ELSEVIER

Biophysical Chemistry 104 (2003) 643–654

Biophysical Chemistry

www.elsevier.com/locate/bpc

Binding of Mg^{2+} to single-stranded polynucleotides: hydration and optical studies

Besik I. Kankia*

Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, 1479 Gortner Avenue, St. Paul, Minnesota, MN 55108, USA

Received 12 March 2003; accepted 8 April 2003

Abstract

The binding of Mg^{2+} to single-stranded ribo- and deoxy-polynucleotides, poly(rA), poly(rU), poly(dA) and poly(dT), has been investigated in dilute aqueous solutions at pH 7.5 and 20 °C. A combination of ultrasound velocimetry, density, UV and CD spectroscopy have been employed to study hydration and spectral effects of Mg^{2+} binding to the polynucleotides. Volume and compressibility effects of Mg^{2+} binding to random-coiled poly(rU) and poly(dT) correspond to two coordination bonds probably between the adjacent phosphate groups. The same parameters for poly(rA) + Mg^{2+} correspond to an inner-sphere complex with three–four direct contacts. However, almost no hydration effects are arising in binding to its deoxy analog, poly(dA), indicating mostly a delocalized binding mode. In agreement with hydration studies, optical investigations revealed almost no influence of Mg^{2+} on poly(dA) properties, while it stabilizes and aggregates poly(rA) single-helix. The evidence presented here indicates that Mg^{2+} are able to bind specifically to single-stranded polynucleotides, and recognize their composition and backbone conformation.

Keywords: Poly(rA); Poly(dA); Magnesium binding; Dehydration effects; Inner-sphere complexes; Delocalized binding

1. Introduction

Divalent cations, particularly magnesium, strongly influence the structure and biochemical activity of nucleic acids. Cation binding mode to a nucleic acid can be divided into three modes: (i) diffuse or delocalized binding in which cations interact with the anionic field around the nucleic acids via long-range Coulombic interactions. In this binding mode, hydration shells of cations and the nucleic acids are considered intact [1–3]; (ii) outer-sphere complexes in which the binding occurs through the water bridges and as a result interacting molecules are partly dehydrated; and (iii) inner-sphere complexes in which cation and the nucleic acid make one or more direct contacts [4–7]. These three binding modes can be characterized by hydration techniques. The advantage of a hydration approach becomes more evident if one recalls the difficulty of studying magnesium ions in solutions by other techniques: Mg^{2+} does not possess paramagnetic properties; and heats of bind-

*Tel.: +1-612-624-7468; fax: +1-612-625-5780.
E-mail address: bkankia@umn.edu (B.I. Kankia).

0301-4622/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0301-4622(03)00125-X

ing to nucleic acids are usually small, although, free energy of binding is considerable, which reveals entropic nature of the binding [8–12]. Increase in entropy usually is attributed to release of solvent (water) molecules to bulk state.

The method of volume and compressibility measurements proved to be successful for cation binding to nucleic acids [13–19]. These properties are very sensitive to hydration of solutions, and can follow transfer of water molecules from bulk state to hydration shells and vice versa. The basis of the measurements is that the molar volume and molar compressibility of pure (bulk) water are significantly larger than the same parameters of first shell water molecules. Upon binding, the hydrated water molecules are released to the bulk state, which is accompanied by an increase in volume and compressibility. The magnitude of the effects is determined by the position of cation relative to the surface of a receptor molecule and can characterize the cation binding modes.

Here we report on hydration effects of magnesium binding to single-stranded ribo and deoxy polynucleotides: poly(rA), poly(rU), poly(dA) and poly(dT). These polynucleotides are characterized by their large degree of structural flexibility [20]. At the experimental conditions used in the present work (neutral pH and 20 °C) poly(rA) and poly(dA) occur as right-handed single-stranded helices with ordered secondary structure due to strong stacking interaction between adenine bases [20,21]. The poly(rA) single helix is a member of A-conformation family with C3′-endo sugar puckering [22]; and poly(dA) is characterized by C2′-endo sugar puckering classifying this helix as a B-conformation [23]. At ambient temperature pyrimidine bases of poly(rU) and poly(dT) are exposed to solvent without any interaction between them, leading to random-coiled conformations [20]. Thus, this set of molecules allows us to study the role of secondary structure and chemical composition of polynucleotides in Mg^{2+} binding process.

The present optical studies have demonstrated that magnesium ions increase stacking interaction of poly(rA), while no influence is observed on secondary structure of poly(dA), poly(rU) and poly(dT). We have also found that increase of magnesium concentration in poly(rA) solutions induce an aggregation/condensation process. Other polynucleotides, poly(dA), poly(rU) and poly(dT), do not undergo a transition to any condensed form in the presence or absence of magnesium ions under present experimental conditions.

From acoustic and density measurements the volume and compressibility effects of Mg^{2+} binding to the polynucleotides have been calculated. For both parameters positive changes are observed, which is interpreted in terms of dehydration of magnesium ions and atomic groups of the polynucleotides. Random-coiled poly(rU) and poly(dT) are characterized by similar dehydration effects corresponding to two direct contacts. The highest dehydration effects, corresponding to inner-sphere complex are observed for Mg^{2+} binding to poly(rA). The hydration effects of Mg^{2+} binding to poly(dA) are negligible, indicating mostly a delocalized binding mode.

2. Experimental section

2.1. Materials

All polynucleotides, poly(rA) (450 KDa), poly(rU) (1300 KDa), poly(dA) (420 KDa) and poly(dT) (1400 KDa) were obtained from Sigma. The polynucleotides were converted into sodium salts by dissolving in 100 mM NaCl, 5 mM EDTA, pH 8 and dialyzed against the final buffer (2 mM Na–Hepes, pH 7.5) for 3–4 days at 4 °C. The concentration of the polynucleotides was determined optically using the molar extinction coefficients in M^{-1} cm^{-1} of nucleotide units: $\varepsilon_{257}=$ 10 700 for poly(rA), $\varepsilon_{260}=9100$ for poly(rU), $\varepsilon_{257}=8600$ for poly(dA) and $\varepsilon_{265}=8700$ for poly(dT). The concentrations were determined in 100 mM NaCl, 10 mM Na–Hepes, pH 7.5 at 20 °C. Analytical grade salts were purchased from Fisher and Merck. The amounts of water in the solid salts were determined by measuring ultrasonic velocities in the aqueous solutions at 25 °C and then comparing to literature data [24]. The salt solutions were prepared using the same buffer. No significant differences in pH of the polynucleotide solutions before and after binding were observed.

