Mg^{2+} Recognizes the Sequence of DNA through Its Hydration Shell

Vitaly A. Buckin,[†] B. I. Kankiya,[‡] Dionisios Rentzeperis,[§,∥] and Luis A. Marky*[,§]

Contribution from the Max-Planck-Institute for Biophysical Chemistry, D-37077 Goettingen, Germany, Scientific Center of Radiobiology and Radiation Ecology, Georgian Academy of Sciences, Tbilisi 380003, Republic of Georgia, and Department of Chemistry, New York University, New York, New York 10003

Received January 21, 1994[®]

Abstract: We have studied the interaction of Mg^{2+} with six deoxyoctanucleotide duplexes of known sequence. Specifically, we have measured the resulting hydration changes by following the change in the concentration increment of ultrasonic velocity, δA, of each of these six duplexes, in their Cs^+ salt at 1.2 °C, during a course of a titration with Mg^{2+}. The addition of Mg^{2+} results in the initial lowering of δA that levels off at $[Mg^{2+}]/[P_i]$ molar ratios ranging from 12 to 30, depending on the duplex, and corresponds to a dehydration event from the exchange of Cs^+ counterions by Mg^{2+} in the ionic atmosphere of the duplexes. This is followed by a further lowering of δA at higher $[Mg^{2+}]/[P_i]$ ratios that may result from DNA aggregation and/or conformational change. We obtained a change in the molar concentration increment of ultrasonic velocity per mole of bound Mg^{2+}, $\Delta A_{Mg^{2+}}$, and binding affinities, K_{app}, ranging from –4.4 cm^3 mol^{-1} and 150 M^{-1} for $d(A)_8 \cdot d(T)_8$ to –18 cm^3 mol^{-1} and 40 M^{-1} for $[d(CG)_4]_2$, respectively, by fitting the first portion of each titration curve and assuming an overall binding of 0.5 Mg^{2+} per phosphate. Thus, the overall magnitude of the dehydration effect, which is determined by the structure of the Mg^{2+}–DNA complex, and the K_{app} are functions of the DNA sequence. Furthermore, the dehydration effect of Mg^{2+} binding correlates with the hydration state of the DNA: the higher its hydration state, the lower the dehydration effect of Mg^{2+} binding is. Mg^{2+} recognizes the sequence of DNA through its overall hydration state, probably by forming mostly outer-sphere complexes with oligomers containing exclusively dA·dT base pairs and inner-sphere complexes with dG·dC oligomers.

Introduction

One of the physicochemical properties of DNA that is fundamental to all biological processes is its polyelectrolyte behavior. This behavior is determined by the interaction of counterions with the regular lattice of negatively charged phosphate groups of the sugar–phosphate backbone of the double helix.[1,2] In spite of intensive investigations on the polyelectrolyte behavior of DNA, some questions still remain unanswered. The precise structure of the DNA–counterion atmosphere in solution with monovalent and/or divalent counterions is not known as well as its overall dependence on DNA conformation, composition, and sequence. A complete understanding of these interactions is important for the analysis of how DNA conformation and sequence controls the physicochemical properties and biological function of DNA. In the interaction of DNA with its counterions in aqueous solution, the interatomic distances are comparable to the size of a water molecule; therefore, these interactions are related to the problem of DNA hydration.

The hydration of DNA has been reviewed earlier.[3–8] Most of the results refer to experimental measurements on the physical properties of the hydration shell of helical DNA and its dependence on conformation, composition, and sequence. It has been shown that DNA in the B conformation is the more hydrated one and that A·T base pairs are more hydrated than G·C pairs.[9–14] The effect of sequence on the hydration of DNA has been demonstrated by ultrasound measurements[12,13] as well as by volumetric and calorimetric measurements on the binding of ligands to DNA.[15,16]

The direct observation of the hydration changes that accompany the binding of counterions to DNA can provide a rationale for the specific role of counterion interactions in the stability of the double helix, its conformational plasticity, and other physicochemical properties of DNA duplexes.[1,2,5,14] The investigation of the structural characteristics of the ionic atmosphere of DNA is limited by the sensitivity of traditional physical and chemical methods and the difficulties in the physical interpretation of experimental results. Experimental investigations on counterion binding in solution, for example, Mn^{2+}, Co^{2+}, or Ag^+,[17–23] position them on or near the DNA surface as detected by NMR and optical techniques. Much less is known on the structure of the

[†] Max-Planck-Institute for Biophysical Chemistry. Permanent address: Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region 142292, Russia.
[‡] Georgian Academy of Sciences.
[§] New York University.
[∥] Current address: Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
* To whom correspondence should be addressed.
[®] Abstract published in *Advance ACS Abstracts*, September 15, 1994.

(1) Manning, G. *Q. Rev. Biophys.* **1978**, *11*, 179.
(2) Record, M. T.; Anderson, C. F.; Lohman, T. M. *Q. Rev. Biophys.* **1978**, *11*, 103.
(3) Eagland, D. In *Water*; Franks, F., Ed.; Plenum Press: New York, 1975; Vol. 3, p 305.
(4) Texter, J. *Prog. Biophys. Mol. Biol.* **1978**, *33*, 83.
(5) Saenger, W. In *Principles of Nucleic Acid Structure*; Kantor, G. R., Ed.; Springer-Verlag: New York, 1984.
(6) Buckin, V. A. *Mol. Biol. (Engl. Transl.)* **1987**, *21*, 512.
(7) Westhof, E. *Annu. Rev. Biophys. Biophys. Chem.* **1988**, *17*, 125.
(8) Berman, H. M. *Curr. Opin. Struct. Biol.* **1991**, *1*, 423.
(9) Tunis, M.-J. B.; Hearst, J. E. *Biopolymers* **1968**, *6*, 1325.
(10) Mrevlishvili, G. M. *Dokl. Akad. Nauk. SSSR* **1981**, *260*, 761.
(11) Kopka, M. L.; Fratini, A. V.; Drew, H. R.; Dickerson, R. E. *J. Mol. Biol.* **1983**, *163*, 129.
(12) Buckin, V. A.; Kankiya, B. I.; Bulichov, N. V.; Lebedev, A. V.; Gukovsky, I. Ya.; Chuprina, V. P.; Sarvazyan, A. P.; Williams, A. R. *Nature* **1989**, *340*, 321.
(13) Buckin, V. A.; Kankiya, B. I.; Sarvazyan, A. P.; Uedaira, H. *Nucleic Acids Res.* **1989**, *17*, 4189.
(14) Rentzeperis, D.; Kupke, D. W.; Marky, L. A. *Biopolymers* **1993**, *33*, 117.
(15) Marky, L. A.; Kupke, D. W. *Biochemistry* **1989**, *28*, 9982.
(16) Rentzeperis, D.; Kupke, D. W.; Marky, L. A. *Biopolymers* **1992**, *32*, 1065.
(17) Pezzano, H.; Podo, F. *Chem. Rev.* **1980**, *80*, 365.
(18) Sissoeff, I.; Grisvard, J.; Guille, E. *Prog. Biophys. Mol. Biol.* **1976**, *31*, 165.
(19) Forsen, S.; Drakenberg, T.; Wennerstrom, H. *Q. Rev. Biophys.* **1987**, *19*, 83.
(20) Granot, J.; Feigon, J.; Kearns, D. R. *Biopolymers* **1982**, *21*, 181.
(21) Granot, J.; Kearns, D. R. *Biopolymers* **1982**, *21*, 203.
(22) Kennedy, S. D.; Briant, R. G. *Biophys. J.* **1986**, *5*, 669.
(23) Braunlin, W. H.; Drakenberg, T.; Nordenskiold, L. *Biopolymers* **1987**, *26*, 1047.

