binds to the nucleic acids (RNA and DNA), usually as a fully hydrated cation. To evaluate the ionic radius of  $Ca^{2+}$ , we performed a series of calculations modeling ion exchange equilibrium between DNA fibers and eluent with  $\epsilon = 45$  (65% EtOH) and concentrations of  $CaCl_2$  and  $MgCl_2$  equal to 2 mM each ( $C_{KCl}^0 = 10 \text{ mM}$ ). We varied the value of the  $Ca^{2+}$  ion radius at fixed values of the radii of the other ions (1.62  $\text{\AA}$  for  $K^+$ , 2.00  $\text{\AA}$  for  $Cl^-$ , and 3.00  $\text{\AA}$  for  $Mg^{2+}$ ). At  $\sigma = 2.60 \text{ \AA}$  for  $Ca^{2+}$ , the magnitude of  $K_c^{Ca_{Mg}}$  calculated with the GCMC method with the HB model for the DNA polyion is equal to the experimentally determined value ( $K_c^{Ca_{Mg}} = 2.0 - 2.1$ ). This value of the  $Ca^{2+}$  ion radius adjusted for one experimental point

is then used in all other calculations simulating experimental conditions with different ethanol and salt concentrations.

We have also used values of ionic radii obtained earlier by MSA calculations without correction for the  $\epsilon$  change (Triolo et al., 1976) and Stokes' radii (Robinson and Stokes, 1965). These two sets of ionic sizes gave much higher values of the ion exchange constants, with larger deviations compared to the experimental data than obtained for the metal ion radii listed above. Therefore, we did not report values of ion exchange equilibrium constants calculated with parameters taken from Robinson and Stokes (1965) and Triolo et al. (1976).

The existence of high local concentrations of phosphate groups and counterions in the vicinity of the DNA macromolecules allows us to consider the DNA fiber phase to be similar to a concentrated electrolyte solution. Data of MD studies show that in concentrated electrolyte solutions ( $C_s \approx 2$  M), cations and anions create dynamic ion pairs, mostly of two sorts: contact anion-cation and solvent-separated anion-water-cation (Lyubartsev and Laaksonen, 1997). Consequently, the closest distance between neighboring ions of opposite charges lies somewhere between the values characteristic for these two sorts of ionic pairing, i.e., between the sum of their crystallographic radii and this sum plus the diameter of a water molecule. The above-cited values of  $\sigma_+ + \sigma_-$  lie just within this interval.

We have used ionic sizes determined in water solutions. However, the degrees of ionic solvation can differ in ethanol/water mixtures. Nevertheless, our choice of ionic sizes should be reasonably correct, taking into account the fact that DNA fibers should be considerably enriched with water. Thus water is expected to be the dominant solvating ligand in the "DNA phase" (Korolev et al., 1999).

## RESULTS

### GCMC simulation results for model systems

To evaluate the possibilities of the GCMC model in describing the competition between ions differing in charge and size, and to check the influence of the dielectric constant on the ions' distribution functions around the polyion, we have performed GCMC calculations for some model systems. In our model calculations, the dielectric constant,  $\epsilon$ , is either 45 (65% EtOH) or 80.1 ( $H_2O$  at 20°C); the DNA-DNA distance,  $R$ , is 50 Å (it corresponds to the DNA concentration,  $C_p = 450$  mM); the concentration of  $M2^{2+}$  in the bulk phase is constant and equal to  $C_{2+}^o = 4$  mM; and the concentration of  $M1^+$  is varied between  $C_{+}^o = 10$  and 300 mM (a superscript o means that the value refers to the eluent phase). We have studied the ion distribution functions for different sizes of the divalent counterion,  $M2^{2+}$  (the radius of  $M2^{2+}$ ,  $\sigma_{2+}$ , is either 1 or 3 Å) and the univalent coion,  $A^-$  ( $\sigma_-$  is equal to 2 or 5 Å). The radius of  $M1^+$  was  $\sigma_+ = 2$  Å in all model calculations.

#### RDF of counterions

In Fig. 2, radial (cylindrically averaged) distribution functions (RDFs) for  $C_{+}^o = 10$  mM (Fig. 2, *A* and *C*) and  $C_{+}^o = 300$  mM (Fig. 2, *B* and *D*) are shown. The size of the anion has little influence on the RDF of mono- and divalent cations. For the HB model of the polyion ( $\epsilon = 45$ ,  $C_{2+}^o = 4$  mM, and  $\sigma_{2+} = 1$  Å), the bulkier monovalent cations ( $\sigma_+ = 2$  Å) are not capable of pushing out small divalent counterions from the polyion; the increase in the  $M1^+$

concentration in the bulk phase has only a small influence on the divalent cation's RDF. An increase in the dielectric constant ( $\epsilon = 80.1$ ) leads to a 2.0–2.5 times decrease in the  $M2^{2+}$  concentration in the vicinity of the polyion ( $r \approx 8$ – $10$  Å) and to a higher concentration of  $M2^{2+}$  at  $r > 15$  Å (for  $\sigma_{2+} = 3$  Å; *lines* in Fig. 2, *A* and *B*). A bulkier divalent cation ( $\sigma_{2+} = 3$  Å) is substituted for the monovalent cation ( $\sigma_+ = 2$  Å) with an increase in  $C_{+}^o$ ; the  $M2^{2+}$  concentration at  $r$  between 8 and 13 Å decreases from 1–2 M to 0.2–0.4 M (*open circles* in Fig. 2, *A* and *B*, respectively). An increase in the cation size also leads to a change in the RDF shape: two maxima (near the polyion surface and at  $r = 11$ – $13$  Å) can be seen in the RDF curves. Qualitatively, similar RDF curves have recently been obtained in molecular dynamics simulations for NaDNA oligomers in water (Lyubartsev and Laaksonen, 1998; Young et al., 1997). Our data confirm a conclusion drawn by Montoro and Abascal (1995) that the simplified presentation of the polyion as a cylinder with spherical charges is a satisfactory approximation for describing the electrostatic polyion-small ion interactions.

In the case of small ( $\sigma_{2+} = 1$  Å) divalent ions and at  $C_{+}^o = 10$  mM monovalent cations are almost completely removed from the surface of the polyion; the concentration of  $M2^{2+}$  at  $r = 8$ – $10$  Å is 100 or more times higher than the local  $M1^+$  concentration. However, when the monovalent ion concentration is high ( $C_{+}^o = 300$  mM), the concentration of  $M1^+$  is large, even if the divalent cation is small (*filled circles* in Fig. 2 *D*). The increase in the  $M2^{2+}$  radius ( $\sigma_{2+} = 3$  Å) improves the competitive ability of the monovalent cation: at  $C_{+}^o = 10$  mM,  $C_{+} = 0.2$  M at  $r = 8$ – $10$  Å, which is 10 times higher than for  $\sigma_{2+} = 1$  Å, although still 5–10 times less than the concentration of  $M2^{2+}$  in the same region (*open circles* in Fig. 2, *A* and *C*). An increase in the dielectric constant has a relatively small influence on the RDF of  $M1^+$  (*lines* in Fig. 2, *C* and *D*).