All experiments were conducted in 2 mM Na–Hepes, pH 7.5 at 20 °C.

2.2. Optical studies

All UV absorption experiments were conducted on a GBC 918 spectrophotometer equipped with thermoelectrically controlled six-cell holder. Absorbance vs. temperature profiles (melting curves) were measured at either 257, 260 or 320 nm. The temperature was scanned at a heating rate of 0.5 °C per minute. CD spectra were obtained with a JASCO J710 spectropolarimeter equipped with a water-jacketed cuvette holder. Quartz cells with 0.05- and 1-cm path lengths were used in all studies. The optical titrations were performed by adding salt solutions to polynucleotide solutions in the cuvette. Stirring was carried out directly in the cuvette using a vibrating bar.

2.3. Centrifugation study of polynucleotide aggregation

The polynucleotide solutions of 0.4 ml volume (0.1 mM per nucleotide) were mixed with the appropriate amount of $MgCl_2$ solution, and made up to 0.65 ml with the buffer in microcentrifuge tubes. After vortexing and incubation at ambient temperature, the mixtures were centrifuged for 10 min at 14 000 rev./min. The supernatant (0.5 ml) was carefully transferred into 1-cm cuvette and the remaining amount of nucleic acids were determined by absorbance measurements on a GBC 918 spectrophotometer at 20 °C.

2.4. Ultrasound velocity measurements

Relative ultrasound velocity was measured by the resonator technique [25–27]. The molar increment of ultrasonic velocity, A, was calculated using the equation:

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

where U and U_o are the ultrasound velocities in the solution and solvent, respectively; and C is the molar concentration of the polynucleotides per nucleotide. The change of molar increment of ultrasound velocity, ΔA, accompanying the interaction of cations with the polynucleotides was calculated using the equation:

Table 1
Mg^{2+} binding parameters obtained from the acoustic titration curves

Polynucleotide	n	$K\times10^{-4}$ (M^{-1})
Poly(rA)	0.26±0.04	1.8±0.4
Poly(rU)	0.20±0.03	0.7±0.2
Poly(dT)	0.23±0.03	1.1±0.2

$$\Delta A=A-A_o \quad (2)$$

where A is the molar increment of ultrasound velocity of the polynucleotide+cation solution relative to buffer+cation and A_o is the molar increment of ultrasound velocity of polynucleotide solution relative to buffer. ΔA values per mole of phosphate have been obtained from the ultrasound titration curves at $[Mg^{2+}]/[P]=0.44$. ΔA values per mole of cations have been calculated in two separate ways: (i) from the initial points of the titration curves; and/or (ii) multiplying number of binding sites, n, from Table 1 on the ΔA values per mole of phosphates; for poly(dA) $n=0.5$ have been used.

2.5. Density measurements

The density of solutions was measured with DMA 602 and 5000 densimeters (Anton Paar). The molar apparent volume was calculated using the equation [28]:

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

where ρ_o and ρ are the density of the solvent and solution, respectively; and M is the molecular mass of the polynucleotides per nucleotide unit. The volume changes, ΔV, accompanying cation interaction with the polynucleotides were calculated

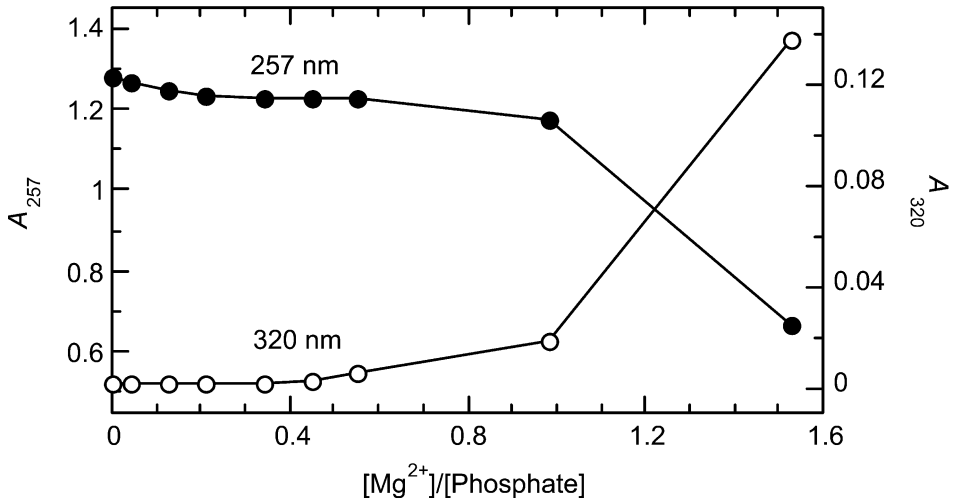

Fig. 1. Optical titration of poly(rA) with Mg^{2+} in 2 mM Na–Hepes, pH 7.5 at 20 °C. The concentration of the polynucleotides was 2.4 mM per phosphate.

using the equation:

$$\Delta V = \Phi V - \Phi V_o \quad (4)$$

where ΦV is the apparent molar volume of polynucleotide + cation solution relative to buffer + cation, and ΦV_o is the apparent molar volume of a polynucleotide solution relative to buffer. The volume effects of binding were measured as batch experiments mixing certain amounts of polynucleo-
tide and salt solutions on the balance.

