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Interaction of alkaline–earth metal ions with calf thymus DNA. Volume and compressibility effects in diluted aqueous solutions

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Abstract

The binding of Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} ions to calf thymus DNA in solutions has been investigated by ultrasonic and densimetric techniques. The obtained parameters, the apparent molar volume, ΦV, and the apparent molar adiabatic compressibility, ΦK_S, are very sensitive to hydration of investigated molecules. The interaction between the cations and DNA is accompanied by overlapping their hydration shells and consequently releasing the water molecules from hydration shells to bulk state. The change in the hydration is reflected in the measured parameters, ΦV and ΦK_S. The magnitude of these hydration changes is determined by the position of the cation relative to DNA atomic groups involved in the binding, and thus can characterize the structure of cation–DNA complexes. The values of the dehydration effects of the binding, $\Delta\Phi V$ and $\Delta\Phi K_S$, correspond to two direct or higher number of indirect contacts between calf thymus DNA and the cations.

Keywords: Alkaline–earth metal ions; Binding to DNA; Hydration effects; Ultrasound; Volume; Compressibility

1. Introduction

The interaction between DNA and cations is one of the most important factors determining the structural stability and biochemical activity of DNA [1–6]. To understand the mechanisms of such activity, it is necessary to define the structure of cation–DNA complexes. In spite of intensive studies in this field, there is still no clear picture of the complexes of many biologically important metal ions with DNA duplexes. It is particularly true for alkaline–earth metal ions,

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Mg^{2+} and Ca^{2+}, which are generally believed to interact non-specifically with phosphate residues of DNA. However, there are experimental evidences that the interaction process is not so simple. For example, NMR works on the Mg^{2+} and Ca^{2+} interaction with calf thymus DNA showed that at small amounts of the cations, $[Me^{2+}]/[\text{phosphate}] < 0.1$, the interaction is specific and cannot be described by simple electrostatic association [7]. The ultrasonic investigation of the Mg^{2+} interaction with DNA of different sequences revealed quite an unusual result, that the metal ion recognizes the nucleotide sequence of the DNA through its hydration shell [8]. X-Ray examination of the DNA decamer d(CGATCGATCG) [9] and dodecamer d(CGCGAATTCGCG) [10] duplexes revealed that hydrated Mg^{2+} is located in the major groove of the duplexes [11].

Another factor that influences the physical and biological properties of nucleic acids is hydration. The importance of water molecules in stability of nucleic acid duplexes is emphasized in many experimental and theoretical works [2,12–15]. The water molecules are creating an ordered structure into the minor groove of AT content B-DNA [16–19]; they create bridges between adjacent phosphate groups of A- and Z-DNA [20–23]. The hydration of double helixes depends on their nucleotide content, sequence and type [24–29]. Thus, nucleic acids are heavily and specifically hydrated as well as cations [30], and therefore, any interaction between them is accompanied by an overlapping of their hydration shells and the release of water molecules to bulk state. The magnitude of these hydration changes is determined by the actual position of the cation relative to the atomic groups at the surface of DNA and can characterize the structure of the complexes. Such hydration effects can be detected by acoustic and densimetric techniques which yield two independent thermodynamic parameters: the apparent molar volume and the apparent molar adiabatic compressibility. These parameters are very sensitive to hydration of DNA [24,25,31,32] and hydration changes that accompany DNA binding with cations [8,25,33,34]. For proper analysis of cation–DNA complexes the effect of a contact in measured parameters should be known. This value can be estimated from volume and compressibility effects of complex formation of the same cations with an organic chelating molecule with well-characterized structure of the complex. The recent paper on volume and compressibility changes in the formation of cation–EDTA complexes [35] represents such example and provides the basis for analysis of data on DNA interaction with the alkaline earth metal ions.

The present paper describes the ultrasonic, densimetric and CD experiments on Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} binding to calf thymus DNA. The binding process is accompanied by release of water molecules from hydration shells to bulk state. The hydration effects of the binding correspond to two direct contacts between DNA and the cations. From the ultrasonic titration curves the binding constants and number of binding sites on the DNA were also obtained.