Our simulations show that for mono- and divalent counterions with radii close to those of hydrated ions, and for the discretely charged polyion model (HB model in Fig. 1), the RDF of the cations has two maxima, which correspond to the accumulation of the ions in the minor groove of the B-DNA and near the vertexes of the phosphate groups. These results also give some justification for the "two-state" approximation used in the well-known counterion condensation theory (Manning, 1996) or in interpretations of NMR counterion relaxation measurements (which postulates a division of the counterions into two classes, condensed on the polyion and "free"). The RDF of cations calculated for the HB model has two distinct areas: 1) the 4–6-Å layer of rather high concentration near the polyion and 2) all other cations at  $r > 12$ – $14$  Å. In the uniformly charged cylinder approximation (CB model in Fig. 1), the RDF is just decaying with increasing the distance, and it is impossible to find such a distinct border that separates the condensed and free counterions.

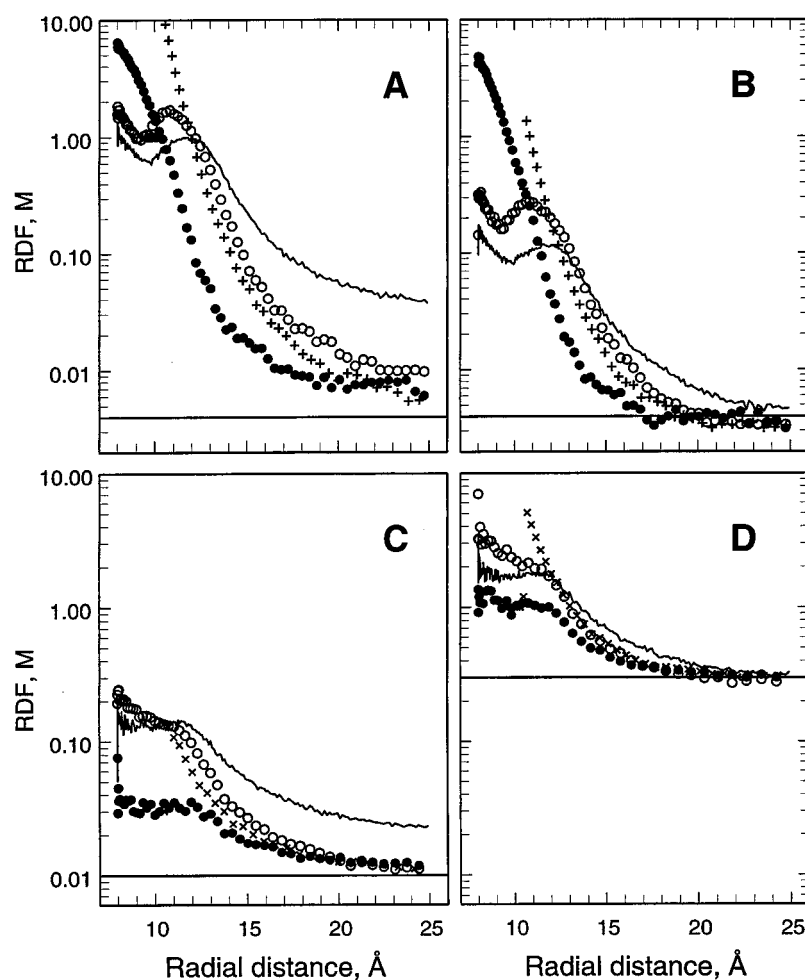


FIGURE 2 RDF of the divalent (*A* and *B*) and monovalent (*C* and *D*) counterions calculated with the GCMC method for the concentration of 1-1 salt in the bulk phase 10 (*A* and *C*) or 300 mM (*B* and *D*) and constant (4 mM) concentration of the divalent cation. Points are for dielectric constant,  $\epsilon = 45$  (65% EtOH at 20°C), lines are for  $\epsilon = 80.1$  (H<sub>2</sub>O at 20°C); the DNA-DNA distance is 50 Å ( $C_p = 450$  mM); if not specified, the HB model of the polyion is used. The radius of monovalent cation  $\sigma_+ = 2$  Å; the radii of divalent cation ( $\sigma_{2+}$ ) and monovalent anion ( $\sigma_-$ ) are varied: ●, ×,  $\sigma_{2+} = 1$  Å,  $\sigma_- = 2$  Å; ○, +, and lines,  $\sigma_{2+} = 3$  Å,  $\sigma_- = 5$  Å; + and ×, CB model. Horizontal bars in the graphs are drawn to show the bulk concentration of ions. Note the logarithmic scale of the ordinate.

*RDFs of coions*

The RDFs of the coions are shown in Fig. 3. The most interesting feature of these curves is the maximum at  $r = 11-13$  Å (*crosses* and *filled circles* in Fig. 3 *A*). At this maximum, the concentration of anions exceeds the bulk value by two to three times ( $C_{-}^o = 18$  mM). This maximum appears for small sizes of the divalent counterions ( $\sigma_{2+} = 1$  Å) and can be explained by ion-ion correlations. For the uniformly charged cylinder (CB model), the effect is much more pronounced than for the HB model. Analysis of the angular distribution shows that coions form a compact cloud, which is concentrated around the areas of the minor groove and near the vertexes of phosphate groups, in the region where also most of the  $M1^+$  and  $M2^{2+}$  ions are accumulated. An increase in the 1-1 salt concentration (at constant  $C_{2+}^o = 4$  mM) leads to a decrease in the anion accumulation near the polyion, but the maximum on the corresponding RDF is still observed at  $C_{+}^o = 100$  mM (data not shown). The maximum (obtained for  $\sigma_{2+} = 1$  Å,  $\sigma_- = 2$  Å, and  $\epsilon = 45$ ) disappears only at  $C_{+}^o = 300$  mM (*filled circles* in Fig. 3 *B*). The RDFs of coions are in agreement with the results on the mobile ion distributions obtained by the MC and the hypernetted chain methods

(Bacquet and Rossky, 1988) and by the modified Poisson-Boltzmann approach (Das et al., 1997).

The clear maxima on the coions' RDFs are observed only for small values of the dielectric constant and for small radii of the coions and counterions; under other conditions (i.e., at  $\epsilon = 80.1$  and for  $\sigma_{2+} = 3$  Å), the maximum on the coion RDF is either absent or insignificant. The coion radial and angular distributions are strongly dependent on the dielectric permittivity of the solvent. In Fig. 3, *A* and *B*, RDF curves calculated for  $\sigma_- = 2$  Å and  $\sigma_{2+} = 3$  Å (the radii close to the size of hydrated ions) are shown for  $\epsilon = 45$  (*crosses*) and  $\epsilon = 80.1$  (*lines*). One can see that a lower dielectric constant makes anions come closer to the polyion surface. An increase in the coion radius results in a shift of the coions from the polyion: at  $\epsilon = 45$  and  $\sigma_- = 5$  Å, the steep increase in the coion concentration begins 4–6 Å further away from the polyion axis than for the anions with  $\sigma_- = 2$  Å.