2.6. Calculation of the change in apparent molar adiabatic compressibility

The apparent molar adiabatic compressibility, $\Delta\kappa_S$, was determined as a function of changes in the increment of ultrasonic velocity and apparent molar volume [29,30]:

$$\Delta\kappa_S = 2\beta_o(\Delta\Phi V - \Delta A) \quad (5)$$

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:

$$\beta_o = (\rho_o U_o^2)^{-1} \quad (6)$$

3. Results and discussion

3.1. Effects of Mg^{2+} on the secondary structure of the polynucleotides

No effects were seen arising in UV or CD spectra of poly(dA), poly(dT) and poly(rU) from magnesium chloride (data not shown). However, both optical techniques detect some stabilization effect on the poly(rA) single-helix. Fig. 1 shows optical absorbance of poly(rA) at 257 and 320 nm as a function of the concentration ratio, $[Mg^{2+}]/[P]$. The latter wavelength has been used for monitoring the condensation/aggregation process that can accompany multivalent cation binding to nucleic acids. At the initial stage of binding, up to $[Mg^{2+}]/[P] = 0.3$, Mg^{2+} induces a slight decrease in absorption at 257 nm, approximately 4%. This result demonstrates that additional base stacking takes place in single-stranded helical structure of poly(rA). Similarly, CD titration of poly(rA) by Mg^{2+} showed an increase of the signal at both positive (264 nm) and negative (249 nm) peaks

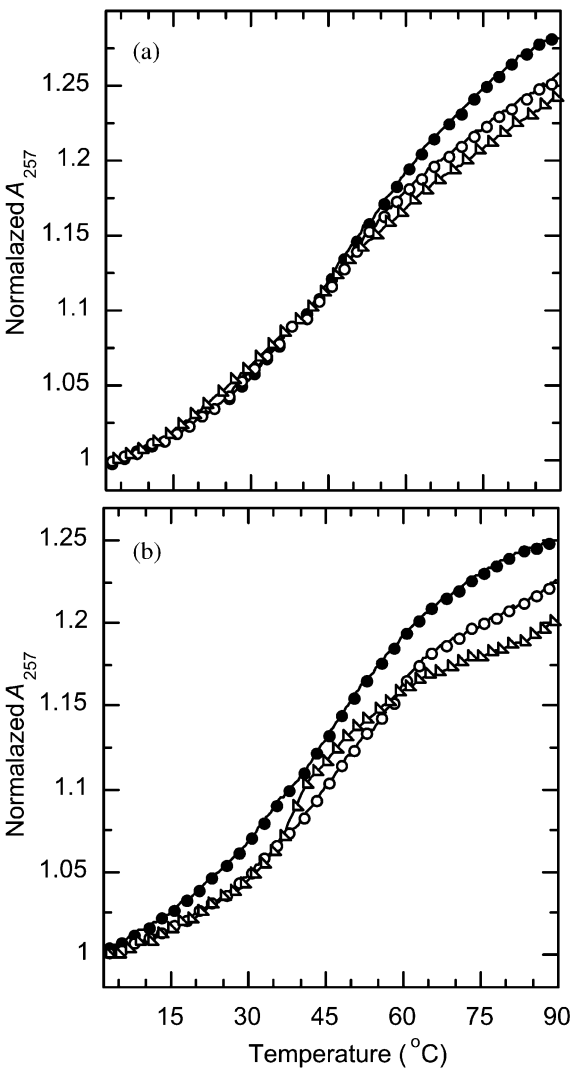

Fig. 2. UV melting profiles of poly(dA) (a) and poly(rA) (b) without Mg^{2+} (filled circles), with 1 mM Mg^{2+} (open circles) and with 4 mM Mg^{2+} (triangles). The concentration of the polynucleotides was 0.10 mM per phosphate. In the case of poly(rA), melting curves with Mg^{2+} have been relocated for clarity.

by ~10% (data not shown) also indicating a stabilization effect. Total unfolding of the single-helical structure of poly(rA) is accompanied by ~25% increase in UV signal at 257 nm (Fig. 2b) and ~80% drop of CD signal at 264 nm [31]. Thus, 4% decrease in UV and 10% increase of CD signals in our experiments agree well with each other and correspond to 10–15% of total folding effect of single-helical structure of poly(rA).

After $[Mg^{2+}]/[P] = 0.5$, the absorbance at 257 nm drops (Fig. 1), accompanied by increase in absorption at 320 nm; this corresponds to aggregation and/or precipitation of Mg^{2+}-poly(rA). No aggregation was observed for the other polynucleotides under the same experimental conditions.

3.2. UV melting curves

Fig. 2 shows the thermal unfolding profiles of poly(dA) and poly(rA) single helices with and without magnesium ions. The presence of magnesium ions has little influence on melting behavior of poly(dA). The only observable effect is the decrease of the overall hyperchromic effect with increasing Mg^{2+} concentration, which can be explained by non-specific electrostatic stabilization of the single-helical structure by the divalent cations. In poly(rA) solutions, the stabilization effect of Mg^{2+} is profound: the melting curves are shifted to higher temperatures (by 7 °C). In addition, the curves with magnesium ions exhibit complicated shapes (Fig. 2b). For instance, at 4 mM concentration of magnesium ions, one can clearly see some transition process at approximately 40 °C, which levels off at approximately 70 °C. A further increase of temperature is again accompanied by an increase in A_{257}. Additional experiments revealed that the melting profiles are perturbed by condensation of poly(rA) in the presence of magnesium ions: the effects at 257 nm are accurately accompanied by changes at 320 nm (Fig. 3). Both heating and magnesium ions favor the aggregation process: at higher concentrations of Mg^{2+} lower temperature is needed to initiate the aggregation process. For instance, at 1.0 mM, Mg^{2+} aggregation starts after 55 °C, while at 8.1 mM is approximately 25 °C (Fig. 3). At all three Mg^{2+} concentrations, a further increase in temperature results in the dropping of A_{320} value to zero level indicating resolubilization of poly(rA) molecules. In the presence of 1.0 mM Mg^{2+} this happens at approximately 80 °C, and at higher concentrations at approximately 95 °C (Fig.