2. Materials and methods

2.1. Materials

The sodium salt of calf thymus DNA and analytical grade salts ($MgCl_2$, $CaCl_2$, $SrCl_2$, $BaCl_2$) were obtained from Merck (Darmstadt, Germany). DNA was dissolved in 200 mM NaCl, 2 mM Hepes, 2 mM EDTA, pH 7.5 and sonicated in 2 min bursts with a 1-min waiting period between bursts. The sonication was performed at 2–4°C temperature under nitrogen atmosphere with a Branson sonifier. After the sonication the DNA solution was filtered through a 0.45-μm filter. The concentration of the DNA for sonication was approximately 1.5 mg/ml in a volume of 5 ml. The UV melting curves of the duplexes before and after ultrasound treatment show no significant change in melting behavior. Agarose gel electrophoresis showed that the length of this sonicated DNA was in the range of 500–3000 base pairs. DNA then was dialyzed against 2 mM Hepes, pH 7.5 for 3–4 days at 4°C. The concentration of DNA in nucleotide units was determined optically using a molar extinction coefficient 6550 M^{-1} cm^{-1} at 260 nm in the buffer

100 mM NaCl, 10 mM Na–Hepes, pH 7.5 at 20°C. The amount of water in the solid salts were determined by measuring ultrasonic velocities in the aqueous salt solutions at 25°C and then comparing the value with literature data [36]. The concentration of calf thymus DNA in all experiments was 2.6 mM/nucleotide unit.

2.2. Absorption and circular dichroism measurements

Absorption spectra were obtained with a HP 8452A spectrophotometer and circular dichroism (CD) spectra with a Jasco J-600 spectropolarimeter using a quartz cuvette with a 0.05-cm path length.

2.3. Ultrasound velocity measurements

Relative ultrasonic velocity measurements were made by the resonator method [37–39] at 7–8 MHz frequency with cells of 0.8 cm^3 volume with a built-in magnetic stirrer [39]. To avoid the influence of temperature fluctuations on ultrasonic velocity, a differential method was used in which two identical cells (reference and sample) were connected to a common thermostat. The molar increment of ultrasonic velocity, A, for a dilute solution with the solute concentration, C, was calculated using the equation:

$$A = (U - U_o)/(U_o C) \quad (1)$$

where U and U_o are the ultrasonic velocities in the solution and solvent, respectively. The relative experimental error in the measurement of $(U - U_o)/U_o$ was 2×10^{-5}%.

Acoustic titration experiments were performed by adding salt solutions to the DNA solutions in the sample cell. Stirring was performed directly in the sample cell with a built-in stirrer [39]. In order to estimate the net effects of the interactions of divalent ions with the DNA, a parallel titration of the buffer in the same sample cell was carried out. The change of molar increment of ultrasonic velocity, ΔA, accompanying the interaction of Me^{2+} with the DNA was calculated using the equation $\Delta A = A - A_o$, where A is the molar increment of ultrasonic velocity of the DNA + Me^{2+} solution relative to buffer + Me^{2+}, and A_o is the molar increment of ultrasonic velocity of Me^{2+}-free DNA solution relative to buffer.

2.4. Calculating the binding parameters

The acoustic titration curves of Me^{2+} binding to DNA were used to obtain the number of binding sites, n, and the binding constants, K. The fitting was based on the model with non-cooperative one type of binding sites, which is similar to standard Scatchard procedure [40]:

$$\upsilon/[Me^{2+}]_{free} = K\,(n - \upsilon) \quad (2)$$

where υ is the number of Me^{2+} ions bound to DNA per nucleotide unit and is described by the equation: $\upsilon = \Delta A_n/\Delta A$. In this equation ΔA_n is the change of ultrasound velocity increment during titration of DNA by Me^{2+}, and ΔA is the change of ultrasound velocity increment at saturation level.

2.5. Density measurements

The density of solutions was measured with a DMA-602 densimeter (Anton Paar, Graz, Austria) using a 0.2 cm^3 cell. As in the case of the acoustic measurements, a differential system consisting of two cells was employed. The apparent molar volume, ΦV, was calculated using the equation [30]:

$$\Phi V = M/\rho_o - (\rho - \rho_o)/(\rho_o C) \quad (3)$$

where ρ_o and ρ are the density of the solvent and solution, respectively, and M is the molecular mass of DNA per nucleotide unit. The volume changes, ΔV, accompanying Me^{2+} binding to the DNA were calculated using the equation:

$$\Delta V = (m_1\rho_1 + m_2\rho_2 - m_{12}\rho_{12})/(m_1\rho_1 C_1) \quad (4)$$

where m_1 and m_2 are the masses of components 1 (DNA) and 2 (Me^{2+}); $m_1 + m_2 = m_{12}$; ρ_1, ρ_2 and ρ_{12} are the densities of component 1, compo-

nent 2 and their mixture. The mixtures of the DNA with metal chlorides were made in microcentrifuge tubes by weighing.