Large anions cannot be accumulated near the polyion, and this leads to stronger neutralization of the negative charge from the polyion by the counterions. Under certain conditions (e.g., high concentration of salt in the eluent) this may even cause "overneutralization." Thus, for  $\sigma_- = 5$  Å,

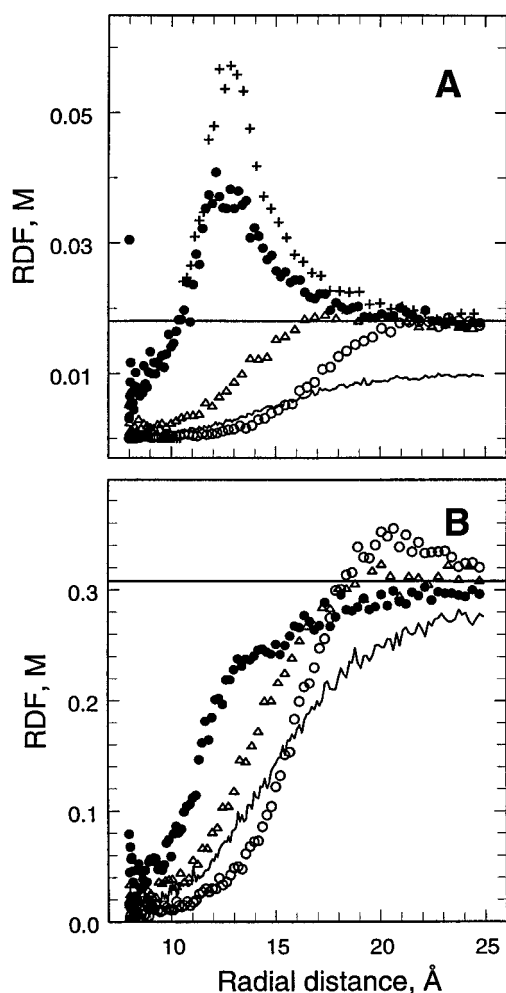


FIGURE 3 RDF of the monovalent coions calculated with the GCMC method for the concentration of the 1-1 salt in the bulk phase: (A) 10 mM or (B) 300 mM and constant (4 mM) concentration of the divalent cation. Points are for dielectric constant,  $\epsilon = 45$  (65% EtOH at 20°C), lines are for  $\epsilon = 80.1$  (H<sub>2</sub>O at 20°C). The DNA-DNA distance is 50 Å ( $C_p = 450$  mM); if not specified, the HB model of the polyion is used. Radius of the monovalent cation  $\sigma_+ = 2$  Å; the radii of divalent cation ( $\sigma_{2+}$ ) and monovalent anion ( $\sigma_-$ ) are varied: ●, +,  $\sigma_{2+} = 1$  Å,  $\sigma_- = 2$  Å; △, —,  $\sigma_{2+} = 3$  Å,  $\sigma_- = 2$  Å; ○,  $\sigma_{2+} = 3$  Å,  $\sigma_- = 5$  Å; +, CB model. Horizontal bars in the graphs are drawn to show the bulk concentration of ions.

the electrostatic potential changes its sign at  $r \approx 11$ –20 Å (this distance varies, depending on the salt concentration, radius of  $M2^{2+}$ , and the polyion model). The effect of potential inversion also correlates with the appearance of the maximum on the coion RDF. This effect was also observed in other works (Lyubartsev and Nordenskiöld, 1997; Montoro and Abascal, 1995).

A salt accumulation effect in the DNA ordered phase (overequivalent sorption) has also been found both in experiment and in the model GCMC calculation in our previous paper (Korolev et al., 1999). This effect is below the limit of experimental error at low salt concentration in the eluent. However, at high concentrations of salts ( $C_+^o > 100$  mM), the experimental data definitely show that the amount

of salts in the DNA fibers is several times higher than that calculated by the GCMC method for DNA-DNA distances characteristic for oriented fibers. Results of the present study demonstrate some overequivalent sorption of KCl and/or NaCl only for  $C_+^o = 300$  mM (data not shown).

#### Modeling ion exchange

In Fig. 4 we show an example of the RDF curves obtained in mimicking the ion exchange equilibrium between DNA fibers and eluent (65% EtOH with a mixture of MgCl<sub>2</sub> and CaCl<sub>2</sub>, 2 mM of each salt, and the following concentrations of NaCl and KCl: 5 mM of each salt (Fig. 4 A) and 150 mM of each salt (Fig. 4 B); the choice of specific ion radii for K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> is discussed in Materials and Methods). An increase in the 1-1 salt concentration in the eluent leads the divalent counterions being pushed out of the ordered phase and to their replacement by the monovalent ions. The RDFs of K<sup>+</sup> and Na<sup>+</sup> do not differ significantly. These ions can penetrate the minor groove of B-DNA; the second maximum on their RDF (at  $r = 10$ –11 Å) is weakly shaped. The difference between the Ca<sup>2+</sup> and Mg<sup>2+</sup> curves is more noticeable: the larger Mg<sup>2+</sup> ion penetrates the minor

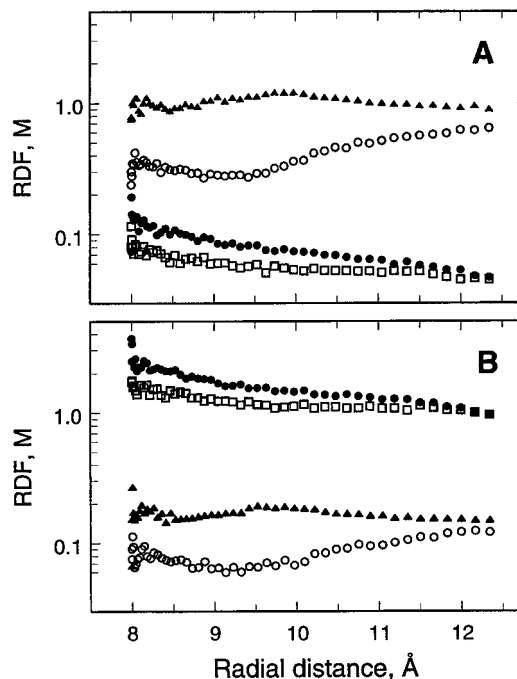


FIGURE 4 RDF of divalent (Ca<sup>2+</sup> and Mg<sup>2+</sup>) and monovalent (K<sup>+</sup> and Na<sup>+</sup>) counterions calculated with the GCMC method, modeling the conditions of the ion exchange experiment. The concentration of the 1-1 salt (NaCl + KCl, K/Na = 1/1) in the bulk phase is 10 mM (A) or 300 mM (B); the concentration of MgCl<sub>2</sub> + CaCl<sub>2</sub> (Ca/Mg = 1/1) is constant (4 mM). Ions are marked as follows: ▲, Ca<sup>2+</sup>, ionic radius,  $\sigma = 2.6$  Å; ○, Mg<sup>2+</sup>,  $\sigma = 3.0$  Å; ●, Na<sup>+</sup>,  $\sigma = 1.88$  Å; □, K<sup>+</sup>,  $\sigma = 1.62$  Å. Other parameters are as follows: HB model of the DNA polyion; DNA-DNA distance 25 Å; dielectric constant  $\epsilon = 45$  (65% EtOH at 20°C). Note the inversion of the monovalent/divalent cation RDF with the increase in the bulk concentration of the 1-1 salt and logarithmic scale of the ordinate.



groove to a lesser extent than does Ca<sup>2+</sup>, which is the major reason for the higher selectivity of DNA (modeled as a HB polyion) for Ca<sup>2+</sup>.

The GCMC simulation procedure conforms ideally with the modeling of the ion exchange experiments because the number of ions in the simulation cell is automatically adjusted to the specified value of the chemical potential, which in turn is linked to the concentration of ions in the bulk phase. Integration of the ions' RDF over the whole cell volume gives the total amount of ions of each type. This quantity can be directly compared with the experimentally determined amount of ions in the DNA fibers.