0002-7863/94/1516-9423$04.50/0 © 1994 American Chemical Society

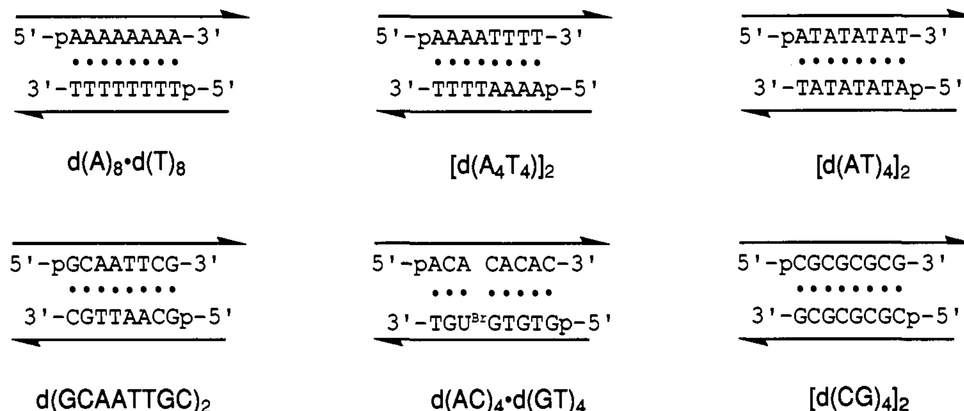


Figure 1. Sequences of deoxyoligonucleotides and their designations used throughout the text.

ionic atmosphere consisting of counterions like Li^+, Na^+, K^+, Cs^+, and Mg^{2+}, which do not possess paramagnetic properties and exert small influences on the DNA optical and NMR characteristics in solution, see, for example, Na-NMR investigations[24–28] or Mg–DNA investigations.[29,30] The information on the counterion distribution around DNA from small-angle X-ray scattering in solution was only obtained for heavy metals.[31] The X-ray data on the actual positions of small counterions in oligonucleotide crystals constitute a serious problem.

Hydration changes in DNA–counterion interactions are determined by the position of the counterion relative to the atomic groups at the surface of DNA. The measurement of these changes gives general information on the structural characteristics of the ionic atmosphere of DNA. High-precision ultrasonic velocity measurements are very sensitive to the hydration of a DNA–counterion molecule. The ability of high-precision ultrasonic velocity measurements to measure the hydration of a DNA molecule and to determine the dependence of DNA hydration on sequence and composition,[12,13] as well as on the binding of metal ions and ligands to DNA,[13,32] has been previously demonstrated.

In this work, we use ultrasonic velocity measurements to follow the hydration changes that accompany the binding of Mg^{2+} to oligonucleotide duplexes with different compositions and sequences. Specifically, we measure the change in the concentration increment of ultrasonic velocity of each of six octameric duplexes in their Cs^+ salt during a course of a titration with Mg^{2+}. The results show that the formations of Mg^{2+}–DNA complexes are accompanied by a dehydration effect that correlates with the overall hydration state of the DNA, and they suggest that Mg^{2+} is able to recognize the sequence of a DNA through its hydration shell. These results can be rationalized in terms of the formation of Mg^{2+}–outer-sphere complexes with dA·dT base pairs and Mg^{2+}–inner-sphere complexes with dG·dC base pairs.

Materials and Methods

Materials. All oligonucleotides (Figure 1) were synthesized and purified using standard procedures as described previously,[33] and their sequences were confirmed by Maxam–Gilbert analysis.[34] Oligonucleotides were desalted by double elution through a Sephadex G-10 column and lyophilized. All solutions were prepared in a buffer consisting of 2 mM Hepes and 0.2 M CsCl, at pH 7.8, using double distilled water with a conductivity of <10 mho cm^{-1}. Prior to acoustical experiments, the oligonucleotides were dissolved in buffer and their concentration was determined using extinction coefficients obtained by digestion with snake-venom phosphodiesterase. Equimolar solutions of complementary strands for a given duplex were prepared by weighing the required volumes of each strand solution. To reduce evaporation, the mixing was performed in closed vessels by operating with Hamilton syringes through small holes. CsCl was used as the supporting electrolyte; this was selected because of its small overall contribution to the value of the ultrasonic velocity. For instance, at 1 °C, its contribution is ~8 times smaller than that of NaCl. To select the proper temperature and CsCl concentration for the acoustical measurements, for which all oligonucleotides are in the double-stranded helical state, UV melting curves were obtained for all oligomer duplexes at 260 nm as a function of CsCl concentration and at strand concentrations similar to the ones used in acoustical measurements.

Background for the Interpretation of Ultrasonic Velocity Measurements. In high-precision ultrasonic velocity measurements in solution, the main experimental observable that characterizes the physical properties of the solute is the concentration increment of ultrasonic velocity, A, which is determined by the relation

$$A = (u - u_o)/(u_o c \rho_o) \tag{1}$$

where u and u_o are the ultrasonic velocities of the solution and pure solvent, respectively, c is the molal concentration of the solute, and ρ_o is the density of pure solvent. In relatively dilute solutions, the value of A is related to the values of the apparent molar volume, Φ_v, and apparent molar adiabatic compressibility, Φ_{ks}, by the equation[35]

$$A = \Phi_v - \Phi_{ks}/(2\beta_o) - M/(2\rho_o) \tag{2}$$

where β_o is the coefficient of adiabatic compressibility of the solvent and M is the molar mass of the solute. The change in the concentration increment of ultrasonic velocity, δA, as a result of solute–solute interactions is determined by both the change of the apparent molar adiabatic compressibility, $\delta\Phi_{ks}$, and the change of the apparent molar volume, $\delta\Phi_v$, according to[13,32]

$$\delta A = \delta\Phi_v - \delta\Phi_{ks}/2\beta_o \tag{3}$$

In dilute solutions, the values of the apparent molar adiabatic compressibility, Φ_{ks}, and the apparent molar volume, Φ_v, of the solute are related to its molecular physical characteristics by the relations[35]

$$\Phi_{ks} = K_m + \Delta K_h + K_r \quad \text{and} \quad \Phi_v = V_m + \Delta V_h \tag{4}$$

where V_m is the intrinsic volume of the solute molecule, which is inaccessible to surrounding molecules of water, K_m is the intrinsic compressibility (compressibility of volume V_m), ΔK_h (or ΔV_h) represents the hydration contribution equal to the difference between the compressibility (or