2.6. Determination of the apparent molar adiabatic compressibility

The molar adiabatic compressibility, ΦK_S, is determined as the function of increment of ultrasonic velocity and apparent molar volume [41,42]:

$$\Phi K_s = 2\beta_o(\Phi V - A - M/2\rho_o) \quad (5)$$

where β_o is the adiabatic compressibility coefficient of the solvent. The value of β_o was calculated from our data on density, ρ_o, and the ultrasonic velocity, U_o, in the solvent using the equation $\beta_o = (\rho_o\ U_o^2)^{-1}$. The change of molar adiabatic compressibility, ΔK, was calculated from ΔV and ΔA according to the relationship:

$$\Delta K = 2\beta_o\ (\Delta V - \Delta A) \quad (6)$$

2.7. Molecular interpretation of ΦV and ΦK_S values

The following relationships can represent the apparent molar volume, ΦV, and the apparent molar adiabatic compressibility, ΦK_S [43]:

$$\Phi V = V_m + \Delta V_h \quad (7)$$

$$\Phi K_s = K_m + \Delta K_h \quad (8)$$

where V_m and K_m are the intrinsic molar volumes of a solute molecule that is inaccessible to the surrounding solvent, and the intrinsic molar compressibility of this volume, respectively. ΔV_h represents the hydration contribution and consists of the volume change of water around the solute molecule as a result of the solute–water interactions, and the void volume between the solute molecule and the surrounding water. ΔK_h is the hydration contribution to the apparent molar adiabatic compressibility, consisting of the changes in the compressibility of water around the solute molecule and the compressibility of the voids between the solute molecules and the surrounding water. The values of V_m and K_m for DNA duplexes are small relative to their hydration terms, ΔV_h and ΔK_h. Moreover, the calculations of intrinsic molar volumes for A- and B-DNA demonstrated that even this significant rearrangement in the three-dimensional structure of the double helix has invisible effects on its intrinsic volume and intrinsic compressibility [25]. Since our CD experiments show no significant changes in B-conformation due to Me^{2+} binding to calf thymus DNA (Fig. 1 and Section 3), the volume and compressibility changes due to the binding can be represented as

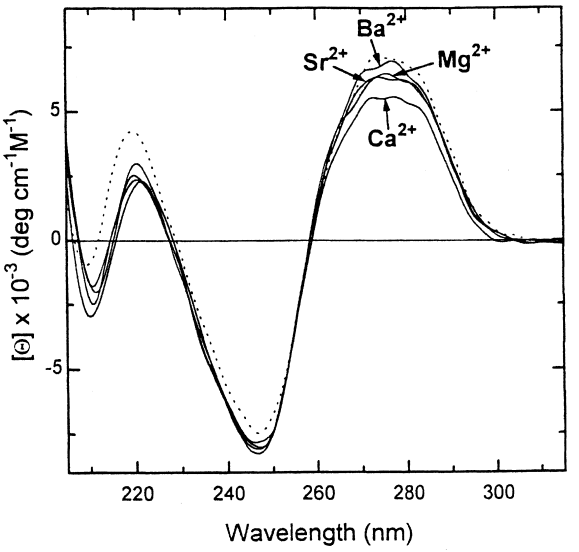

Fig. 1. CD profiles of DNA before (dashed line) and after adding the alkaline–earth metal ions. The concentration ratios for all cations are the same, $[Me^{2+}]/[DNA] = 077$. Buffer: 2 mM Hepes, pH 7.5 at 20°C.

$$\Delta V = \Delta\Delta V_h \quad (9)$$

$$\Delta K_s = \Delta\Delta K_h \quad (10)$$

3. Results

3.1. CD spectra

Fig. 1 demonstrates the influence of the alka-

line–earth metal ions upon the CD spectrum of DNA. As in case of density measurements Me^{2+}–DNA mixtures were measured at $[Me^{2+}]/[DNA] = 0.77$. All cations act in a qualitatively similar way, the positive CD band of approximately 275 nm is diminished while the amplitude of the negative signal of approximately 245 nm is increased. The changes in the negative CD band are relatively small. These CD effects are similar to ones obtained earlier by different investigators at low concentrations of alkaline metal ions and alkaline–earth metal ions [44–46].