### Comparison of the ion exchange and GCMC simulation results

In this section we compare experimental and theoretical results on the competition between K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup> for binding to DNA in oriented DNA fibers. In experimental studies the oriented fibers of DNA are in equilibrium with ethanol/water mixtures (65% and 52% EtOH). The concentration of MgCl<sub>2</sub> and/or CaCl<sub>2</sub> in the eluent is constant and is equal to 4 mM, while the concentration of potassium and/or sodium chlorides is varied between 3 and 300 mM. In cases where both Ca<sup>2+</sup> and Mg<sup>2+</sup> or K<sup>+</sup> and Na<sup>+</sup> are present in the eluents simultaneously, the ratios  $C_{K^+}^o/C_{Na^+}^o$  and  $C_{Mg^{2+}}^o/C_{Ca^{2+}}^o$  are 1:1.

Experimental results of the ion exchange measurements are summarized in Table 1.

### Competition between monovalent and divalent ions

The ion exchange data (*points*) on the competition of monovalent ions (Na<sup>+</sup>, K<sup>+</sup>, or Na<sup>+</sup> + K<sup>+</sup>) with the divalent ones (Mg<sup>2+</sup>, Ca<sup>2+</sup>, or Ca<sup>2+</sup> + Mg<sup>2+</sup>) are compared with the GCMC simulation results (*curves*) in Figs. 5 and 6. In Fig. 5, the percentage of charges from M2<sup>2+</sup> in DNA fibers is shown; in Fig. 6, the values of the ion exchange constant  $D_c^{1/2}$  calculated with Eq. 5 are displayed.

One can see from Table 1 and Fig. 5 that the real competition between monovalent and divalent ions begins after the concentration of M1<sup>+</sup> in the eluent exceeds 30 mM (at constant  $C_{2+}^o = 4$  mM). Most noticeable changes in the amounts of M2<sup>2+</sup> and M1<sup>+</sup> bound to DNA are observed at  $C_+^o$  between 50 and 300 mM. Our data are in agreement with the qualitative estimations of mono/divalent counterion competition based on the Poisson-Boltzmann equation (Rouzina and Bloomfield, 1997). For example, an estimate of the region where almost complete dominance of M2<sup>2+</sup> on DNA reverts to the full substitution of these ions for M1<sup>+</sup> yields approximately  $C_+^o = 10$ –300 mM (at  $C_{2+}^o = 4$  mM), which is borne out in the experimental data on  $P_{M2}$  (Fig. 5).

Both ion exchange and GCMC simulation data show the weak dependence of the M2<sup>2+</sup> binding degree,  $P_{M2}$ , and ion exchange constant,  $D_c^{1/2}$ , on the ethanol concentration (dielectric constant of solvent) for ethanol/water mixtures. At the same time, M2/M1 exchange equilibrium is quite sensitive to the nature of the divalent cation: Ca<sup>2+</sup> ions “agree”

**TABLE 1** Results of the experimental determination of the DNA selectivity for Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, and Na<sup>+</sup> in oriented DNA fibers

Competing ions	$C_+^o$ (mM)	$D_c^{1/2}$		Percentage of M2 <sup>2+</sup> charges on DNA, $P_{M2}$		$K_{c_{Mg}}^{Ca}$		$K_{c_{Na}}^K$	
		65% EtOH	52% EtOH	65% EtOH	52% EtOH	65% EtOH	52% EtOH	65% EtOH	52% EtOH
Ca <sup>2+</sup> , Mg <sup>2+</sup> , K <sup>+</sup>	10	0.00481	0.00553	95.4	95.2	2.08	1.58	—	—
	30	0.0102	0.0109	87.2	86.8	2.00	1.56	—	—
	100	0.0298	0.0313	61.5	59.9	1.96	1.46	—	—
	300	0.086	0.0973	21.3	20.2	1.59	1.35	—	—
Ca <sup>2+</sup> , Mg <sup>2+</sup> , Na <sup>+</sup>	10	0.00480	0.00467	95.3	95.1	1.97	1.52	—	—
	30	0.00844	0.00948	87.8	87.5	2.00	1.55	—	—
	100	0.0293	0.0351	61.1	58.3	1.83	1.31	—	—
	300	0.137	0.137	15.0	15.2	1.49	1.22	—	—
Ca <sup>2+</sup> , Mg <sup>2+</sup> , K <sup>+</sup> , Na <sup>+</sup> *	3	0.0138; —	0.00560; —	96.0; —	97.6; —	2.00; —	1.49; —	1.80; —	0.50; —
	10	0.00645; 0.00647	0.00482; 0.00675	92.9; 94.3	95.5; 94.6	1.97; 2.02	1.49; 1.54	1.50; 1.44	0.90; 0.62
	30	0.0144; 0.00959	0.0133; 0.0112	82.1; 87.2	85.5; 86.5	1.93; 2.00	1.48; 1.50	—; 1.07	0.98; 0.86
	100	0.0382; 0.0311	0.0387; 0.0364	56.5; 59.2	56.2; 57.2	1.83; 1.94	1.39; 1.35	1.30; 1.13	0.93; 0.90
K <sup>+</sup> , Na <sup>+</sup> , Mg <sup>2+</sup>	300	0.124; 0.109	0.129; 0.110	16.4; 17.5	16.4; 17.1	1.53; 1.56	1.37; 1.25	0.93; 1.03	0.92; 0.95
	10	0.00562	0.00579	95.1	95.2	—	—	0.93	0.94
	30	0.0171	0.0138	84.1	85.3	—	—	1.03	0.99
	100	0.0479	0.0490	53.2	52.7	—	—	0.93	0.91
K <sup>+</sup> , Na <sup>+</sup> , Ca <sup>2+</sup>	300	0.155	0.148	14.3	14.6	—	—	0.83	0.90
	10	0.00504	0.00408	95.1	94.6	—	—	0.70	0.72
	30	0.00758	0.00735	88.1	88.6	—	—	0.90	1.00
	100	0.0181	0.0233	67.5	63.7	—	—	0.99	0.97
300	0.0808	0.0857	21.6	19.5	—	—	0.87	0.91	

\*Results of the two series of measurements are listed.

$C_+^o$ , Total concentration of KCl and/or NaCl in the eluent;  $D_c^{1/2}$ , monovalent-divalent ion exchange equilibrium constant (Eq. 5);  $K_{c_{Mg}}^{Ca}$ , Ca/Mg ion exchange equilibrium constant (Eq. 3);  $K_{c_{Na}}^K$ , K/Na ion exchange equilibrium constant (Eq. 3).