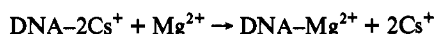
(24) Anderson, C. F.; Record, M. T.; Hart, P. A. *Biophys. Chem.* **1978**, *7*, 301.
(25) Bleam, M. L.; Anderson, C. F.; Record, M. T. *Biochemistry* **1983**, *22*, 5418.
(26) Braunlin, W. H.; Anderson, C. F.; Record, M. T. *Biochemistry* **1987**, *26*, 7724.
(27) Dijk, L. V.; Gruwel, M. L. H.; Jesse, W.; De Bleijser, J.; Leyte, J. C. *Biopolymers* **1987**, *26*, 261.
(28) Paulsen, M. D.; Anderson, C. F.; Record, M. T. *Biopolymers* **1988**, *27*, 1249.
(29) Braunlin, W. H.; Nordenskiöld, L.; Drakenberg, T. *Biopolymers* **1991**, *31*, 1343.
(30) Berggren, E.; Nordenskiöld, L.; Braunlin, W. H. *Biopolymers* **1992**, *32*, 1339.
(31) Chang, S.-L.; Chen, S.-H.; Rill, R. L.; Lin, J. S. *J. Phys. Chem.* **1990**, *94*, 8025.
(32) Buckin, V. A.; Tselikova, S. V. *Mol. Biol. (Engl. Transl.)* **1984**, *18*, 1185.
(33) Ivanova, E. M.; Kutiavin, I. V.; Pletnev, A. G.; Shamanin, V. A. *Bioorg. Khim.* **1982**, *8*, 1501.
(34) Maxam, A. G.; Gilbert, W. *Methods Enzymol.* **1986**, *68*, 499.
(35) Buckin, V. A. *Biophys. Chem.* **1988**, *29*, 283.

volume) of the hydration shell and the compressibility (or volume) of the same amount in the hydration shell of pure water, and K_r is the relaxation compressibility, which may exist if any relaxation process occurs in the system such as the changes in conformer distribution of the solute with temperature and pressure perturbations.[36,37] Experimental results indicate that the K_r values for double-helical DNA are small and can be neglected as compared with the other terms of eq 4 (especially if the interest is in the relative changes of A due to the binding of counterions to DNA and not in its absolute value[13]). The K_m values for small molecules, such as metal ions and nucleic acids bases, are within the experimental error in the determination of Φ_{ks}.[35] For a DNA double helix during the course of a titration with Mg^{2+}, its K_m and V_m values are not negligible but remain constant in the absence of conformational transitions.[13] In addition, the difference in the V_m values for the A and B forms of DNA is only 1 cm^3/mol,[38] which is close to the experimental uncertainty of our measurements. Therefore, the changes in the Φ_{ks} and Φ_v values for double-stranded DNA helices that result from the binding of counterions in the absence of conformational transitions should be determined primarily by their hydration contributions: ΔK_h and ΔV_h ($\delta\Phi_{ks} = \delta(\Delta K_h)$ and $\delta\Phi_v = \delta(\Delta V_h)$). The change of the concentration increment of ultrasonic velocity in this case is also determined mainly by its hydration contribution, δA_h:

$$\delta A = \delta A_h = \delta(\Delta V_h) - \delta(\Delta K_h)/2\beta^\circ \qquad (5)$$

The literature values of ΔK_h, Φ_{ks}, ΔV_h, and Φ_v for different electrolytes, small organic molecules, nucleic acid components, and DNA polymers[35,37,39-42] can be used to predict the overall $\delta(\Delta V_h)$, $\delta(\Delta K_h)$, and δA_h values for different types of counterion–DNA complexes. The second term in eq 5 which is determined by the compressibility of the hydration shell is the dominant term because, in these types of intermolecular interactions, the sensitivity of the compressibility is higher than that of the volume. At ~1 °C, the ΔK_h values for the majority of aqueous solutions of organic and inorganic compounds are negative because the compressibility of water in their hydration shell is less than that of bulk water. The reason for this is the abnormally high structural contribution to the compressibility of bulk water that results from the unique structure of pure water and its changes with pressure. The introduction of a solute molecule normally destroys this structure and therefore decreases the structural compressibility (for a detailed discussion, see ref 35). The higher the hydration state of a particular solute, the lower its ΔK_h value is. For instance, this decrease can be observed in the following changes: hydrophobic organic molecules (or atomic groups) to hydrophilic molecules, atomic groups of sugars (forming hydrogen bonds with water) to charged organic molecules, and electrolytes to multivalent electrolytes. Therefore, one can formulate the following rule: the negative value of δA in eq 5, resulting from the binding of counterions to DNA, is determined by dehydration of the DNA plus its counterion system. The smaller δA, the higher the dehydration effect is.

Previous δA measurements for the Mg^{2+}–Cs^+ exchange in the ionic atmosphere of natural DNAs and synthetic polynucleotides aimed at distinguishing hydration differences as a function of the DNA sequence and conformation[13] have shown that these measurements are sensitive to the hydration changes that take place in this counterion exchange according to the reaction



$$\text{DNA–2Cs}^+ + \text{Mg}^{2+} \rightarrow \text{DNA–Mg}^{2+} + 2\text{Cs}^+$$

High-Precision Differential Acoustical Titrations. The changes of ultrasonic velocity were measured using the resonator technique described earlier.[13,35,43] The ultrasonic resonators, manufactured of titanium, and the electronic block RADA-2 were developed at the Institute of Biological Physics, USSR Academy of Sciences. This instrument operates in the differential mode and consists of two acoustical resonators, measuring and reference cells, each with a total volume of ~1 mL and each containing a magnetic stirrer for the complete mixing of solutions. The changes in ultrasonic velocity are determined from the frequency shift of the preset resonance harmonic according to the expression $(u - u_o)/u_o = [(f - f_o)/f_o](1 + \gamma)$, where f and f_o are the frequency values that correspond to the maxima of the resonance peaks of the preset resonance harmonic of the cells filled with solution and solvent, respectively, that have the same phase shift between input and output signals of the resonator cell and γ is a small constant ~0.01 that is determined by calibration with NaCl. These changes were converted to those of the concentration increment of ultrasonic velocity using eq 1. All measurements were done in the 7.0–7.2 MHz frequency range. The relative error in the measurement of $(u - u_o)/u_o$ is 5×10^{-5}%. All acoustical titrations were performed at 1.2 °C in the differential mode by filling the measuring and reference cells with 677 μL of oligomer solution and buffer, respectively. Under these experimental conditions, all oligonucleotides are in the duplex state. The initial oligomer solution, with concentration ranging from 2.3 to 3.5 mM (in phosphate), was titrated with a ~1.5 M solution of $MgCl_2$ in buffer by adding 4–10 μL stepwise with a 10 μL Hamilton syringe through small holes in the lids of the cells until a $[Mg^{2+}]/[P_i]$ ratio of ~50 was obtained. The temperatures of the measuring and the reference cells were maintained by means of a water thermostat with a large thermal buffer vessel between the thermostat compartment and the cells. The temperature stability of this thermostat was better than 0.02 °C while the differential temperature stability between the two cells was better than 0.1 mdeg, checked by measuring the resonance frequencies of the cells filled with water. To further improve the overall temperature stability of the cells and to decrease the errors caused by evaporation, if any, all titration measurements were performed in a thermostated room at 4 °C.

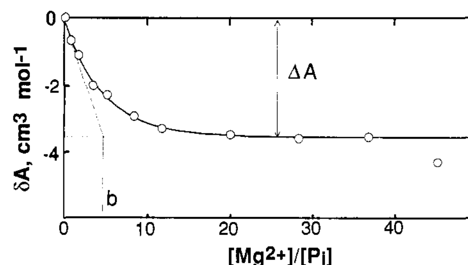


Figure 2. Illustration of the ΔA and b parameters obtained from the fit of the ultrasonic titration curves; this nonlinear least square fitting uses the exponential equation $\delta A = \Delta A[1 - e^{(\{[Mg^{2+}]/[Pi]\}/b)}]$. The solid line is the resulting fitted line of the experimental data for the $[d(AT)_4]_2$ duplex.

