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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

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ABSTRACT

Apparent molar adiabatic compressibilities and apparent molar volumes of poly[d(A-T)]·poly[d(A-T)], poly(dA)·poly(dT), DNA and poly(A)·poly(U) in aqueous solutions were determined at 1°C. The change of concentration increment of the ultrasonic velocity upon replacing counter ion Cs^+ by the Mg^{2+} ion was also determined for these polymers. The following conclusions have been made: (1) the hydration of the double helix of poly(dA)·poly(dT) is remarkably larger than that of other polynucleotides; (2) the hydration of the AT pair in the B-form DNA is larger than that of the GC pair; (3) the substitution of Cs^+ for Mg^{2+} ions as counter ions results in a decrease of hydration of the system polynucleotide plus Mg^{2+}, and (4) the magnitude of this dehydration depends on the nucleotide sequence; the following rule is true: the lesser is a polynucleotide hydration, the larger dehydration upon chaning Cs^+ for Mg^{2+} ions in the ionic atmosphere of polynucleotide.

INTRODUCTION

The interaction between nucleic acids and water (hydration) is one of the important factors, which determine the structure and physico-chemical properties of nucleic acids. There are many experimental and theoretical studies on their hydration.[1-4] The most interesting results were obtained from the investigations on the hydration of moist samples: fibers, sheets, and crystals. But it is little known about the hydration of nucleic acids in solution.

The dependence of the hydration of the double helix upon its shape, nucleotide sequence and composition, and ionic environment is the most important point in the problem of hydration of nucleic acids. Data on the hydration of crystalline DNA show the difference in hydration of the B, A, and Z forms of DNA,[5-7] and also the dependence of hydration of[5]

the B form on the base composition. In the case of the DNA solution, only the dependence of the DNA hydration on the base composition has been investigated.[8,9]

In this work, the hydration of poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)], which have the B-form of the double helix and the same nucleotide composition but a different nucleotide sequence, was studied. The hydration of poly(A)·poly(U), which have the A form of the double helix, and the double-stranded salmon sperm DNA was also investigated. Moreover, we studied the changes of hydration resulting from the change in the ionic strength and the substitution of the counterion Cs^+ for Mg^{2+} ion in the ionic atmosphere of the double helix.

By measuring the density and ultrasonic velocity, we determined the apparent molar volume ϕ_v, apparent molar adiabatic compressibility ϕ_{ks}, and the change of concentration increment of ultrasonic velocity occurring upon the exchange of counter ions in the ionic atmosphere of polymer, ΔA. The ϕ_v and ϕ_{ks} values are defined by the following relations

$$\phi_v = (V - n_1\bar{V}_1^o)/n_2; \qquad \phi_{ks} = (K - n_1\bar{K}_1^o)/n_2,$$

where V and K are the volume and adiabatic compressibility of a solution which contains n_1 moles of the solvent and n_2 moles of the solute (in this work, n_2 is the molar amount of nucleotide in solution); $\bar{V}_1^o$ and $\bar{K}_1^o$ are the molar volume and molar compressibility of the pure solvent, respectively. The values of ϕ_v have been calculated from the solution density data. The values of ϕ_{ks} have been calculated by the equation which is true for dilute solutions[10]

$$\phi_{ks} = 2\beta_o(\phi_v - A - M/2\rho_o) \qquad (1)$$

where M is the molecular weight of the solute (in the case of polynucleotide it will be the mean molecular weight of the nucleotide), and ρ_o and β_o are the density and the adiabatic compressibility coefficient of the pure solvent, respectively. The concentration increment of ultrasonic velocity A is defined by

$$A = (u-u_o)/(u_o c\rho_o) \qquad (2)$$

where u and u_o are the ultrasonic velocities in the solution and pure solvent, respectively, and c is the molal concentration of the nucleotide.

For a dilute solution, ϕ_v is given by[11]

$$\phi_v = V_M + \Delta V_h$$

where V_M is the intrinsic molar volume of a solute molecule which is inaccessible to surrounding molecules of the solvent and is determined by its stereochemical structure; ΔV_h represents the hydration contribution and consists of the volume change of the solvent around the solute molecule as a result of the solute-solvent interactions and the void volume between the solute molecule and the surrounding solvent. Similarly, ϕ_{ks} is given by[11]

$$\phi_{ks} = K_M + \Delta K_h + K_r$$

where K_M is the intrinsic molar compressibility of the solute, ΔK_h represents the hydration contribution and, like ΔV_h, depends on the solute-solvent interaction. K_r is the relaxation compressibility, which may exist if any relaxation processes occur in the system, e.g. the changes in the distribution between different conformations with temperature and pressure. We can estimate the value of K_r from the data of the frequency dependence of ultrasonic absorption. At present, there are data of ultrasonic absorption on solution of nucleosides,[12-14] nucleotides,[15,16] polynucleotides and DNA.[17-19] Our estimations based on these data show that the value of K_r for the polynucleotide double helix is so small that it may be neglected in comparison with the experimental error of ϕ_{ks}.