3.2. Concentration dependence of calf thymus DNA

In the present work, experiments on cation binding to DNA are accompanied by 10–15% dilution of DNA solutions. It is important to see whether the dilution alters the A, ΦV and ΦK_S values of the DNA and consequently influence resulting hydration effects, ΔV and ΔK_S. It should be emphasized that we discuss here the concentration dependence of A, ΦV and ΦK_S values and not the actual drop in DNA concentration due to titration. The latter one, of course, is taken into account in Eqs. (1) and (3). The concentration dependencies of A and ΦV of calf thymus DNA were examined and are shown in Fig. 2a,b. From these results, according to Eq. (5), the concentration dependence of ΦK_S was calculated (Fig. 2c). No effects were seen arising from the change in DNA concentration from 0.2 to 13 mM/nucleotide. Therefore, the obtained volume and compressibility effects of cation binding to DNA, ΔV and ΔK_S, do not need any corrections due to decreases in DNA concentration. The absolute values of A, ΦV and ΦK_S are 59.5 cm^3 mol^{-1}, 182.5 cm^3 mol^{-1} and -38.8×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively, which are in good agreement with previous measurements on natural DNA duplexes under similar experimental conditions [47].

3.3. Acoustic experiments of Me^{2+} binding to calf thymus DNA

The acoustic titration curves are shown in Fig. 3. The shapes of the titrations are similar: increasing the Me^{2+} concentration is accompanied by the decrease in ΔA value. The decrease is the result of the exchange of sodium ions for alkaline–earth metal ions in the ionic atmosphere of the DNA. This exchange process is accompanied by overlapping the hydration shells of the cations and DNA and subsequently by the release of

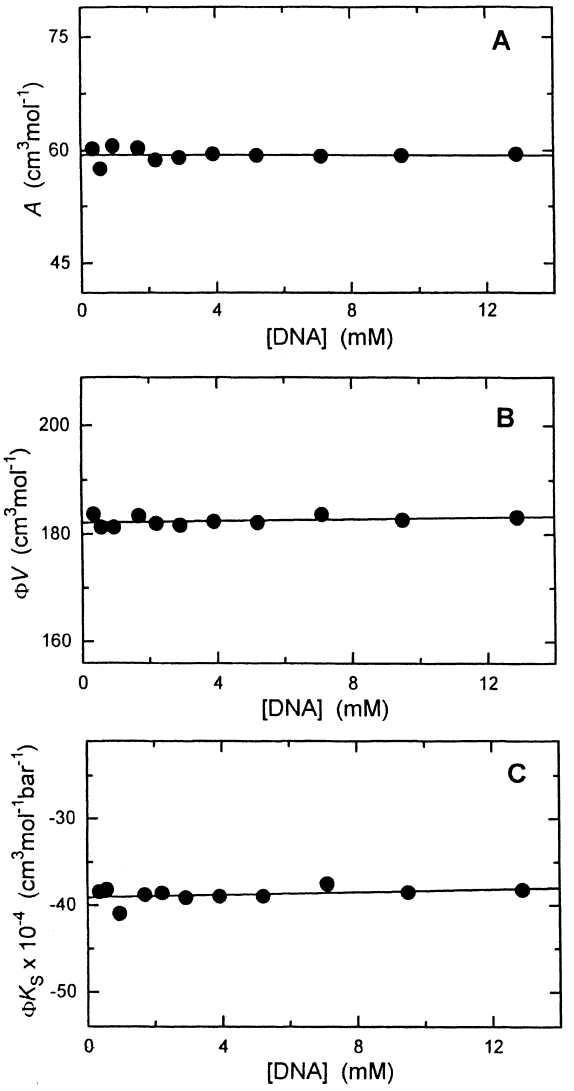

Fig. 2. The increment of ultrasound velocity (A), apparent molar volume (B) and apparent molar adiabatic compressibility (C) of calf thymus DNA as a function of DNA concentration. The concentration of DNA is indicated per nucleotide. Buffer: 2 mM Hepes, pH 7.5 at 20°C.