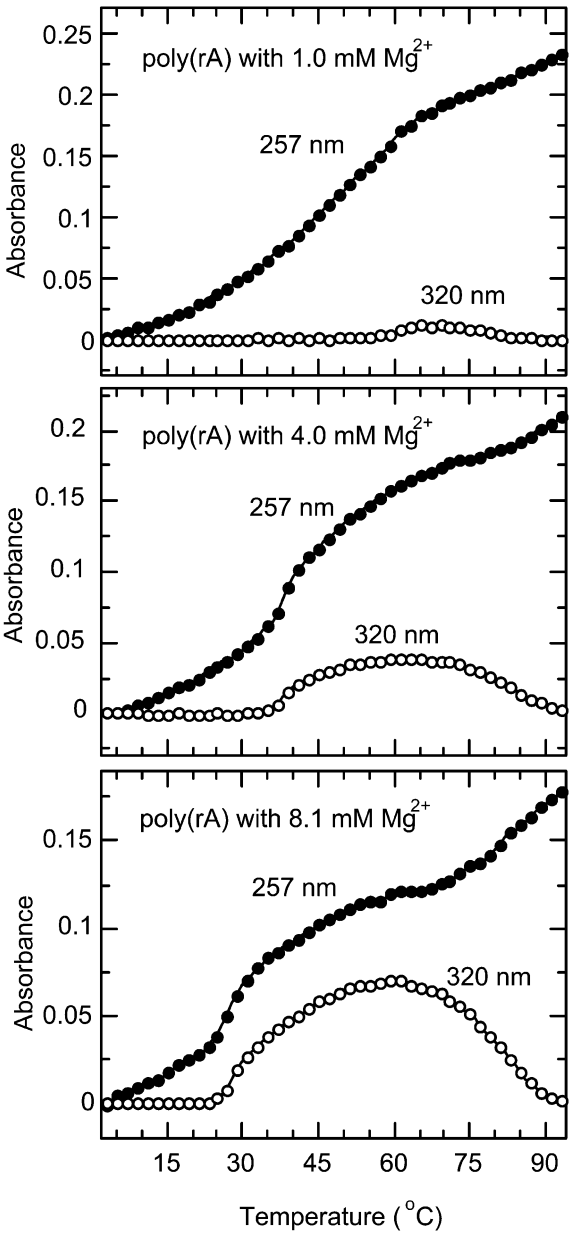

Fig. 3. UV melting curves of poly(rA) with different concentration of Mg^{2+} at 257 and 320 nm. The concentration of poly(rA) was 0.10 mM per phosphate. The melting curves at 257 nm have been relocated to zero level for clarity. Note simultaneous changes at both wavelengths due to aggregation and resolubilization of poly(rA).

3). Heat-induced aggregation of DNA was observed earlier in presence of different cations, including Mg^{2+}, however, at significantly higher temperature and concentrations [32].

No aggregation was seen for poly(rA) in the absence of magnesium ions, or in the absence or presence of similar amounts of Mg^{2+} in poly(dA), poly(dT) and poly(rU) solutions (data not shown). A comprehensive study of the condensation and the resolubilization process, and its dependence on temperature, is beyond the objective of the present paper. We would only like to emphasize here that for the heat-induced condensation of poly(rA) some particular interaction with Mg^{2+} is required, which is absent in the case of poly(dA), poly(dT) or poly(rU).

3.3. Precipitation curves

Fig. 4 demonstrates the effect of Mg ions on the precipitation of the polynucleotides. The presence of Mg ions has no visible effect on precipitation of poly(dA), poly(dT) and poly(rU) up to physiologically unreasonably high concentration of the cation, ~1 M. In contrast, the precipitation of poly(rA) molecule starts at around physiological concentrations of magnesium ions, 0.015 M. Approximately 0.030 M of magnesium ions almost all poly(rA) is collapsed, and a further increase in magnesium concentration does not induce resolubilization of the nucleic acid, which is characteristic for highly charged condensing agents, such as spermine and spermidine [33].

3.4. Acoustic titration curves

In the acoustic experiments, the sodium salts of the polynucleotides have been titrated by magnesium chloride (Fig. 5). The shapes of all curves are similar: increasing the titrant concentration is accompanied by a decrease in increment of ultrasound velocity, which is the result of the hydration effects of binding [14,16–19]. No significant changes in ΔA value are observed after $[Mg^{2+}]/[P] \approx 0.4$. This indicates the saturation of the polynucleotides by Mg^{2+} or further interaction without detectable hydration effects. The hydration effects of the binding will be discussed in the forthcoming

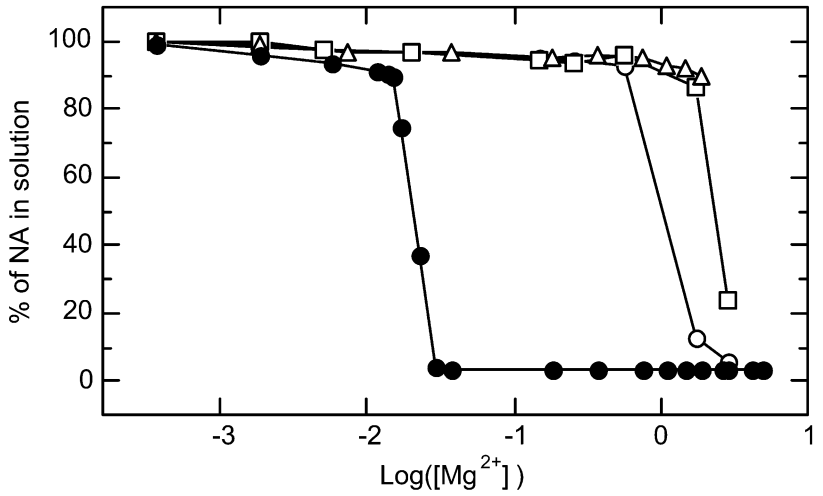

Fig. 4. Precipitation of poly(rA) (filled circles), poly(dA) (open circles), poly(rU) (triangles) and poly(dT) (squares) by Mg ions in 2 mM Na–Hepes, pH 7.5 at 20 °C. The concentration of the polynucleotides was 0.08–0.1 mM phosphate.

sections. In the present section, only binding parameters obtained from acoustic experiments are discussed.