Fitting of Acoustical Titration Curves. All titrations were analyzed by fitting the resulting curves to two different functions. In the first fitting procedure, we used the exponential function $\delta A = \Delta A[1 - \exp(\{[Mg^{2+}]/[P_i]\}/b)$, where ΔA is the overall change of the ultrasonic velocity increment per phosphate at saturation and b is the intercept of the initial slope of each curve with the saturation line that is drawn parallel to the $[Mg^{2+}]/[P_i]$ axis (see Figure 2). This type of fitting is independent of any thermodynamic model of Mg^{2+} binding and allow us to obtain the ΔA and b (and initial slope $\Delta A/b$) values for each curve. This exponential function contains a minimum number of independent parameters and fits the experimental data well within the limits of experimental error. The ΔA value at saturation is determined by the relationship $\Delta A = \sum(\Delta A_{Mg_i^{2+}}) \cdot n_i$, where $\Delta A_{Mg_i^{2+}}$ is the change in ultrasonic velocity increment per mole of bound Mg^{2+} for the i-type of complex and n_i is the maximum number of bound Mg^{2+} ions per phosphate forming an i-type of complex. Therefore, ΔA is the total hydration change from the contributions of all types of complexes. For the physical meaning of b, we use a simple thermodynamic model of identical and independent sites, in which b is determined by the relation $b = n + (K'[P_i])^{-1}$, where n is the maximum number of counterions bound (per phosphate) to DNA, $[P_i]$ is the concentration of DNA in phosphate, and K' is the limiting value of the binding constant at zero concentration of magnesium. In our titration curves, the value of $(K'[P_i])^{-1}$ is much greater than the maximum potential value of $n = 0.5$; therefore, the value of b is mainly determined by the limiting value of the binding constant: $b \approx (K'[P_i])^{-1}$.

The second fitting procedure is based on the binding model where Mg^{2+} binds to a DNA lattice containing one type of identical and independent binding site. These sites are characterized by an equilibrium constant, K_{app}, molar ultrasonic velocity change, $\Delta A_{Mg^{2+}}$, and apparent number of Mg^{2+} counterions per binding site, n. The above three parameters may be determined from the resulting ultrasonic titration curves interactively using the Marquardt algorithms as described

(36) Buckin, V. A.; Sarvazyan, A. P.; Dudchenko, E. I.; Hemmes, P. *J. Phys. Chem.* **1980**, *84*, 696.
(37) Stuehr, J.; Yeager, E. In *Physical Acoustics*; Mason, W. R., Ed.; Academic Press: New York, 1965; Vol. 2A, Chapter 6, pp 351–462.
(38) Pavlov, M. Yu.; Fedorov, B. A. *Biofizika* **1983**, *28*, 931.
(39) Buckin, V. A.; Kankiya, B. I.; Kazaryan, R. L. *Biophys. Chem.* **1989**, *34*, 211.
(40) Conway, B. E. In *Ionic Hydration in Chemistry, Studies in Physical and Theoretical Chemistry*; Elsevier: Amsterdam, 1981; Vol. 12.
(41) Roy-Chowdhury, P. *Macromol. Chem. Phys.* **1987**, *C27*, 219.
(42) Hoiland, H. In *Thermodynamic Data for Biochemistry and Biotechnology*; Springer-Verlag: Berlin-Heidelberg, 1986; pp 129–147.
(43) Sarvazyan, A. P. *Ultrasonics* **1982**, *20*, 151.

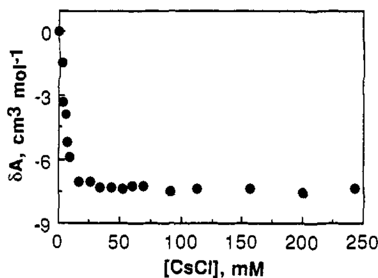


Figure 3. Ultrasonic titration curve of the least stable duplex with CsCl: 677 μL of a $[d(AT)_4]_2$ solution, 3.40 mM in phosphate, in 2 mM Hepes buffer at pH 7.8 and 1.2 °C titrated with a concentrated solution of CsCl in the same buffer with aliquots ranging from 4 to 20 μL.

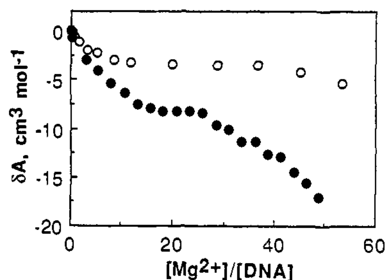


Figure 4. Typical ultrasonic titration curves of oligonucleotide duplexes in 2 mM Hepes buffer and 0.2 M CsCl at pH 7.8 and 1.2 °C: $[d(AT)_4]_2$ (open circles), 3.55 mM in phosphate; $[d(CG)_4]_2$ (closed circles), 3.24 mM in phosphate. Each oligomer solution is titrated with ~1.5 M $MgCl_2$, in the same buffer, by the incremental addition of few microliters at a time.

previously.[44] The fitting procedure is to let all three parameters float or to fix $\Delta A_{Mg^{2+}}$ or n or K_{app} parameters until the lowest standard deviation of the fit is obtained.

Circular Dichroism Spectra (CD). As an additional control, we obtained the CD spectra of each duplex to check both for the conformation of the duplex in the absence of Mg^{2+} and for any DNA conformational changes that might result from the addition of Mg^{2+}. All CD spectra were recorded with a Jasco Model J-41A spectrophotometer at 1 °C, oligomer concentrations of 1.7–2.7 mM in phosphate, and $[Mg^{2+}]/[DNA]$ molar ratios of 0 to 20–30. We used a 0.5 mm pathlength cell that allowed us to measure the spectra of the oligomer solutions used in the acoustical experiments.

Results

To determine the optimum temperature and CsCl concentration for the acoustical titrations of each oligomer duplex, we obtained absorbance vs temperature profiles (data not shown) in buffer solution at several CsCl concentrations up to 0.6 M. These melting profiles indicate that in 0.2 M CsCl all oligomers are in the duplex state at temperatures below 5 °C and melt in increasing order of stability with increasing percentage of dC·dG content. In addition, we have obtained the dependence of δA on CsCl concentration, shown in Figure 3, for the $[d(AT)_4]_2$ duplex that has the lowest stability. We observed a decrease in δA with increasing CsCl concentration up to 0.05 M that levels off at higher CsCl concentrations. Under these solution conditions, the initial decrease corresponds to a decrease in the overall hydration that accompanies the formation of a DNA duplex from its component single strands. At higher CsCl concentrations, the value of δA remains constant and indicates no further conformational changes as detected from the hydrational perturbations. Therefore, all acoustical titrations were carried out in a buffer solution containing 0.2 M CsCl at 1.2 °C.