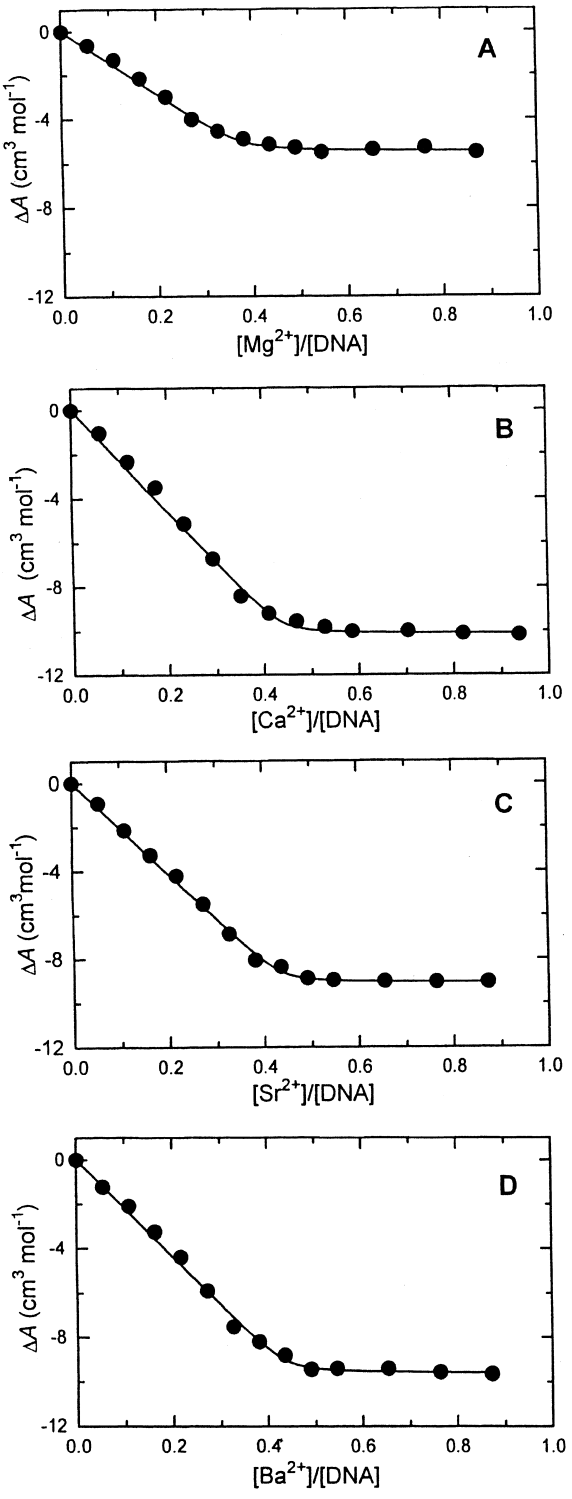

Fig. 3. Ultrasonic titration curves of DNA with alkaline–earth metal ions in 2 mM Hepes, pH 7.5 at 20°C.

water molecules from the hydration shells [8,25,34]. Introduction of additional metal ion to DNA solution ($[Me^{2+}]/[DNA] > 0.5$) does not induce any change in ΔA value, indicating the saturation of DNA by the cation.

The acoustic titration curves can be considered as Me^{2+}–DNA binding isotherms and one can obtain the information on binding parameters: association constants, K; number of binding places, n; and also overall change in the increment of ultrasound velocity, ΔA. For this reason we used a non-linear fitting procedure based on the model with non-cooperative one type of binding sites [see Eq. (2)]. The results of the fitting are listed in Table 1. The binding constants, K, are in the range of $(1–3) \times 10^5$ M^{-1}. They are slightly less than the binding constants predicted by Manning, 2×10^6 (see Eq. 57 in [1]). The number of binding places, $n = 0.37–0.44$, are somewhat lower than $n = 0.5$, the value expected from simple electrostatic interaction. However, $n < 0.5$ were obtained in earlier studies on Me^{2+} interaction with different nucleic acids [48]. For example, Clement et al. reported n between 0.4 and 0.5 from centrifugation and potentiometric data on Mg^{2+} binding to calf thymus DNA at low ionic strength 1–5 mM NaCl [44]. It should be noted that the careful examination of acoustic titration curves reveals that the decrease of ΔA value in Fig. 3 is not homogeneous. In the region $[Me^{2+}]/[DNA] = 0.2–0.4$ the decrease gets more profound than before. However, it must be recognized that the non-linear fitting procedure with one type of binding sites describes the titration curves quite well (solid lines in Fig. 3) and therefore, we did not analyze this slight diversity of the acoustic curves.