Similar to calorimetric or optical titrations, the ultrasonic titrations can be considered as binding isotherms and one can estimate the binding parameters: number of binding sites, n, and binding constants, K. It was found that a simple model with a single type of non-cooperative binding sites accurately describes the acoustic titration curves. The model is described elsewhere [17,18]. The solid lines in Fig. 5 are results of the non-linear fitting based on this model. The obtained n and K values are collected in Table 1. For poly(dA) molecule, the fitting revealed physically unreasonable values because of insignificant changes in the increment of ultrasound velocity and, therefore, are not listed in Table 1.

The number of binding sites per phosphate is between 0.20 and 0.26. They are well below of $n=0.5$, which is expected from a simple charge compensation. However, similarly small values of n were obtained earlier. For instance, potentiometric studies on Mg^{2+} binding to poly(rA) and poly(rU) showed $n=0.3$ [34]. Porschke [35] in his photometric experiments also revealed $n=$ 0.19–0.29 for different divalent cations and polyribonucleotides. Our binding constants are in the range of $(0.7–1.8)\times10^4$ M^{-1} (Table 1), which are also in good agreement with earlier measured values [34,35].

3.5. Molecular interpretation of volume and compressibility effects

For a dilute solution the molecular interpretations of the apparent molar volume, ΦV, and the apparent molar adiabatic compressibility, $\Phi\kappa_S$, are based on the following simple relationships [36]:

$$\Phi V = V_m + \Delta V_h \tag{7}$$

$$\Phi\kappa_S = \kappa_m + \Delta\kappa_h \tag{8}$$

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. $\Delta\kappa_h$ is the hydration contribution to the apparent molar adiabatic compressibility, consisting of the changes in the compressibility of water around the solute

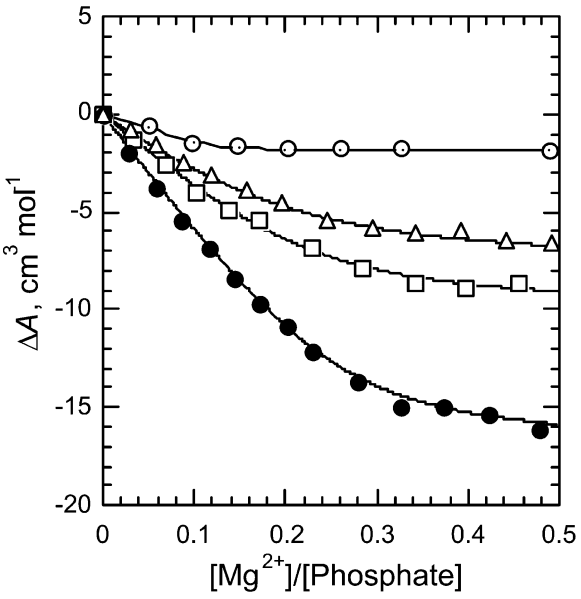

Fig. 5. Ultrasonic titration curves of the polynucleotides with Mg^{2+} in 2 mM Na–Hepes, pH 7.5 at 20 °C. Solid lines are the result of non-linear three parameter fitting. The concentration of each polynucleotide was 2–2.5 mM per phosphate.

molecule and the compressibility of the voids between the solute molecules and the surrounding water. Overall, volume and compressibility effects of cation binding to the polynucleotides, ΔV and $\Delta\kappa_S$, can be expressed as:

$$\Delta V = \Delta V_m + \Delta\Delta V_{h,b} \quad (9)$$

$$\Delta\kappa_S = \Delta\kappa_m + \Delta\Delta\kappa_{h,b} \quad (10)$$

where ΔV_m and $\Delta\kappa_m$ are the effects on intrinsic molar volume and intrinsic molar adiabatic compressibility, respectively, due to structural changes of the polynucleotides and $\Delta\Delta V_{h,b}$ and $\Delta\Delta\kappa_{h,b}$ are the hydration effects of the binding process. Thus, to get net effect of the binding process one should estimate ΔV_m and $\Delta\kappa_m$ terms in Eqs. (9) and (10). Since no structural perturbations of poly(dA), poly(dT) and poly(rU) have been observed, ΔV_m and $\Delta\kappa_m$ terms for these polynucleotides are equal to zero. As it was shown earlier, in the case of poly(rA) we see 10–15% stabilization effect or, in other words, improved stacking interactions between adenine bases. It appears that the stabilization effect cannot contribute significantly in the volume and compressibility effects. The stacking interactions between purine bases decrease volume by 4 $cm^3\ mol^{-1}$ and increase compressibility by 7×10^{-4} $cm^3\ mol^{-1}\ bar^{-1}$ [37]. Table 2 shows volume and compressibility effects of poly(rA) + Mg^{2+} before and after correction.

The volume and compressibility effects in our experiments is the result of exchange of monovalent sodium ions for divalent magnesium in ionic atmosphere of the polynucleotides. However, the hydration contribution from release of sodium ions is considered negligible, due to the fact that monovalent cations maintain a full hydration shell in the ionic atmosphere of nucleic acids [1].

In the present work, ultrasonic and density experiments on Mg^{2+} binding to the polynucleotides are accompanied by 10% dilution of the polynucleotide solution, and the aggregation of poly(rA). It is important to see whether these processes alter the hydration parameters. The concentration dependence of A of poly(rA) were examined and no effects were seen between 1 and 5 mM phosphate (data not shown). This is in very good agreement with earlier measurements on calf thymus DNA, which did not show any concentration dependence between 0.2 and 13 mM phosphate [17]. As one can see from Fig. 1, performed at high concentration of poly(rA) (2.4 mM phosphate), aggregation starts only after $[Mg^{2+}]/[P] = 0.5$, while ultrasonic and density values are collected before this point.

Table 2
Hydration effects of Mg^{2+} binding to the polynucleotides calculated per mole of the cation

Polynucleotide	ΔV ($cm^3\ mol^{-1}$)	$\Delta\kappa_S\times10^4$ ($cm^3\ mol^{-1}\ bar^{-1}$)
Poly(rA)	29.6±3	79.9±7
	31.9[a]	75.9[a]
Poly(rU)	14.8±3	39.5±6
Poly(dA)[b]	1.9±3	4.5±6
Poly(dT)	16.1±2	49.5±5

[a] Corrected for stacking contribution (see text).
[b] For poly(dA) the values are calculated using $n = 0.5$.