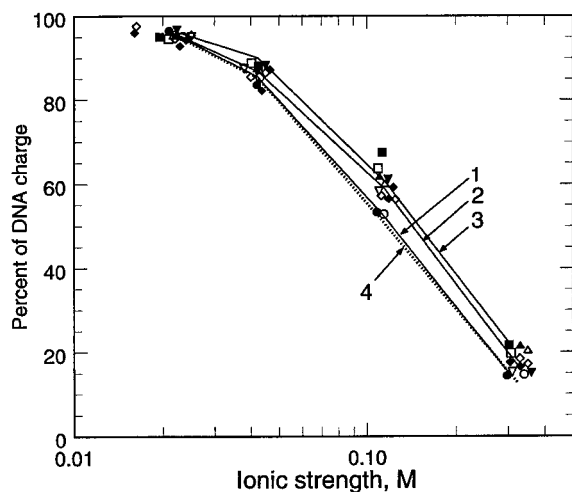


FIGURE 5 Dependencies on the ionic strength in the eluent of experimental values (points) and values calculated with the GCMC method (curves), for the charge fraction of divalent cations,  $P_{M2}$ , determined in the phase of oriented DNA. The concentration of 1-1 salt is varied from 3 to 300 mM at a constant (4 mM) concentration of the divalent cation salt. Solid points are for 65% EtOH; the open ones are for 52% EtOH in the eluent. Competing ions are as follows:  $\circ$ ,  $\bullet$  and curve 1, Mg/K/Na;  $\square$ ,  $\blacksquare$  and curve 3, Ca/K/Na;  $\triangle$ ,  $\blacktriangle$ , Ca/Mg/K;  $\nabla$ ,  $\blacktriangledown$ , Ca/Mg/Na;  $\diamond$ ,  $\blacklozenge$  and curves 2 and 4 (.....), Ca/Mg/K/Na (two series of experiments). Theoretical curves are for the dielectric constant  $\epsilon = 45$  (65% EtOH at 20°C) —, GCMC data for the HB model (curves 1-3); ....., GCMC data for the CB model (curve 4).

to be replaced by  $M1^+$  ( $K^+$  or  $Na^+$ ) to a lesser extent than  $Mg^{2+}$  ions (compare the values of  $P_{M2}$  obtained at  $C_{+}^0 = 100$  and 300 mM in the systems K/Na/Ca and K/Na/Mg). The difference in  $P_{M2}$  values is most noticeable at  $C_{+}^0 = 100$  mM:  $P_{M2}$  is equal to 53% for  $Mg^{2+}$  and 64–67% for  $Ca^{2+}$ . This means that the amount of  $Ca^{2+}$  on DNA is  $\sim 20\%$  higher than that of  $Mg^{2+}$  under conditions when about half of the DNA charge is neutralized by the monovalent ions.  $Na^+$  ions have a slightly higher efficiency in replacing  $M2^{2+}$  on DNA than  $K^+$  ions. This can be seen only at relatively high concentrations of monovalent cations in the eluent (Table 1). In addition, the  $Na^+$  ions preferentially substitute  $Ca^{2+}$  from  $Ca^{2+}/Mg^{2+}$  mixtures.

Because our data have been obtained in a broad range of 1-1 salt concentration and M1/M2 ratio, we have found that the values of  $D_c^{1,2}$  are dependent on the  $M1^+$  concentration, contrary to previous work (Braunlin et al., 1986; Paulsen et al., 1988). The fact that in previous work (Paulsen et al., 1988) values of  $D_c^{1,2}$  did not depend on concentration was most likely a result of the relatively high concentrations of  $MgCl_2$  in the NMR experiments. Under these conditions, the “real” (i.e., comparable degree of  $M1^+$  and  $M2^{2+}$  binding) competition between mono- and divalent cations does not take place, and  $Mg^{2+}$  ions are the dominant counterions in the vicinity of the DNA polyion.

Some discrepancy can be observed between theoretical curves and experimental data at low salt concentrations ( $C_{+}^0 = 3$  and 10 mM). This may be due to the uncertainty in the experimental values of  $Ca^{2+}$  and  $Mg^{2+}$  ion quantities

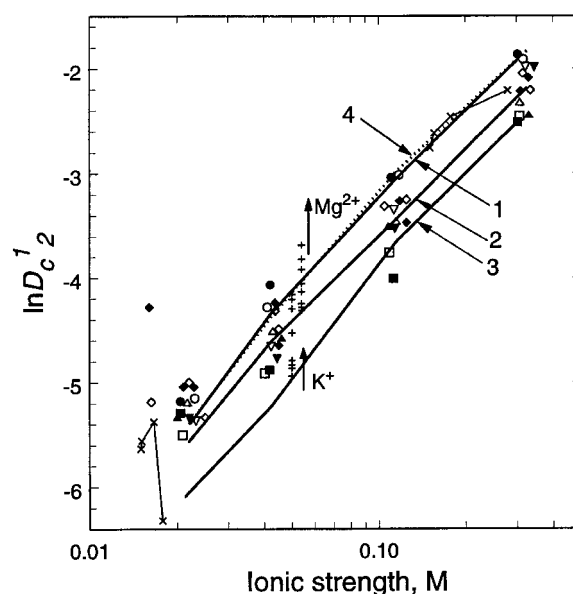


FIGURE 6 Dependencies on the ionic strength in the eluent of experimental values (points) and values calculated with the GCMC method (curves), for the monovalent-divalent cation exchange equilibrium constant,  $D_c^{1,2}$ , determined in the phase of oriented DNA. The concentration of 1-1 salt is varied from 3 to 300 mM at constant (4 mM) concentration of the divalent cation salt. Data are marked as in Fig. 5. Values of  $D_c^{1,2}$  calculated from literature data are also shown in the figure:  $\times$  with lines, theoretical results by Bacquet and Rossky (1988) (two groups of data);  $+$ , experimental data from Soldatov and Bichkova (1988).

in DNA fibers. The experimental error can be caused by the interference from DNA phosphate groups in the analyzed solutions or by small differences in viscosity between the calibration solutions and the analyzed samples in the AAS analyses. Possible underestimation of the amount of  $Ca^{2+}$  and  $Mg^{2+}$  in the DNA samples is confirmed by the fact that the total charge, calculated as a sum of the cation concentrations, is slightly (1–5%) lower than the amount of phosphate groups determined in the acid-hydrolyzed DNA fibers (this difference is observed only at a low concentration of salts in the eluent). This little misbalance, however, can substantially influence the values of  $D_c^{1,2}$  when the amount of univalent cations in the DNA is small, resulting in an overestimation of the monovalent cations' binding at low concentrations of KCl and/or NaCl.

The nature of the divalent cation ( $Ca^{2+}$  or  $Mg^{2+}$ ) is a factor that influences the  $D_c^{1,2}$  values more than other experimental variables. Magnesium ions (circles and upper curves in Figs. 5 and 6) are weaker competitors for binding to DNA with  $K^+$  and/or  $Na^+$  than  $Ca^{2+}$  (for  $Ca^{2+}$ , experimental and GCMC data are shown, respectively, as squares and lower curves in Figs. 5 and 6). For the mixtures of  $Ca^{2+}$  and  $Mg^{2+}$ , we have obtained intermediate values of  $D_c^{1,2}$  and  $P_{M2}$ .

At all concentrations,  $Na^+$  and  $K^+$  do not differ significantly in the competition with  $M2^{2+}$ . Only at  $C_{+}^0 = 300$  mM does  $Na^+$  (down triangles) push out from DNA larger

amounts of  $Ca^{2+}$  and/or  $Mg^{2+}$  than does  $K^+$  (*up triangles*). This difference is better displayed in Fig. 6 than in Fig. 5.

Some values of  $D_c^{1_2}$ , calculated from the theoretical (Bacquet and Rossky, 1988) and experimental (Soldatov and Bichkova, 1988) literature data, are also drawn in Fig. 6. Theoretical values of  $D_c^{2_1}$  reported by Paulsen et al. (1988) deviate substantially from other data and are not shown in Fig. 6. Two groups of small crosses, connected by thin lines, are the results by Bacquet and Rossky (1988) obtained in Monte Carlo and hypernetted chain calculations for a uniformly charged cylindrical polyion (the charge density is equal to that of B-DNA, counterions are point charges, and  $\epsilon = 78$ ). In these calculations counterions were considered bound to DNA if they are closer than 17.5 Å to the polyion axis. Bacquet and Rossky (1988) also performed two series of calculations for low and high ionic strengths and varying M1/M2 ratios. Despite the differences in the parameters and in the models of our work and the cited paper, the coincidence between the data can be considered as very good.