The binding of Mg^{2+} to calf thymus DNA is characterized by $\Delta A = -5.5$ cm^3 mol^{-1}. Our previous investigation of Mg^{2+} binding to the calf thymus DNA in 20–30 mM CsCl and at 1.2°C has shown similar data, $\Delta A = -4.7$ cm^3 mol^{-1} [25]. The present value is in excellent agreement with this study, keeping in mind that the measurements were performed under different conditions. The binding effects in the increment of ultrasound velocity for Ca^{2+}, Sr^{2+} and Ba^{2+} ions are close to each other and are almost twice as high than for the Mg^{2+} ion: $-(9–10)$ cm^3 mol^{-1}.

Table 1
The binding parameters obtained from the acoustic titration curve

Me^{2+}	n	$K \times 10^{-5}$ M^{-1}	ΔA (cm^3 mol^{-1})
Mg^{2+}	0.37	1.0	−5.5
Ca^{2+}	0.44	3.0	−10.1
Sr^{2+}	0.44	2.9	−9.1
Ba^{2+}	0.44	2.4	−9.6

3.4. Volume and compressibility effects of Me^{2+} binding to calf thymus DNA

The volume and compressibility changes of Me^{2+} binding to the DNA are listed in Table 2. These effects are positive, indicating the release of water molecules from hydration shells to bulk state. It should be mentioned that the water molecules occupy less space and are less compressible in the hydration shell then in the bulk state and therefore the effects are positive.

The volume changes are calculated from density measurements of Me^{2+} and DNA solutions and their mixtures according to Eq. (4). The density of the mixtures were measured at $[Me^{2+}]/[DNA] = 0.77$, which corresponds to saturation level from the ultrasonic titration curves (see Fig. 3). The compressibility effects were calculated from ΔV and ΔA values according to Eq. (6). The volume effects, ΔV, are similar for all metal ions, ~ 10 cm^3 mol^{-1} calculated per mole of phosphate. To our knowledge there are only two studies on volume changes of Mg^{2+} binding to DNA. Both of these works used tetramethylamonium (TMA) salts of DNA. Measurements were done at different concentrations of TMA, 0.001 M [44] and 0.2 M [49]. The volume effects at high salt concentration are much smaller, $\Delta V =$ 3–4 cm^3 mol^{-1} [49], then at lower ionic strength, $\Delta V = 10$ cm^3 mol^{-1} [44]. The latter result is in excellent agreement with our data, which was done at low sodium concentration. This observation demonstrates that the volume effect of Mg^{2+} binding to DNA does not depend on the type of initial counterion (TMA^+ or Na^+), but strongly depends on its concentration.

4. Discussion

Hydration contributions in the apparent molar volume and apparent molar compressibility, ΔV_h and ΔK_h, are mainly determined by the first coordination layer of water molecules around solute molecules [32,50,51]. Therefore, any visible dehydration effect in volume or compressibility due to Me^{2+} binding to DNA should involve the release of water molecules from the first hydration shells of the interacting molecules. Initially, one can assume that all effects in molar adiabatic compressibility correspond only to direct contacts, and in that case, the number of the direct contacts, N_c, can be obtained from the following equation:

$$\Delta K_{Me} = N \Delta K_c \tag{11}$$

where ΔK_c is the dehydration effect of a direct contact between DNA and Me^{2+}, and can be estimated from the similar investigations on Me^{2+} binding to EDTA. This molecule has six chelating atomic groups: four negatively-charged oxygens and two polar nitrogens. These atomic groups are

Table 2
Volume and compressibility effects of Me^{2+} binding to the calf thymus DNA