3.6. Dehydration effects and binding modes

Volume and compressibility effects of Mg^{2+} interaction with all polynucleotides are positive (Table 2). The interaction is accompanied by overlapping the hydration shells of the interacting molecules (Mg ions and atomic groups of the polynucleotides) and thereby releasing water molecules to the bulk state.

The hydration contributions to the apparent molar volume and apparent molar adiabatic compressibility are mainly determined by the first coordination layer of water around solute molecules [38,39]. Therefore, if adding Mg^{2+} to nucleic acid solution is not accompanied by visible volume and compressibility effects, the interaction can be interpreted in terms of delocalized mode. A good example of such binding is poly(dA)+Mg^{2+}, which is characterized by small dehydration effects, less than the experimental uncertainties (Table 2). Thus, in poly(dA)+Mg^{2+} system both interacting molecules keep intact most of their hydration shells and binding attributed to diffuse type or delocalized mode. However, it must be recognized that the small dehydration effects can be indicative of a fraction of outer-sphere binding.

For quantitative analysis of the dehydration effects of poly(rA), poly(rU) and poly(dT) (see Table 2), one should estimate volume and compressibility effects of a coordination bond, or dehydration effect due to replacement of a water molecule from its first hydration shell by a ligand group of the polynucleotides. These values can be estimated from the overall hydration parameters of magnesium ion and peripheral atomic groups of the nucleic acids. The values for Mg^{2+} are -33.4 cm^3 mol^{-1} and -63.4×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively [40]. Keeping in mind that the hydration number of cations is usually equal to the total coordination number in cation–chelator complexes [41,42], replacement of hydrated water molecules by another ligand (a direct contact) will cause an increase in volume and compressibility by 5.6 cm^3 mol^{-1} and 10.6×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively. Note positive signs of the parameters due to release of water molecules from hydration shells. Similar or somewhat less dehydration effects are expected from atomic groups of nucleic acids [43]. Thus, one direct contact should cause increase in volume and compressibility not more than 11 cm^3 mol^{-1} and 21×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively.

More precise estimation can be done from volume and compressibility effects of Mg^{2+} binding to a chelator with well-characterized structural properties. An example is ethylenediaminetetraacetic acid (EDTA), which has six atomic groups involved in coordination to magnesium ion: four negatively charged oxygens and two polar nitrogens [41]. The use of $EDTA^{4-}$ as a model system is well justified. Its ligand groups are similar to peripheral residues of the nucleic acids, phosphate oxygens and nucleic base nitrogens, which are the most plausible binding sites for divalent cations [20,44–50]. Binding of Mg^{2+} to $EDTA^{4-}$ leads to compressibility and volume effects of 131.1×10^{-4} cm^3 mol^{-1} bar^{-1} and 46.6 cm^3 mol^{-1}, respectively [51]. Keeping in mind that six atomic groups of $EDTA^{4-}$ are involved in Mg^{2+} chelaton, dehydration effects of one direct contact will be equal to 8 cm^3 mol^{-1} and 22×10^{-4} cm^3 mol^{-1} bar^{-1}, respectively. Thus, dehydration effects less than these values should correspond to outer-sphere complex. However, the dehydration effects of Mg^{2+} binding to poly(rA), poly(rU) and poly(dT) (see Table 2) are significantly higher than dehydration effects of one direct contact, indicating on inner-sphere complexes. In the case of random-coiled poly(rU) and poly(dT), volume and compressibility effects correspond to two direct coordination, while the same parameters for poly(rA) correspond to three–four direct contacts.

Porschke [52] in his relaxation studies by electric field jump techniques, found a slow relaxation process, typical for inner-sphere complexes, for an aqueous solution of oligo(rA) in the presence of magnesium ions. However, the same technique revealed outer-sphere complexes with poly(rA) [5,35]. Melting studies also suggested direct coordination of Mg^{2+} to the ribosomal RNA [53].

The present observations, with exception of poly(rA), can be explained by flexibility of the polynucleotide backbone. Due to base stacking interactions, the ribose–phosphate backbone of single-stranded polypurine molecules is known to exhibit considerably more rigidity than that of

polypyrimidines [54,55]. Larger dehydration effects can be expected to occur for the polypyrimidine molecules with larger backbone flexibility, allowing divalent magnesium ions to attract two phosphate groups at sufficiently close distances to create coordination bonds. Conversely, much smaller dehydration effects would be expected for polypurine (more rigid) molecules with unsuitably large interphosphate distances. The experimental data in Table 2, except for the Mg^{2+}-poly(rA) system, bear out this expectation. Mg^{2+} binding to unstacked polypyrimidines, poly(rU) and poly(dT), is accompanied by dehydration effects corresponding to two direct contacts, while rigid poly(dA) with interphosphate distance of 7 Å[20] is not favorable for such binding. Similarly, one should not expect significant dehydration effects from Mg^{2+} binding to the polyribopurine molecule, poly(rA). Nevertheless, it shows unusually high dehydration effects (Table 2). A similar picture was observed in an earlier study of Mg^{2+} binding to single-stranded ribo and deoxy oligomers with different purine–pyrimidine composition and, consequently, different flexibility [16]. The dehydration effect of Mg^{2+} binding to the flexible deoxy oligomer was twice as high as that of the less flexible complementary deoxy strand, while for the ribo analogs the opposite effect was observed: the most rigid oligomer showed the largest dehydration effects [16]. Thus, the dehydration effects of the binding indicate specific binding of Mg^{2+} to nucleic bases of poly(rA).

Acknowledgments

I am indebted to Prof. Victor Bloomfield for his support, stimulating discussions and reading the manuscript. I thank Prof. Thomas Record and Dr Michael Capp of the University of Wisconsin for use of the densimeter. I would also like to thank Salome Boroda for technical assistance in the precipitation experiments. This investigation was supported by NIH grant GM28093 to Victor Bloomfield.