The two groups of points at ionic strength 0.05 M in Fig. 6 are the experimental results on the competition of  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $Na^+$ , and  $K^+$  for binding to the sulfonic groups of the polystyrenesulfonate ion exchange resin (Soldatov and Bichkova, 1988). The eluent is water at 25°C. Two series of measurements were carried out: in the first one, the relative amount of  $K^+$  ions in the eluent was varied at a constant Ca/Mg ratio; in the second series, the K/Na ratio was fixed and the amounts of  $Ca^{2+}$  and  $Mg^{2+}$  were varied. The tendency of  $D_c^{1_2}$  to change with increasing  $K^+$  and  $Mg^{2+}$  content in the eluent is shown in Fig. 6 by arrows, with comments near the respective group of points. Again the data for the polystyrenesulfonate ion exchange resin are in general agreement with our results, despite the difference in the chemical nature of the polyion exchange group and the conditions in the eluent. Comparing our results with literature data in more detail, one can see that the influence of the nature of the divalent cation on the  $D_c^{1_2}$  value is similar for DNA and a sulfonic ion exchanger ( $Mg^{2+}$  is a weaker competitor with M1<sup>+</sup> than  $Ca^{2+}$  is). At the same time we have not found any significant influence of the nature of the M1<sup>+</sup> cation ( $K^+$  or  $Na^+$ ) on the  $D_c^{1_2}$  values obtained for DNA fibers in ethanol/water eluents. This is in contrast with the sulfonic cation exchanger, where  $K^+$  exhibits a higher capacity than  $Na^+$  in replacing  $M2^{2+}$  from the ion exchanger phase. Furthermore, at  $C_+^o = 300$  mM, we have observed an increase in  $D_c^{1_2}$  after substituting  $K^+$  for  $Na^+$ . This means that DNA exhibits a tendency that is the opposite that of the sulfonic ion exchanger in binding  $K^+$  and  $Na^+$ .

In general, the data presented in Fig. 6 demonstrate that the binding of  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Na^+$ , and  $K^+$  to DNA has mostly electrostatic character. The only exception is that DNA (unlike sulfonic groups of cation exchange resins) has some specific nonelectrostatic binding mode in its interactions with  $Na^+$  (see K/Na Equilibria in the Discussion).

In Figs. 5 and 6, the dotted lines 4 were calculated by the GCMC method for the CB model of the DNA polyion, K/Na/Ca/Mg competition, and for  $\epsilon = 45$  (65% EtOH). The differences between the CB and HB models in the evaluation of  $D_c^{1_2}$  are clearly seen (one should compare curves 4 with the corresponding curves 2 for the HB model). The HB model reproduces experimental dependencies much better than the CB model does (corresponding experimental data are rhombi in Figs. 5 and 6).

#### Competition between $Ca^{2+}$ and $Mg^{2+}$ ions

$Ca^{2+}$  always wins in competition with  $Mg^{2+}$  for binding to DNA in oriented DNA fibers:  $K_c^{Ca_{Mg}}$  exceeds unity under all of the conditions we have studied. A decrease in the ethanol concentration results in a lowering of the  $K_c^{Ca_{Mg}}$  value (from 2.0–2.1 to 1.5–1.6 in 65% and 52% EtOH, respectively, and at low concentrations of monovalent cations). Consequently, in water the difference in binding of  $Ca^{2+}$  and  $Mg^{2+}$  to DNA is likely to be very small. Actually, some disagreement still exists on whether  $Ca^{2+}$  or  $Mg^{2+}$  has a higher affinity for DNA in water. Direct determination of  $K_c^{Ca_{Mg}}$  for DNA immobilized in a polyacrylamide gel in water gave  $K_c^{Ca_{Mg}} = 1.2$  (both from direct measurement of  $K_c^{Ca_{Mg}}$  and from comparison of  $K_c^{Ca_K}$  and  $K_c^{Mg_K}$  values) (Kuznetsov et al., 1984). In these experiments the Ca/Mg ratio was equal to 1:1. NMR relaxation measurements of the Ca/Mg competition for binding to DNA reported in a number of works (Braunlin et al., 1987, 1989; Manzini et al., 1990; Rose et al., 1982) were performed with varying Ca/Mg ratios. It is possible that the dependence of  $K_c^{Ca_{Mg}}$  on the Ca/Mg ratio could be the reason for contradictions in findings on whether  $Ca^{2+}$  or  $Mg^{2+}$  is more strongly bound to DNA; according to Rose et al. (1982),  $Mg^{2+}$  binds selectively ( $K_c^{Ca_{Mg}} < 1$ ) at high Ca/Mg ratios, but for  $Ca/Mg \ll 1$ ,  $Ca^{2+}$  is the preferentially bound cation ( $K_c^{Ca_{Mg}} > 1$ ) (Braunlin et al., 1987, 1989). In ion exchange chromatography this phenomenon is called preferential sorption of the minor component.

In Fig. 7, results of the comparison of experimental (*points*) and theoretical (*curves*) values of the DNA selectivity for  $Ca^{2+}$  and  $Mg^{2+}$  are presented. An increase in the  $Na^+$  or  $K^+$  concentration in the eluent leads to a reduction in the DNA selectivity for  $Ca^{2+}$ . Thus  $K_c^{Ca_{Mg}}$  decreases from 2.0–2.1 to 1.5–1.6 in 65% EtOH and from 1.5–1.6 to 1.2–1.4 in 52% EtOH for  $C_+^o = 3–30$  mM and 300 mM, respectively (see Table 1). This effect, caused by a change in the ratio of the  $Ca^{2+}$  and  $Mg^{2+}$  activity coefficients ( $\gamma_{Ca}/\gamma_{Mg}$ ) with increasing ionic strength, is reproduced qualitatively by the GCMC simulations. The values of the corrected selectivity coefficient,  $K_a^{Ca_{Mg}}$  (calculated with Eq. 4), remain approximately constant in the whole range of salt concentration we have simulated in the GCMC calculations. The tendency of the apparent ion exchange equilibrium constant to depend on the ionic strength is in agreement with similar dependencies that we have found in studies of the DNA selectivity for  $Li^+$ ,  $Na^+$ , and  $K^+$  in a