Figure 4 shows typical acoustical titration curves for two duplexes with 0 and 100% dG·dC base pairs (δA as a function of the $[Mg^{2+}]/[P_i]$ ratio), increasing the $[Mg^{2+}]$ results in the overall decrease in δA. These curves show two parts: (i) an initial exponential decrease that levels off at $[Mg^{2+}]/[P_i]$ ratios of 20–30 that corresponds to the overall hydration changes in the ionic atmosphere of each oligonucleotide duplex, due to the Mg^{2+}–Cs^+ counterion exchange, and (ii) a further and almost linear decrease at higher $[Mg^{2+}]/[P_i]$ ratios which may be the result of conformational changes and/or aggregation of the Mg^{2+}–DNA complexes. For these two duplexes, the initial slopes of the curves are similar but the overall drop in the δA values at $[Mg^{2+}]/[P_i]$ saturation ratios of 20–30 for the $[d(CG)_4]_2$ duplex is almost twice as much as the drop for the $[d(AT)_4]_2$ duplex. The addition of more Mg^{2+} results in a further decrease in δA for both duplexes: small for $[d(AT)_4]_2$ and large for $[d(CG)_4]_2$. In the case of $[d(CG)_4]_2$, this may correspond to the familiar conformational transition to the Z form.

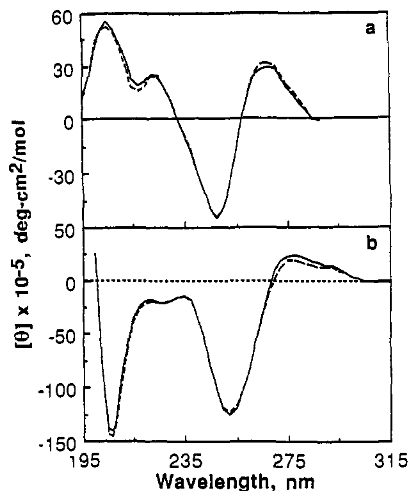


Figure 5. Typical CD spectra of oligonucleotide duplexes in 2 mM Hepes buffer and 0.2 M CsCl at pH 7.8, 1.2 °C, and $[Mg^{2+}]/[P_i]$ ratios of 0 (solid lines) and 25 (dotted lines). Panel a: $[d(AT)_4]_2$, 2.14 mM in phosphate. Panel b: $[d(CG)_4]_2$, 1.72 mM in phosphate.

In Figure 5, we show the CD spectra of these two oligomer duplexes at strand concentrations used in the acoustical titrations and at two $[Mg^{2+}]/[P_i]$ ratios of 0 and 20–30. These latter $[Mg^{2+}]/[P_i]$ ratios correspond to the plateau of the acoustical titration curves. At these ratios, the CD spectra of each oligomer correspond to the typical spectra of DNA duplexes in the B conformation and show no significant changes with the increase in Mg^{2+} concentration; the other four duplexes follow a similar behavior.

In this report, we concentrate on the dehydration changes that occur just in the first part of each titration curve, at $[Mg^{2+}]/[P_i]$ ratios below 20–30 that correspond to the concentration range of 0–0.1 M for $MgCl_2$. Figure 6 shows the resulting acoustical titrations for all six duplexes. In this range, the observed changes are determined only by the exchange of Cs^+ ions by Mg^{2+} in the ionic atmosphere of each oligonucleotide. We note that each curve levels off at different δA values: the higher the dG·dC content, the lower the final δA value.

Comparison of Fitting Procedures. The resulting fitted curves using the exponential function are shown in Figure 6, see solid lines, and the parameters derived from both types of fits are shown in Table 1. The values of ΔA at saturation are obtained better with the exponential fit, while the second type of fit determines K_{app} more directly. In the second fitting procedure and for the fits of each duplex, the value of the root mean square deviation (RMS) which characterizes the quality of the fitting is nearly independent of n, for n ranging from 0.05 to 1; also, the product $\Delta A_{Mg^{2+}} \times n$ (= ΔA) and K_{app} remains constant in this range of n. This means that, within the limits of the experimental uncertainties, the only parameters that can be evaluated are K and ΔA, as is the case of the exponential fits. The maximum

(44) Wiseman, T.; Williston, S.; Brandts, J. F.; Lin, L. N. *Anal. Biochem.* **1989**, *179*, 131.

Table 1. Parameters for the Binding of Mg^{2+} to DNA Oligomers and Polymers at 1.2 °C

	fits by the function $\delta A = \Delta A\{1 - e^{(-[Mg^{2+}]/[P_i])/b}\}$				fits by a thermodynamic model of one set of identical binding sites		
	b $[Mg^{2+}]/[P_i]$	K'^{a} (M^{-1})	ΔA (cm^3/mol)	RMS (cm^3/mol)	K_{app} (M^{-1})	ΔA (cm^3/mol)	RMS (cm^3/mol)
$d(A)_8 \cdot d(T)_8$	3.9	120	2.2	0.15	180	2.4	0.17
$[d(A_4T_4)]_2$	3.2	110	2.9	0.23	140	3.3	0.10
$[d(AT)_4]_2$	4.8	70	3.5	0.07	85	4.1	0.11
$d(AC)_4 \cdot d(GT)_4$	9.8	30	7.8	0.24	30	11.0	0.23
$[d(GCAATTGC)]_2$	6.0	70	6.6	0.16	80	8.0	0.23
$[d(CG)_4]_2$	8.1	40	8.9	0.19	40	11.7	0.22
poly(dA)·poly(dT)	1.0	1000	2.1	0.11	960	2.4	0.11
poly[d(AT)]·poly[d(AT)]	1.2	710	3.9	0.18	700	4.6	0.23

[a] The limiting values of K' are calculated from the b values by using the relation $b = n + (K'[P_i])$, which is true for the case of one type of binding site; n was assumed equal to 0.5.

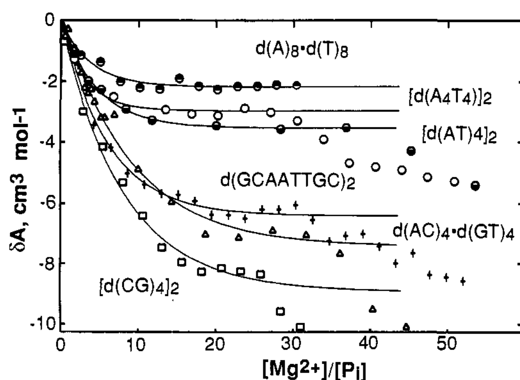


Figure 6. Ultrasonic titration curves of oligonucleotide duplexes with $MgCl_2$ in 2 mM Hepes buffer and 0.2 M CsCl at pH 7.8 and 1.2 °C. Each titration is performed by adding a ~1.5 M solution of $MgCl_2$ to 677 μL of oligonucleotide solution in the resonator sample cell using increments ranging from 4 to 10 μL. The duplexes have the following initial concentrations in phosphate: $d(A)_8 \cdot d(T)_8$, 2.34 mM; $[d(AT)_4]_2$, 3.55 mM; $[d(A_4T_4)]_2$, 3.50 mM; $[d(CG)_4]_2$, 3.24 mM; $d(AC)_4 \cdot d(GT)_4$, 3.40 mM; $[d(GCAATTGC)]_2$, 2.70 mM. The solid lines correspond to the exponential fitted lines.

number of potentially electrostatically bound Mg^{2+} ions per phosphate is ~0.5, which is also the maximum value of bound Mg^{2+} ions that can be detected acoustically and whose binding would lead to hydration changes. Therefore, the resulting $\Delta A_{Mg^{2+}}$ values that were obtained assuming $n = 0.5$ characterize the average hydration effect of bound Mg^{2+} ions or the minimum hydration effect of the bound Mg^{2+} ions that formed acoustically detectable complexes. In Table 1, the ΔA values and the binding constants are given for both fits at $n = 0.5$. The results of both fitting procedures are in good agreement. The fact that we were unable to obtain n values indicates that it is impractical to use more complicated models, such as the one that involves a higher number of binding sites, because these will include an additional number of unknown parameters. Our attempts to fit the data with more complicated fitting models confirmed this.