Me^{2+}	Per mole of nucleotide		Per mole of Me^{2+}	
	ΔV (cm^3 mol^{-1})	$\Delta K \times 10^4$ (cm^3 mol^{-1} bar^{-1})	ΔV_{Me} (cm^3 mol^{-1})	$\Delta K_{Me} \times 10^4$ (cm^3 mol^{-1} bar^{-1})
Mg^{2+}	9.9 ± 0.6	14.0 ± 1	26.5	37.5
Ca^{2+}	9.7 ± 0.6	18.0 ± 1	22.2	41.1
Sr^{2+}	9.4 ± 0.6	16.9 ± 1	21.5	38.7
Ba^{2+}	9.6 ± 0.6	17.5 ± 1	21.9	40.0

similar to peripheral residues of DNA, phosphate oxygens and nucleic base nitrogens, which are most plausible binding sites for divalent cation [2]. The structures of Me^{2+}–EDTA complexes are thoroughly investigated in both solutions and crystals (for references see [35]). These studies suggest that the Me^{2+}–EDTA complexes packed in penta- and hexa-coordinated structures with direct contacts between Me^{2+} and EDTA. The recent volume–compressibility study on the binding of that Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} to EDTA revealed that the compressibility change due to a direct contact, ΔK_c, is $\sim 20 \times 10^{-4}$ cm^3 mol^{-1} bar^{-1}, for all metal ions. Using these values in Eq. (11) reveals that the dehydration effects of Me^{2+} binding to DNA correspond roughly to two direct contacts for all alkaline–earth metal ions, however, one cannot exclude the larger amount of indirect contacts (outer-sphere complexes).

As it was mentioned earlier the volume effects of binding, calculated per mole of phosphate, is almost the same for all four metal ions. The compressibility changes accompanied by Ca^{2+}, Sr^{2+} and Ba^{2+} binding to DNA are also very close to each other, while for Mg^{2+} it is significantly less (see Table 2). These results reveal that the ratio $\Delta V/\Delta K$ for the magnesium ion is equal to 0.71×10^4 bar and significantly differ from the same parameter for other alkaline–earth metal ions, $\Delta V/\Delta K = 0.55 \times 10^4$ bar. What kind of information can be derived from this data? The ratio of volume and compressibility values, defined as k, were used to characterize the water molecules in the hydration shells around different molecules [35,52–55]. In the case of the apparent molar volume and the apparent molar adiabatic compressibility the k value is characteristic to water uptaken into hydration shells of dissolved molecules, while in the case of some association or dissociation processes the ratio of the changes in volume and compressibility is characteristic to hydrated water molecules involved in these processes. The k values for different classes of molecules or the processes are shown in Fig. 4. The smallest k values are characteristic to the ions and ion pair formations, $k = (0.3–0.4) \times 10^4$ bar [35,53,54]. Non-charged molecules with some hydrophobic atomic groups are characterized by

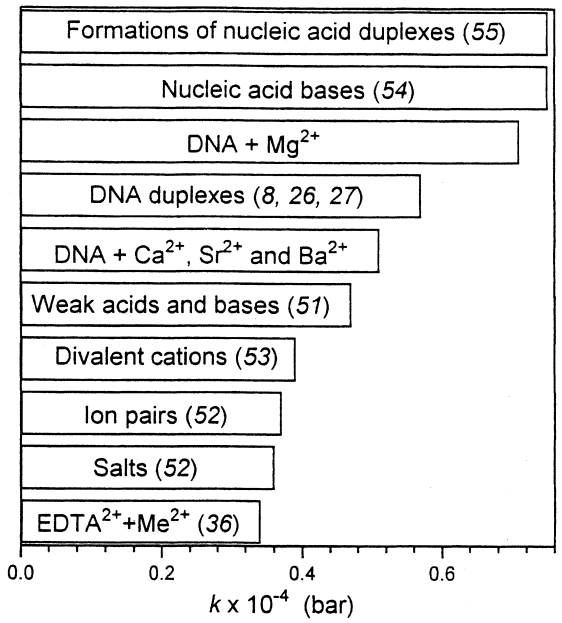

Fig. 4. Ratio of hydration contributions to volume and compressibility, k. See text for details.

higher k values. For instance, $k = 0.75 \times 10^4$ bar for nucleic acid bases [55] and the process of nucleic acid duplex formation, which is accompanied by uptake of water molecules across nucleic bases in the grooves [34].