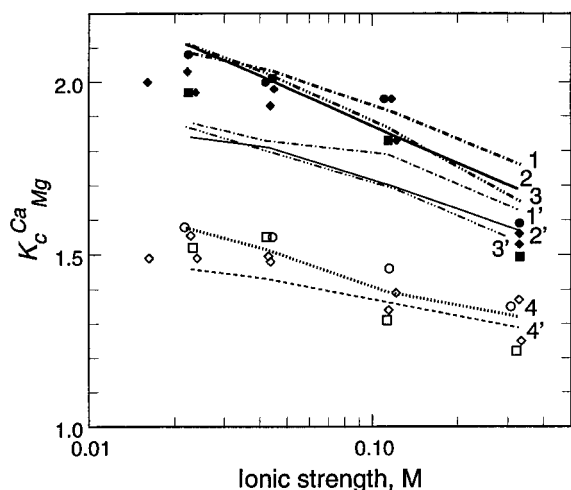


FIGURE 7 Dependencies on the ionic strength in the eluent of experimental values (points) and values calculated with the GCMC method (curves), for the Ca/Mg ion exchange equilibrium constant,  $K_c^{Ca/Mg}$ , determined in the phase of oriented DNA. The concentration of 1-1 salt is varied from 3 to 300 mM at constant (4 mM) concentration of the divalent cation salt. Solid points and thicker lines are for 65% EtOH; the open points and thinner lines are for 52% EtOH in the eluent. Competing ions are as follows:  $\circ$ ,  $\bullet$  and curves 3, 3', Ca/Mg/K;  $\square$ ,  $\blacksquare$  and curves 1, 1', Ca/Mg/Na;  $\diamond$ ,  $\blacklozenge$  and curves 2, 2', 4, 4', Ca/Mg/K/Na (two experimental series). Theoretical curves are as follows: for the HB model, curves 1-3, dielectric constant  $\epsilon = 45$  (65% EtOH at 20°C); curves 1', 2', 3',  $\epsilon = 53$  (52% EtOH at 20°C); and for the CB model, curve 4,  $\epsilon = 45$ ; curve 4',  $\epsilon = 53$ .

broad range of ethanol and salt concentrations (Korolev et al., 1999). In that work we have shown that the GCMC simulation method can correctly evaluate these ionic activities. For the case of mixtures of alkali and alkali earth metal halides in ethanol/water solutions, we could not find corresponding data on the activities of the cations. The only experimental data we are aware of (Butler, 1968) have shown satisfactory agreement (within 10%) with our GCMC calculations of activities of  $Ca^{2+}$  in a water solution of  $CaCl_2 + NaCl$  salt (data not shown).

The simulation results on  $K_c^{Ca/Mg}$  determined for  $\epsilon = 53$  deviate from the experimental values obtained in 52% EtOH. This means that the value  $\sigma_{Ca} = 2.6 \text{ \AA}$  that we use in the GCMC calculations is not a proper choice for the  $Ca^{2+}$  ion size in both 52% and 65% EtOH. It has to be remembered here that the value  $\sigma_{Ca} = 2.6 \text{ \AA}$  has been adjusted to get a  $K_c^{Ca/Mg}$  value similar to the experimental one at  $C_+^0 = 10 \text{ mM}$  and  $\epsilon = 45$ , i.e., in 65% EtOH (see Materials and Methods). It is possible that the degree of  $Ca^{2+}$  hydration in the DNA fibers equilibrated with 52% EtOH eluent is higher than that in 65% EtOH.

The GCMC simulation results also show that  $K_c^{Ca/Mg}$  is weakly dependent on the nature of the third (univalent) competing cation. At high concentration of M1Cl ( $C_+^0 > 100 \text{ mM}$ ), GCMC data show that smaller monovalent cations cause a decrease in  $K_c^{Ca/Mg}$ , i.e., the small  $M1^+$  ions may replace the smallest of the divalent cations near the DNA surface. The GCMC method gives values for  $K_c^{Ca/Mg}$

in the presence of  $Na^+$  that are higher than those in the presence of  $K^+$  (respectively, lines 1 or 1' and 3 or 3' in Fig. 7). Experimental values of  $K_c^{Ca/Mg}$  display an opposite dependence:  $Na^+$  ions replace divalent counterions more effectively (than  $K^+$ ) near the DNA at high concentrations of M1Cl in the eluent. This causes the  $D_c^{1/2}$  values to be higher in the presence of  $Na^+$  than in the presence of  $K^+$  (see Fig. 6). Our data also reveal that  $Na^+$  competes for the same specific binding sites on DNA as  $Ca^{2+}$ , and this reduces the values of  $K_c^{Ca/Mg}$  in the presence of a high concentration of NaCl in the eluent.

#### DNA selectivity for $Na^+$ and $K^+$ ions

The experimental values of  $K_c^{K/Na}$  determined in the presence of divalent cations show a significant spread in the magnitude, especially at low salt concentration in the eluents (Table 1 and Fig. 8). The uncertainty of  $K_c^{K/Na}$  is probably a result of experimental errors in the analysis of these ions at the very low contents in the DNA fibers under conditions of competition with divalent ions (see above). At the same time, the  $K_c^{K/Na}$  values found in two series of measurements in 65% EtOH indicate some DNA selectivity for  $K^+$  for all salt concentrations except  $C_+^0 = 300 \text{ mM}$  (solid rhombi in Fig. 8).

The GCMC simulation results show that within this model the identity of the divalent cation and the concentration of ethanol in the eluent do not effect the values of  $K_c^{K/Na}$ . All theoretical curves are close to each other (in Fig. 8, only the  $K_c^{K/Na}$  values calculated for four-cation competition are shown). Within experimental uncertainty the experimental values of  $K_c^{K/Na}$  also do not show any applicable

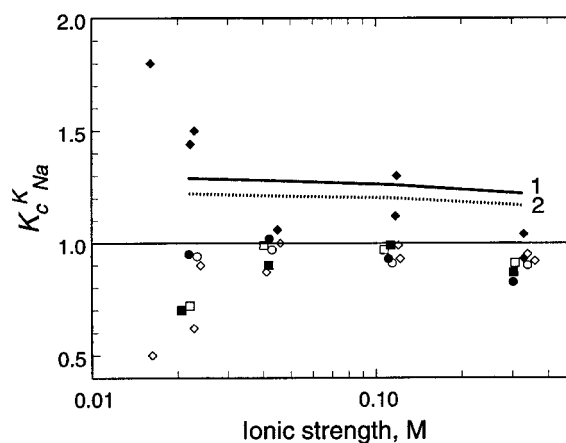


FIGURE 8 Dependencies on the ionic strength in the eluent of experimental values (points) and values calculated with the GCMC method (curves), for the K/Na ion exchange equilibrium constant,  $K_c^{K/Na}$ , determined in the phase of oriented DNA. The concentration of 1-1 salt is varied from 3 to 300 mM at constant (4 mM) concentration of the divalent cation salt. Curves and solid points are for 65% EtOH; the open points are for 52% EtOH in the eluent. Competing ions are as follows:  $\circ$ ,  $\bullet$ , Mg/K/Na;  $\square$ ,  $\blacksquare$ , Ca/K/Na;  $\diamond$ ,  $\blacklozenge$ , curves 1 and 2, Ca/Mg/K/Na (two experimental series). The theoretical curves are as follows: for the HB model, curve 1; for the CB model, curve 2. Dielectric constant  $\epsilon = 45$  (65% EtOH at 20°C).



dependence on the ethanol and  $KCl + NaCl$  concentrations or on the presence of  $Ca^{2+}$  and/or  $Mg^{2+}$ .