References

[1] G.S. Manning, The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides, Q. Rev. Biophys. 11 (1978) 179–246.

[2] C.F. Anderson, M.T. Record Jr., Ion distributions around DNA and other cylindrical polymers: theoretical descriptions and physical implications, Ann. Rev. Biophys. Biophys. Chem. 19 (1990) 423–465.

[3] C.F. Anderson, M.T. Record Jr., Salt-nucleic acid interactions, Ann. Rev. Phys. Chem. 46 (1995) 657–700.

[4] J.A. Cowan, The biological chemistry of magnesium, in: J.A. Cowan (Ed.), Biological Chemistry of Magnesium Ions with Physiological Metabolites, Nucleic Acids, and Drug Molecules, VCH Publishers, New York, 1995, p. 185.

[5] D. Porschke, The biological chemistry of magnesium, in: J.A. Cowan (Ed.), Models and Dynamics of Mg^{2+}-Polynucleotide Interactions, VCH Publishers, New York, 1995, p. 85.

[6] V.K. Misra, D.E. Draper, On the role of magnesium ions in RNA stability, Biopolymers 48 (1999) 113–135.

[7] V.K. Misra, D.E. Draper, A thermodynamic framework for Mg^{2+} binding to RNA, Proc. Natl. Acad. Sci. USA 98 (2001) 12456–12461.

[8] H. Krakauer, The binding of Mg^{2+} ions to polyadenylate, polyuridylate, and their complexes, Biopolymers 10 (1971) 2459–2490.

[9] H. Krakauer, A calorimetric investigation of the heats of binding of Mg^{2+} to poly A, to poly U, and their complexes, Biopolymers 11 (1972) 811–828.

[10] H. Krakauer, A thermodynamic analysis of the influence of simple mono- and divalent cations on the conformational transitions of polynucleotide complexes, Biochemistry 13 (1974) 2579–2589.

[11] P.D. Ross, J.T. Shapiro, Heat of interaction of DNA with polylysine, Spermine and Mg^{2+}, Biopolymers 13 (1974) 415–416.

[12] T. Ohyama, J.A. Cowan, An approach to the evaluation of RNA solution structure and metal coordination by titration calorimetry, J. Biol. Inorg. Chem. 2 (1997) 553–554.

[13] V.A. Buckin, B.I. Kankiya, A.P. Sarvazyan, H. Uedaira, Acoustical investigation of poly(dA).poly(dT), poly[d(A-T)].poly[d(A-T)], poly(A).poly(U) and DNA hydration in dilute aqueous solutions, Nucleic Acids Res. 17 (1989) 4189–4203.

[14] V.A. Buckin, B.I. Kankiya, D. Rentzeperis, L.A. Marky, Mg^{2+} recognizes the sequence of DNA through its hydration shell, J. Am. Chem. Soc. 116 (1994) 9423–9429.

[15] V.A. Buckin, H. Tran, V. Morozov, L.A. Marky, Hydration effects accompanying the substitution of counterions in the ionic atmosphere of poly(rA).poly(rU) and poly(rA).2poly(rU) helices, J. Am. Chem. Soc. 118 (1996) 7033–7039.

[16] B.I. Kankia, L.A. Marky, DNA, RNA and DNA/RNA oligomer duplexes: a comparative study of their stability, heat, hydration and Mg^{2+} binding properties, J. Phys. Chem. B 103 (1999) 8759–8767.