The Hydration Effect of Mg^{2+} Binding Depends on the DNA Sequence. The ΔA values that characterize the overall change of the ultrasonic velocity increment in the titration of oligonucleotides by Mg^{2+} are different for all six oligonucleotides (see Table 1 and Figure 6). Since the ΔA value is determined by the hydration effect, we conclude that the resulting hydration changes for the interaction of magnesium with these duplexes are a function of the nucleotide sequence. The Mg^{2+} binding affinities also depend on the oligonucleotide sequence: the higher the value of K_{app}, the lower the overall dehydration effect is. These observations are in agreement with previous NMR results[25] that the binding of $^{25}Mg^{2+}$ to a particular DNA environment depends on the sequence of DNA.[31,32]

The Overall Hydration Effect of Mg^{2+} Binding Is Independent of the Length of DNA. We have also fitted the acoustical titration curves of Mg^{2+} with poly[d(AT)]·poly[d(AT)] and poly(dA)·poly(dT) that were obtained earlier under similar solution conditions but at the lower CsCl concentration of 20 mM,[13] and the results are included in Table 1. At saturation, the ΔA values for these polynucleotide sequences are similar to those of the corresponding oligomer duplexes $dA_8 \cdot dT_8$ and $[d(AT)_4]_2$. This indicates that the total hydration effect of Mg^{2+} is similar despite the fact that these oligomers may contain a percentage of frayed ends.[45,46] We thus conclude that for these sequences the total hydration effect upon Mg^{2+} binding, which is determined primarily by the structure of the Mg^{2+}–DNA complex, is independent of the DNA length. This observation is interesting from the point of view of the polyelectrolyte properties of DNA because in our oligomer duplexes the length and the diameter have similar dimensions. Therefore, the counterion condensation phenomenon inherent in linear polyelectrolytes such as DNA should be absent. However, we obtained larger K_{app} values for the association of Mg^{2+} to the polydeoxynucleotides, which is not surprising because of the stronger electrostatic contribution to the binding affinity of Mg^{2+} that may result from the polyelectrolyte effect and/or the lower Cs^+ concentration used in the polymer titrations.

Discussion

The Structure of the Mg^{2+}–DNA Complexes Depends on the DNA Sequence. For all oligonucleotides the values of ΔA are negative. This means that the exchange of Cs^+ for Mg^{2+} in the ionic atmosphere of each duplex is accompanied by a dehydration or release of water molecules. The absolute values of the ΔA ranges from −2.2 to −9 cm^3/mol (per phosphate), in terms of per mole of potential binding sites (0.5 per phosphate), yield $\Delta A_{Mg^{2+}}$ values ranging from −4.5 cm^3 mol^{-1} for $d(A)_8 \cdot d(T)_8$ to −18 cm^3 mol^{-1} for $[d(CG)_4]_2$ (see Table 1). Do these differences result from a particular structure of the Mg^{2+}–DNA complexes? Let us assume that the actual structure of the Mg^{2+}–DNA complexes does not depend on the nucleotide sequence and that the differences in the hydration effects of Mg^{2+} binding are determined by other factors such as differences in oligonucleotide conformation; if true, then one should expect a higher dehydration effect for the binding of Mg^{2+} to DNA sequences that are more highly hydrated. Table 2 shows reported values of the apparent molar adiabatic compressibilities and related parameters that characterize the overall hydration of each oligomer duplex.[12] The lower the Φ_{ks} value, the higher the hydration state of the oligonucleotide duplex is.[6,12,13] Figure 7 shows the dependence of $\Delta A_{Mg^{2+}}$ on the apparent molar adiabatic compressibilities of these oligonucleotide duplexes. As seen in this figure and in Table 2, the $\Delta A_{Mg^{2+}}$ value decreases with a decrease of Φ_{ks}. This means that the higher the hydration state of the oligonucleotide is, the lower the dehydration effect of Mg^{2+} binding is. Therefore, the particular structure of a Mg^{2+}–DNA complex depends on the nucleotide sequence of DNA and

(45) Otting, G. G.; Grutter, R.; Leupin, W.; Minganti, C.; Ganesh, K. N.; Sproat, B. S.; Gait, M. J.; Wuthrich, K. *Eur. J. Biochem.* **1987**, *166*, 215.
(46) Celda, B.; Widmer, H.; Leupin, W.; Chazin, W. J.; Denny, W. A.; Wuthrich, K. *Biochemistry* **1989**, *28*, 1462.

Table 2. Concentration Increments of Ultrasonic Velocity A, Apparent Molar Volume Φ_v, and Apparent Molar Adiabatic Compressibility Φ_{ks}, the Difference between the Apparent Molar Volume of Oligonucleotides and the Intrinsic Molar Volume V_m, and the Change of Ultrasonic Velocity Increment $\Delta A_{Mg^{2+}}$ of Oligonucleotides[a]

DNA	A (cm³/mol)	Φ_v (cm³/mol)	$10^{-4}\Phi_{ks}$ (cm³/[mol·bar])	$(\Phi_v - V_m)$[b] (cm³/mol)	$\Delta A_{Mg^{2+}}$[c] (cm³/mol)
$d(A)_8 \cdot d(T)_8$	112.5 ± 0.5	145.3 ± 1.6	−127.9 ± 2.1	−16.4	−4.4
$[dA_4T_4)]_2$	102.9 ± 0.4	155.0 ± 1.5	−110.0 ± 1.9	−6.7	−5.8
$[d(AT)_4]_2$	107.0 ± 0.4	156.0 ± 1.5	−112.1 ± 1.9	−5.7	−7.0
$d(AC)_4 \cdot d(GT)_4$	98.6 ± 0.5	156.1 ± 1.5	−101.0 ± 2.0	−4.4	−15.6
$[d(GCAATTGC)]_2$	96.5 ± 0.6	153.9 ± 1.5	−105.7 ± 2.1	−6.5	−13.2
$[d(CG)_4]_2$	92.0 ± 0.5	162.0 ± 1.4	−92.0 ± 1.9	3.0	−17.8
poly(dA)·poly(dT)[d]	92.4 ± 0.6	140.6 ± 1.5	−112.7 ± 2.1	−21.1	−4.2
poly[d(AT)]·poly[(d(AT)][d]	87.9 ± 0.5	147.5 ± 1.5	−101.8 ± 2.0	−14.2	−7.8

[a] These values are from ref 12. [b] These values are from ref 34; in these calculations, a correction for the intrinsic volume of Br but not for that of Na^+ has been included. [c] These values are calculated per mole of potential binding sites (0.5) from the ΔA values given in Table 1. [d] These polymer values are from ref 13.

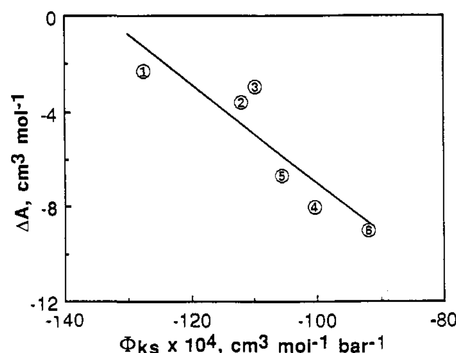


Figure 7. Correlation of the ultrasonic velocity change, ΔA, of oligonucleotide duplexes as a result of magnesium binding with the apparent molar adiabatic compressibilities of the Mg^{2+} free duplexes: 1, $d(A)_8 \cdot d(T)_8$; 2, $[d(AT)_4]_2$; 3, $[d(A_4T_4)]_2$; 4, $d(AC)_4 \cdot d(GT)_4$; 5, $[d(GCAATTGC)]_2$; 6, $[d(CG)_4]_2$.

most likely it is in this way that Mg^{2+} is able to recognize the nucleotide sequence.