## DISCUSSION

### Theoretical modeling of the DNA interactions with charged ligands

The GCMC simulation approach, employing quite rough approximations for the ion exchange experiment conditions (dielectric continuum, empirical values of mobile ion radii, simplified presentation of the DNA polyion), nevertheless produces a satisfactory quantitative agreement with experimental results for the monovalent-divalent ion competition (Figs. 5 and 6). We have adjusted only a single parameter, the  $\sigma_{Ca}$  value, by fitting  $K_c^{Ca_{Mg}}$  at  $C_{+}^0 = 10$  mM in 65% EtOH for the Ca/Mg/K competition. Other ion radii were taken from the literature (see Materials and Methods). This success of the electrostatic model supports experimental (Black and Cowan, 1994; Braunlin et al., 1987, 1991) and theoretical (Jayaram and Beveridge, 1996; MacKerell, 1997; York et al., 1992) results, indicating that alkali metals, as well as  $Mg^{2+}$  and  $Ca^{2+}$  ions, interact with DNA as hydrated ions in a delocalized dynamic manner.

However, the agreement between the GCMC simulations and the ion exchange results for the description of competition between counterions of similar charge ( $K^+$  with  $Na^+$  or  $Ca^{2+}$  with  $Mg^{2+}$ ) is only qualitative. Obviously, this kind of competition is much more sensitive to the molecular details of the DNA-ion-solvent interaction. Molecular dynamics simulations are currently in progress in our laboratory. The aim is to investigate the effects of a molecular description of the water/ethanol solvent on the hydration of counterions and on the selectivity of DNA for counterion binding in the presence of competition.

### Implications of the metal ion-nucleic acid interactions in vivo

Our ion exchange measurements yield the following selectivity series of DNA for the metal ions in oriented DNA fibers:



(we have included a result for  $Li^+$  obtained in our previous paper; Korolev et al., 1999). Undoubtedly, for the systems in vivo, thermodynamic affinities of DNA for counterions can be substantially changed by interactions with numerous DNA-binding proteins, by effects of DNA bending in the nucleosome, and by a number of other factors. Our data obtained in studies of oriented DNA fibers reveal that the  $K_c^{K_{Na}}$  value is approximately constant, being independent of the ethanol content (Korolev et al., 1999) and the presence of  $Mg^{2+}$  and/or  $Ca^{2+}$  (this paper) or trivalent  $Co(NH_3)_6^{3+}$  cations (Korolev et al., manuscript in preparation). This shows that the general thermodynamic prefer-

ences in the “crude DNA phosphate” selectivity for counterions are quite “conservative.”

### Balance of divalent and monovalent cations

Our data (Table 1, Fig. 5) show that real competition (similar degrees of binding to DNA) between divalent and monovalent cations occurs just at the “physiological” concentrations of  $K^+$ ,  $Na^+$ ,  $Mg^{2+}$ , and  $Ca^{2+}$  observed in seawater or blood serum. This means that the salinity of the medium and capacity of the cell for osmotic regulation can govern the composition of inorganic cations in chromatin. It also means that  $Ca^{2+}$  can be most effectively replaced by  $K^+$  just at the concentrations of cations observed in cytosol. Our data confirm recent suggestions about the role of  $Ca^{2+}$ - $K^+$  ion exchange in an “anionic matrix” for controlled (by ionic channels) release of  $Ca^{2+}$  during Ca-dependent signaling in the cell (Nguyen et al., 1998). (One can consider the DNA in the cell nucleus as some kind of “anionic matrix.”)

For a 0.1–0.3 M concentration of monovalent cations in the eluent, the part of  $M2^{2+}$  bound to DNA corresponds roughly to the M2/M1 proportion determined in the analyses of the total content of ions in the cell. This proportion is  $\sim 160$  mM of  $K^+ + Na^+$  to 40 mM of  $Mg^{2+} + Ca^{2+}$  (the amount of ions is expressed in a mean cell concentration) (Collins, 1997). Thus, for such organisms as bacteria (where most of the polyelectrolyte charge is on nucleic acids; Record et al., 1998a, b), the  $M2^{2+}/M1^+$  ratio is quite close to the equilibrium thermodynamic value of the dominant ion exchanger.

### Differences in the binding of $Ca^{2+}$ and $Mg^{2+}$ to DNA

At a given concentration of  $K^+$  and/or  $Na^+$ , the degree of  $Mg^{2+}$  and  $Ca^{2+}$  binding to DNA depends mainly on the nature of divalent cation and is not influenced significantly by the dielectric constant of the solvent or by the nature of the monovalent cation. The type of cation ( $Ca^{2+}$  or  $Mg^{2+}$ ) is especially important when nearly half of the DNA charge is neutralized by these ions, i.e., at a “natural” concentration of salts (100–300 mM). At the same time, DNA shows weaker selectivity for  $Ca^{2+}$  in Mg/Ca mixtures compared with the carboxylic and sulfonic ion exchangers.

Charge neutralizing or nonspecific and, in particular, selective (by specialized proteins) binding of  $Ca^{2+}$  to other natural “cation exchangers” show much higher affinity for  $Ca^{2+}$  than for  $Mg^{2+}$  and monovalent cations. Combining our results with data on Ca/Mg binding to proteins, one can conclude that to compete for binding with  $Ca^{2+}$ , the DNA concentration should be very high. In this context, we doubt that DNA in vivo can influence  $Ca^{2+}$  binding by such a Ca-selective protein as calmodulin, as has been suggested recently (Dobi and Agoston, 1998).



### K/Na equilibria

The absence of a significant DNA selectivity for either  $K^+$  or  $Na^+$  ( $K_c^{K/Na} \approx 1$  under all conditions we have studied) indicates that DNA has some specific (nonelectrostatic) binding mode in its interaction with  $Na^+$  and that this mode compensates for the preferential electrostatic binding of  $K^+$  to the polyion, which is predicted by polyelectrolyte theories and confirmed in experiments on systems with "purely electrostatic" binding of ions. We also have found that in experiment,  $K_c^{K/Na}$  is usually slightly lower than 1.0 (Table 1). This result has been obtained for a K/Na ratio equal to 1:1 in the eluent. It might be possible that for  $K/Na \gg 1$  (e.g.,  $\sim 15:1$ , as in the living cell), the selectivity of DNA and RNA phosphate groups for  $Na^+$  can be substantially enhanced (as reported, for example, for carboxylic ion exchangers; Gregor et al., 1956) in accordance with the ion exchange chromatography rule of the preferential sorption of the minor component. This phenomenon could be connected with the observation that the distribution of  $K^+$  and  $Na^+$  is uneven inside the cell, with  $Na^+$  being concentrated in the nucleus (Dawson and Smith, 1986). In the cited reference it has been shown that the sequestration of  $Na^+$  from cytosol to the nucleus influences the energetics of the nutrient transport by the specialized  $Na^+$ -gradient-dependent pumps on the cell membrane.

On the whole, our experimental data and theoretical calculations reveal that DNA fibers do not possess any particular selectivity for  $K^+$  or  $Na^+$ . In this context it is of interest to consider this result in relation to the distribution of  $Na^+$  and  $K^+$  in vivo.  $Na^+$  and  $K^+$  are slightly different in ionic size and behave differently in their interaction with water molecules. These differences have been suggested to be the origin of the selective permeability of the living cell membrane (Collins, 1997; Doyle et al., 1998). If one considers a cell as a small grain of ion exchange resin, then the value of  $K_c^{K/Na}$  for this "ion exchanger" is equal to 600 or higher, when calculated from the mean concentrations of  $Na^+$  and  $K^+$  in extracellular liquids (seawater, blood serum) and intracellular fluid. Our data do not support the hypothesis (Ling, 1994) that cation exchange groups of biological polyelectrolytes can exhibit high selectivity for  $K^+$  under special conditions of the cell cytosol.

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