[17] B.I. Kankia, Interaction of alkaline-earth metal ions with calf thymus DNA. Volume and compressibility effects in diluted aqueous solutions, Biophys. Chem. 84 (2000) 227–237.
[18] B.I. Kankia, Hydration effects of Ni^{2+} binding to synthetic polynucleotides with regularly alternating purine–pyrimidine sequences, Nucleic Acids Res. 28 (2000) 911–916.
[19] B.I. Kankia, V.A. Buckin, V.A. Bloomfield, Hexamminecobalt(III)-induced condensation of calf thymus DNA: circular dichroism and hydration measurements, Nucleic Acids Res. 29 (2001) 2795–2801.
[20] W. Saenger, Principles of Nucleic Acid Structure, Springer-Verlag, New York, 1984.
[21] V.A. Bloomfield, D.M. Crothers, I.J. Tinoco, Nucleic Acids: Structures, Propeties and Functions, University Sciences Books, Sausalito, 2000.
[22] W. Saenger, J. Riecke, D. Suck, A structural model for the polyadenylic acid single helix, J. Mol. Biol. 93 (1975) 529–534.
[23] C.S.M. Olsthoorn, L.J. Bostelaar, J.H. van Boom, C. Altona, Conformational characteristics of the trinucleoside diphosphate dApdApdA and its constituents from nuclear magnetic resonance and circular dichroism studies, Eur. J. Biochem. 112 (1980) 95–110.
[24] F.J. Millero, G.K. Ward, P.V. Chetirkin, Relative sound velocities of sea salts at 25 °C, J. Acoust. Soc. Am. 61 (1977) 1492–1498.
[25] F. Eggers, T. Funck, Ultrasonic measurements with milliliter liguid samples in the 0.5–100 MHz range, Rev. Sci. Instrum. 44 (1973) 969–977.
[26] F. Eggers, U. Kaatze, Broad-band ultrasonic measurement techniques for liquids, Meas. Sci. Technol. 7 (1996) 1–19.
[27] A.P. Sarvazyan, Development of methods of precise ultrasonic measurements in small volumes of liquids, Ultrasonics 20 (1982) 151–154.
[28] F.J. Millero, Water and aqueous solutions, in: R.A. Horne (Ed.), The Partial Molar Volumes of Electrolytes in Aqueous Solutions, Wiley-Interscience, New York, 1972, p. 519.
[29] S. Barnatt, The velocity of sound in electrolytic solutions, J. Chem. Phys. 20 (1952) 278–279.
[30] B.B. Owen, H.L. Simons, Standard partial molal compressibilities by ultrasonics. I. Sodium chloride and potassium chloride at 25 °C, J. Phys. Chem. 61 (1957) 479–482.
[31] J. Brahms, A.M. Michelson, K.E. Van Holde, Adenylate oligomers in single- and double-strand conformation, J. Mol. Biol. 15 (1966) 467–488.
[32] D.A. Knoll, M.G. Fried, V.A. Bloomfield, Structure and expression. DNA and its drug complexes, in: R.H. Sarma, M.H. Sarma (Eds.), Heat-Induced DNA Aggregation in the Presence of Divalent Metal Salts, Adenine Press, Albany, 1988, p. 123.
[33] M. Saminathan, T. Antony, A. Shirahata, L.H. Sigal, T. Thomas, T.J. Thomas, Ionic and structural specificity effects of natural and synthetic polyamines on the aggregation and resolubilization of single-, double- and triple-stranded DNA, Biochemistry 38 (1999) 3821–3830.
[34] C. Sander, P.O.P. Tso, Interaction of nucleic acids. VIII. Binding of magnesium ions by nucleic acids, J. Mol. Biol. 55 (1971) 1–21.
[35] D. Porschke, Thermodynamic and kinetic parameters of ion condensation to polynucleotides. Outer sphere complex formed by Mg^{2+} ions, Biophys. Chem. 4 (1976) 383–394.
[36] H. Shio, T. Ogawa, H. Yoshihashi, Measurement of the amount of bound water by ultrasonic interferometer, J. Am. Chem. Soc. 77 (1955) 4980–4982.
[37] H. Hoiland, A. Skauge, I. Stokkeland, Changes in partial molar volumes and isentropic partial molar compressibilities of stacking of some nucleobases and nucleosides in water at 298.15 K, J. Phys. Chem. 88 (1984) 6350–6353.
[38] V.A. Buckin, A.P. Sarvazyan, D.P. Kharakoz, Water in disperce systems, in: B.V. Deryagin, N.V. Churayev, F.D. Ovcharenko (Eds.), Water Around Biological Molecules, Kmimiya, Moscow, 1989, p. 45.
[39] T.V. Chalikian, A.P. Sarvazyan, K.J. Breslauer, Hydration and partial compressibility of biological compounds, Biophys. Chem. 51 (1994) 89–109.
[40] A. Lo Surdo, A.F. Millero, Apparent molal volumes and adiabatic compressibilities of aqueous transition metal chlorides at 25 °C, J. Phys. Chem. 84 (1980) 710–715.
[41] J.J. Stezowski, R. Countryman, J.L. Hoard, Structure of the ethylendiaminetetraacetato-aquomagnesate(II) ion in a crystalline sodium salt. Comparative stereochemistry of the seven-coordinate chelates of magnesium(II), manganese(II), and iron(III), Inorg. Chem. 12 (1973) 1749–1754.
[42] B.L. Barnett, V.A. Uchtman, Structural investigations of calcium-binding molecules. 4. Calcium binding to aminocarboxylates. Crystal structures of Ca(CaEDTA).7H_2O and Na(CaNTA), Inorg. Chem. 18 (1979) 2674–2678.
[43] V.A. Buckin, B.I. Kankiya, R.L. Kazaryan, Hydration of nucleosides in dilute aqueous solutions. Ultrasonic velocity and density measurements, Biophys. Chem. 34 (1989) 211–223.
[44] S. Hanlon, A. Chan, S. Berman, Specific cation effects on conformational transitions of DNA in aqueous solutions, Biochim. Biophys. Acta 519 (1978) 526–536.
[45] K. Grzeskowiak, K. Yanaga, G.G. Prive, R.E. Dickerson, The structure of B-helical C-G-A-T-C-G-A-T-C-G and comparison with C-C-A-A-C-G-T-T-G-G. The effect of base pair reversals, J. Biol. Chem. 266 (1991) 8861–8883.
[46] H.A. Tajmir-Riahi, Interaction of guanilic acid with the Mg(II), Ca(II), Sr(II), and Ba(II) ions in the crystalline solid and aqueous solution: evidence for the ribose C2′-endo/anti and C3′-endo/anti conformational changes, Biopolymers 31 (1991) 101–108.

[47] J. Duguid, J.M. Benevides, V.A. Bloomfield, G.J.J. Thomas, Raman spectroscopy of DNA-metal complexes. I. Interactions and conformational effects of the divalent cations: Mg, Ca, Sr, Ba, Mn, Co, Ni, Cu, Pd, and Cd, Biophys. J. 65 (1993) 1916–1928.
[48] J.G. Duguid, V.A. Bloomfield, J.M. Benevides, G.J.J. Thomas, Raman spectroscopy of DNA–metal complexes. II. The thermal denaturation of DNA in the presence of Sr^{2+}, Ba^{2+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+} and Cd^{2+}, Biophys. J. 69 (1995) 2623–2641.
[49] I. Rouzina, V.A. Bloomfield, DNA bending by small, mobile multivalent cations, Biophys. J. 74 (1998) 3152–3164.
[50] X. Shui, L. McFail-Isom, G.G. Hu, L.D. Williams, The B-DNA dodecamer at high resolution reveals a spine of water on sodium, Biochemistry 37 (1998) 8341–8355.
[51] B.I. Kankia, T. Funck, H. Uedaira, V.A. Buckin, Volume and compressibility effects in the formation of metal–EDTA complexes, J. Sol. Chem. 26 (1997) 877–888.
[52] D. Porschke, The mode of Mg^{2+} binding to oligonucleotides. Inner sphere complexes as markers for recognition?, Nucleic Acids Res. 6 (1979) 883–898.
[53] L.G. Laing, T.C. Gluick, D.E. Draper, Stabilization of RNA structure by Mg ions. Specific and non-specific effects, J. Mol. Biol. 237 (1994) 577–587.
[54] F.E. Evans, R.H. Sarma, Nucleotide rigidity, Nature 263 (1976) 567–572.
[55] J.B. Mills, E. Vacano, P.J. Hagerman, Flexibility of single-stranded DNA: use of gapped duplex helices to determine the persistence lengths of poly(dT) and poly(dA), J. Mol. Biol. 285 (1999) 245–257.