The present k value of Mg^{2+} binding to DNA is equal to 0.71×10^4 bar, which can be indicative to dehydration only of DNA polar atomic groups. The k values for Ca^{2+}, Sr^{2+} and Ba^{2+} binding to DNA, $(0.54–0.56) \times 10^4$ bar, lie between those of charged molecules and nucleic bases (Fig. 4), indicating the dehydration of the cations in addition to DNA atomic groups. Thus, from the present volume and compressibility effects one can assume that Mg^{2+} binds to DNA in an outer-sphere manner, while larger cations, Ca^{2+}, Sr^{2+} and Ba^{2+}, bind to DNA in an inner-sphere manner. Pörschke [48] has found that Mg^{2+} does not form an inner sphere complex with single helical poly(A). This was done by measuring the rate constants of Mg^{2+} binding to the polynucleotide and then comparing with the rate constant of the water dissociation from the hydration shell of Mg^{2+}, which precedes the formation of an inner sphere complexing. In the case of Mg^{2+} the rate

constant of the binding to poly(A) was higher than the rate constant of the water dissociation, indicating on outer sphere complex. In case of Ca^{2+} these two rates were similar [48], which can be understood as formation of inner sphere complex between poly(A) and Ca^{2+}. Outer sphere complexes for Mg^{2+}–DNA also were suggested from ^{31}P-NMR [56] and crystallographic studies [11]. The existence of outer sphere complex for Mg^{2+} and inner sphere complex for Ca^{2+}, Sr^{2+} and Ba^{2+} can be correlated with hydration energies of the cations [57,58]. Gibbs free energies of hydration decrease with increasing the cation radius and are equal to −1830, −1505, −1380, and −1250 kJ mol^{-1} for Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+}, respectively [57]. Dividing of the data on the typical coordination number for the cations reveals that 305 kJ energy is necessary to strip off a part of hydration shell of Mg^{2+} to create one direct contact, while for other metal ions the energetical barrier is significantly lower, 156–188 kJ. One should also point out the recent study of CGCGAATTCGCG, GGCGAATTCGCG and GCGAATTCGCG crystals in the presence of Mg^{2+} and Ca^{2+} ions [59]. This work reveals that Mg^{2+} binds to the oligonucleotides in an outer-sphere manner, while Ca^{2+} is engaged in more inner-sphere complexes [59].

At the end, we would like to take closer look at CD data (see Fig. 1 and Section 3.1). The influence of the cations on the CD spectrum of DNA is different and the following rule is observed: the influence decreases with increase of the cation radius (Fig. 5). Mg^{2+} is an exception, which is tempting to associate with the distinct nature of the Mg^{2+}–DNA complex (outer sphere against inner sphere for Ca^{2+}, Sr^{2+} and Ba^{2+}). Keeping in mind, that most plausible binding places for alkaline–earth metal ions are in the major groove between N7 of purines and phosphates [9,10,46,60,61], the following explanation might be suggested for the observation: the binding of cations between phosphate oxygens and atomic groups of the nucleic bases is accompanied by attracting the atomic groups toward the cations. The attraction is accompanied by shortening the distance between phosphate groups and the bottom of the major groove, which induces the change

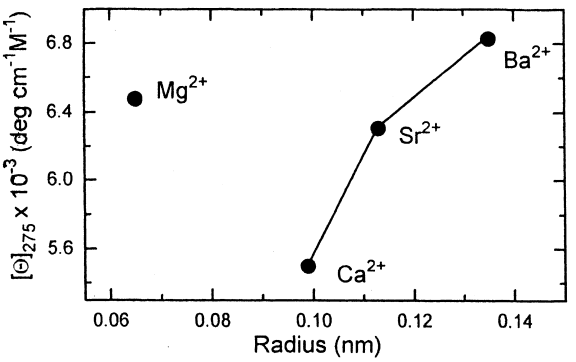

Fig. 5. Ellipticity of calf thymus DNA at 275 nm, $[\Theta]_{275}$, as a function of cation radius.

in CD spectra of DNA [45]. The larger cations occupy more space in the binding distance and consequently induce less structural changes of DNA, while in the case of Mg^{2+} the distance is increased by the diameter of an extra water molecule.

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