Types of Mg^{2+}–DNA Complexes. What type of Mg^{2+}–DNA complexes do the resulting $\Delta A_{Mg^{2+}}$ values correspond to? To have an idea of these $\Delta A_{Mg^{2+}}$ values, one should compare them with the hydration parameters of both the Mg^{2+} ions and DNA molecules. The hydration part of the ultrasonic velocity increment of a solute molecule, A_h, is determined by the volume and compressibility properties of the hydration shell, according to eqs 2–4 by the relation $A_h = \Delta V_h - \Delta K_h/2\beta_o$. This corresponds to the change in the ultrasonic velocity increment as a result of the full dehydration of the solute. The value of A_h can be estimated from experimental values of A, the intrinsic compressibility K_m, and the volume V_m of the solute and its molecular mass M, according to

$$A_h = A - V_m + K_m/(2\beta_o) + M/(2\rho_o)$$

The value of A for Mg^{2+} can be estimated using eq 2 from its partial molar volume Φ_v and partial molar adiabatic compressibility Φ_{ks} values reported earlier.[40,47–50] At 25 °C, these values are −32 cm³ mol⁻¹ and -63×10^{-4} cm³ mol⁻¹ bar⁻¹,[40,47,48] respectively; the V_m value for Mg^{2+}, calculated from the crystal radii,[47] is about 0.7 cm³ mol⁻¹, which is very small and can be neglected, as well as its K_m value,[37,40] resulting in an A_h value for Mg^{2+} of 38 cm³ mol⁻¹. If we assume a total of six water molecules in the coordination sphere of Mg^{2+} [51] then, in its interaction to DNA, we shall expect a decrease in its ultrasonic velocity increment of about 6.5 cm³ mol⁻¹ for the exchange of one water molecule from its coordination sphere plus an additional contribution from the dehydration of the oligonucleotide atomic groups; this latter contribution cannot be more than a few units of cm³ mol⁻¹.[39] We estimate then that the minimum value of $\Delta A_{Mg^{2+}}$ for the formation of a Mg^{2+}–DNA inner-sphere complex is about −10 cm³ mol⁻¹. Alternatively, we can estimate this on the basis of the results of acoustical titrations of tetramethylammonium–EDTA with Mg^{2+} at pH 12 (Kankiya et al., manuscript in preparation). Under these conditions, Mg^{2+} looses all or most of its hydration shell in its binding to EDTA (see, for example, ref 52), resulting in a decrease in the ultrasonic velocity increment of −100 cm³ mol⁻¹ at 25 °C, corresponding to −17 cm³ mol⁻¹ per water molecule substituted in the hydration shell of Mg^{2+}. This latter value includes the contribution of the dehydration of atomic groups of the highly hydrated EDTA anion. At 1 °C, the $\Delta A_{Mg^{2+}}$ value, for these types of Mg^{2+}–DNA complexes should be slightly lower; for instance, the A_h value for $MgCl_2$ at 25 °C is about 17% smaller in magnitude than the value at 0 °C.[49] Therefore, we can expect a $\Delta A_{Mg^{2+}}$ value in the range of −10 to −20 cm³ mol⁻¹ for the formation of an inner-sphere Mg^{2+}–DNA complex containing one coordination bond between Mg^{2+} and DNA. For higher Mg^{2+} coordination numbers with DNA, the $\Delta A_{Mg^{2+}}$ values shall be much lower. In our estimates we have not taken into account any potential changes in the hydration state of Cs^+ ions released on Mg^{2+} binding. Earlier reports have indicated that Cs^+ maintains a full hydration shell in the ionic atmosphere of DNA,[1] and thus, its hydration state upon release should not change.

The validity of the above estimations is supported by the measurements of the volume and compressibility changes for the formation of Mg^{2+} complexes with SO_4^{2-} by Millero and coworkers,[53] who reported a $\Delta A_{Mg^{2+}}$ value of −5 cm³ mol⁻¹. In these experiments the outer-sphere types of complexes predominate. In the case of Ca^{2+}, which has nearly the same A_h value[47,48] as Mg^{2+} but forms inner-sphere complexes with SO_4^{2-}, its ΔA value is about −35 cm³/mol⁻¹ (Millero et al.[54]) and corresponds to the exclusion of ∼6.5 water molecules from the hydration shells of Ca^{2+} and SO_4^{2-} in the formation of this complex. This corresponds to about three coordination bonds between the interacting ions. In addition, the binding of bis(guanidinium) to DNA that is hydrogen bonded to two phosphates has a ΔA value of 24 cm³/mol per guanido group or 12 cm³ mol⁻¹ per hydrogen bond.[32]

All of the above suggest that the average dehydration effect of Mg^{2+} binding to the oligonucleotide duplexes containing 100% dA·dT base pairs corresponds to the formation of outer-sphere complexes in which Mg^{2+} keeps all of its coordinated water. This

(47) Millero, F. J. In *Water and Aqueous Solutions*; Horn, R. A., Ed.; Wiley-Interscience: New York, 1972; pp 519–595.
(48) Millero, F. J.; Ward, G. K.; Chetirkin, P. F. *J. Acoust. Soc. Am.* **1977**, *61*, 1492.
(49) Connaughton, L. M.; Hershey, J. P.; Millero, F. J. *J. Solution Chem.* **1986**, *15*, 989.
(50) Millero, F. J.; Vinokurova, F.; Fernandez, M.; Hershey, J. P. *J. Solution Chem.* **1987**, *16*, 269.
(51) Heizinger, K.; Palinkas, G. In *The Chemical Physics of Solvation*; Dogonadze, R., Kalman, E., Kornyshev, A. A., Ulstrup, J., Eds.; Elsevier: Amsterdam, 1985; pp 313–353.
(52) Eigen, M.; Maas, G. *Z. Phys. Chem. (Munich)* **1966**, *49*, 163.
(53) Millero, F. J.; Ward, G. K.; Lepple, F. K.; van't Hoff, E. *J. Phys. Chem.* **1974**, *78*, 1636.
(54) Millero, F. J.; Combar, F.; Oster, J. *J. Solution Chem.* **1977**, *6*, 269.

does not exclude the possibility that a few Mg^{2+} counterions may be in direct contact with atomic groups of DNA, forming inner-sphere complexes and losing some water molecules from its coordination sphere. In the binding to the $[d(CG)_4]_2$ duplex, nearly all Mg^{2+} ions can form inner-sphere complexes with one coordination bond or partially form complexes with two or more coordination bonds. The other oligonucleotides with mixed sequences have intermediate $\Delta A_{Mg^{2+}}$ values and may form both types of complexes.

The formation of inner-sphere Mg^{2+}–DNA complexes has been suggested from the results of NMR experiments where the equilibrium (inner sphere) ↔ (outer sphere) apparently depended on the total concentration of magnesium.[20,55] The earlier measurements of the volume change, ΔV, for the binding of Mg^{2+} to DNA also support the idea of inner-sphere complex formation. The ΔV obtained in 0.2 M (TMA)Cl by Skerjanc and Strauss[56] is equal to 6.4–8.7 cm^3/mol of Mg^{2+}, and it is much larger (20 cm^3/mol of Mg^{2+}) if the ionic strength is decreased to 5 mM (TMA)Cl.[57] The ΔV_h value for Mg^{2+} is about −32 cm^3/mol. If we assume six water molecules in the first coordination shell of Mg^{2+}, then in the formation of the Mg^{2+}–DNA complex with one coordination bond, we would expect a volume effect of 5.3 cm^3/mol in addition to the small contribution to the atomic groups of DNA. The latter one is not more than a few units of cm^3 mol^{-1}.[35,39] Therefore, the observed volume effects for Mg^{2+} binding to DNA support the partial formation of inner-sphere complexes. In the original interpretation of Skerjanc and Strauss,[56] these authors compared the volume effects of Mg^{2+} binding to DNA to those of polyphosphates, which were in the range 16–26 cm^3/mol of Mg^{2+}. The smaller value for the Mg^{2+}–DNA system was attributed to the larger phosphate–phosphate distance that makes it impossible for Mg^{2+} to coordinate with more than one DNA phosphate group, in contrast to the Mg^{2+}–polyphosphates system where a higher number of coordinated bonds is possible. This explanation is in agreement with our observations; therefore, the observed volume effect of 6.4–8.7 cm^3/mol of Mg^{2+} bound to DNA corresponds to the formation of a Mg^{2+}–DNA complex with one substituted coordination bond.

Mg^{2+} Recognizes the Nucleotide Sequence through the Hydration State of DNA. We have shown that the overall dehydration effect in the binding of Mg^{2+} to the oligonucleotide duplexes decreases with increasing their hydration levels. Another observation is the increase in the values of K with an increase in the hydration state of the oligomer. It is difficult to explain these effects if only one type of Mg^{2+}–DNA complex forms. On the other hand, we have shown that Mg^{2+} forms two types of complexes (inner sphere and outer sphere) with some oligonucleotides. The presence of two types of Mg^{2+}–DNA complexes has been reported in both NMR investigations[31,32,55] and studies using cation-specific electrodes.[58] A possible explanation for the observed regularities based on the existence of both inner-sphere and outer-sphere complexes is shown in Figure 8. The formation of inner-sphere complexes requires removal of water molecules from the hydration shell of DNA, an effect that may be done easier for less hydrated sequences. Therefore, for these sequences, one would expect a higher amount of inner-sphere complexes and a larger value of the total dehydration of Mg^{2+} binding.

Similarly, to explain the dependence of K_{app} on DNA hydration, we suggest that the formations of inner-sphere complexes are much more "visible" in our ultrasonic titration curves because their formation is accompanied by a release of water molecules or higher dehydration effect. Assuming that the binding constant of Mg^{2+} to DNA for an inner-sphere complex is smaller than for

(55) Rose, D. M.; Bleam, M. D.; Record, M. T., Jr.; Bryant, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 6289.
(56) Skerjanc, J.; Strauss, U. P. *J. Am. Chem. Soc.* **1968**, *90*, 3081.
(57) Clement, R. H.; Sturm, J.; Daune, M. P. *Biopolymers* **1973**, *12*, 405.
(58) Sander, C.; Ts'o, P. *J. Mol. Biol.* **1971**, *55*, 1.

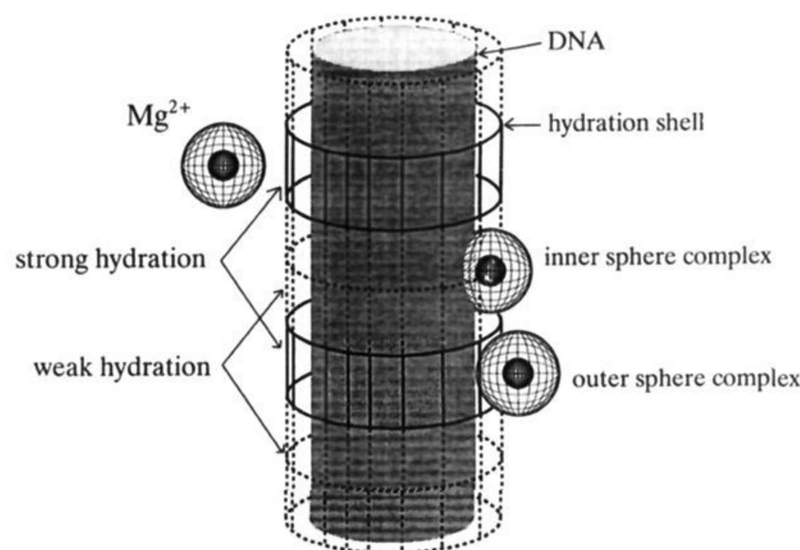


Figure 8. Illustration of the types of complexes that Mg^{2+} forms with the oligonucleotide duplexes: (i) In outer-sphere complexes, Mg^{2+} does not penetrate into the hydration shell of highly hydrated oligomer sequences (dA·dT base pairs). As a result, the dehydration effect of Mg^{2+} binding is small. (ii) In inner-sphere complexes, Mg^{2+} is able to penetrate past the hydration shell of less hydrated oligomer sequences (dC·dG base pairs). As a result, the dehydration effect of Mg^{2+} binding is large.

an outer-sphere complex, then the average value of K_{app} that was obtained using the model with one type of binding site would decrease with an increasing percentage of inner-sphere complexes. These complexes have a higher "visibility". This is exactly what we found and explains the dependence of K on the overall hydration state of the duplexes. If this assumption is true, we can conclude that Mg^{2+} is able to recognize the sequence of DNA through the properties of its hydration shell.

Conclusions

We have used ultrasonic titration measurements to follow the overall hydration changes that occur upon the binding of Mg^{2+} to oligonucleotide duplexes of known sequences. We were able to show that this type of measurement is very convenient for the investigation of metal ion binding to DNA. Our experimental results indicate that the overall dehydration effect of Mg^{2+} binding depends on the DNA sequence. The higher the A·T content of the oligonucleotide, the lower the dehydration effect, an effect that is consistent with the higher hydration state of these duplexes. Thus, Mg^{2+} is able to recognize the sequence of DNA through its overall hydration state probably by forming a combination of two types of complexes: mostly outer-sphere complexes with DNA oligomers containing exclusively dA·dT. For oligomers with dG·dC base pairs, this changes gradually to those of inner-sphere complexes as the percentage of G·C base pairs increases.

Acknowledgment. We are grateful to Dr. N. Bulichov from the Institute of Bioorganic Chemistry, Russian Academy of Sciences, for the synthesis and purification of oligonucleotides; Professor L. De Maeyer and Dr. Th. Funck from the Max-Planck-Institute for Biophysical Chemistry, Professor Sarvazyan from the Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, and Dr. V. Chuprina for helpful discussions; Dr. S. Y. Venyaminov from the Institute of Protein Research, Russian Academy of Sciences (Pushchino), for technical assistance in the CD measurements; Dr. V. Schestimirov of the Institute of Cell Biophysics for technical assistance in the electronics of the ultrasonic equipment; and Professor Louise Pape from NYU for critical reading of the manuscript. This research was supported by the Alexander von Humboldt Foundation in the form of a fellowship to V.B. and by Grant GM-42223 (L.A.M.) from the National Institutes of Health.