The DNA double helix was a very compact structure with a small void volume. The packing coefficient for the A and B forms of the double helix, which is represented by the ratio of the van der Waals volume of nucleotide to its intrinsic volue V_M, is very large. It is equal to 0.87. (The value of the van der Waals volume was calculated from the data of Bondi[20] and Edward,[21] and that of V_M was taken from the work of Pavlov and Fedorov[22]). An organic crystal whose packing

coefficient is approximately the same as that of the polynucleotide double helix (normally, such state is obtained under high pressure) is characterized by the value of the compressibility coefficient, $\sim 3\times10^{-6}$ bar^{-1}.[23] This value correponds to the internal compressibility of polynucleotide, $K_M \cong 5\times10^{-4}$ cm^3·mol^{-1}·bar^{-1} and is much smaller than the absolute value of ϕ_{ks}. Below we will consider only the difference of ϕ_{ks} for different double helices, and not the absolute value. We can suppose that the values of K_M for different double helices are close to each other because even in the B→A transition, the values of the intrinsic volume and packing coefficient for the double helix change are only 1÷2%.[22] Therefore, we can neglect the contribution of K_M to ϕ_{ks}.

From Eq. (1), the change of the A value caused by the exchange of cations in the ionic atmosphere, ΔA, is determined by

$$\Delta A = \Delta\phi_v - \Delta\phi_{ks}/(2\beta_o) \qquad (3)$$

The relation of ΔA to molecular characterisitics is given by

$$\Delta A \cong \Delta(\Delta V_h) - \Delta(\Delta K_h)/2\beta_o \qquad (4)$$

This relationship is deduced from Eqs. (1-3) using an assumpion that the intrinsic volume V_M and compressibility K_M of the double helix do not change significantly with the exchange of the ionic surrounding.

The main characteristics to be used in discussion of the results presented in this paper are ϕ_{ks}, $(\phi_v - V_M)$ and ΔA. As follows from the above, their connection with polynucleotide hydration is determined by Eq. (4) and the relations

$$\phi_{ks} = \Delta K_h + K_M \quad \text{and} \quad (\phi_v - V_M) = \Delta V_h$$

where K_M is a small value which is the same within the experimental error for all the compounds studied. The ΔK_h and ΔV_h values are determined by the water-polynucleotide interaction, i.e. by polynucleotide hydration. They reflect the change in the compressibility and density of water surrounding the polynucleotide. For the majority of aqueous solutions of organic and inorganic compounds, the ΔK_h value

is negative at about 1°C. This circumstance is explained by the fact that the compressibility of water surrounding the solute molecule is smaller than that of pure water. The absolute ΔK_h value decreases upon transition from charged molecules and atomic groups to polar ones and from polar molecules and groups to hydrophobic ones. This gives rise to the regularities revealed in the studies of apparent compressibility of different low molecular weight compounds of inorganic ions,[24] organic hydrophilic and hydrophobic molecules,[25,26] nucleic acid bases,[11] nuclesides,[27] and nucleotides,[4] which at the qualitative level can be formulated as follows: the ϕ_{ks} value decreases with the increase of hydration of the solute molecules or with the increase of its influence on the surrounding water. For ions and ionic groups, ϕ_{ks} has a large negative value, but that for a hydrophobic solute or groups is close to zero. For hydrophilic uncharged atomic groups and molecules, ϕ_{ks} has an intermediate value. Analogous relationship is also valid for ΔV_h; the value of ΔV_h increases with a decrease of hydration. Normally, the change of hydration causes a synchronous change in ϕ_{ks} and ϕ_v at constant V_M, i.e. an increase of ϕ_{ks} increases ϕ_v and vice versa.[11] The dominating term in Eq. (3), however, is $\Delta\phi_{ks}/(2\beta_o)$. Therefore, the negativity of the value of ΔA means the positivity of $\Delta\phi_{ks}$, and so the decrease of hydration in the processes. Thus, we obtain the following empirical rules:

(1) A smaller value of ϕ_{ks} corresponds to greater hydration.

(2) A smaller value of $(\phi_v - V_M)$ also corresponds to greater hydration.

(3) When the cation in ionic atmosphere of polynucleotide is changed for the other, the negativity of ΔA means a decrease of hydration (dehydration) of the atomic group in polynucleotide and cation, and the lesser is ΔA, the greater the dehydration.

EXPERIMENTAL SECTION

Salmon DNA, poly(A)·poly(U), poly(dA)·poly(dT), and poly[d(A-T)]·poly[d(A-T)] were obtained from Sigma. All the

solutions were prepared by redistilled water with specific conductivity less than 10^{-6} Ω^{-1} cm. The samples were dissolved in a 0.2 M NaCl and 0.01 M EDTA solution (pH 8) and dialyzed for 5 days against 2 mM NaCl and 2 mM HEPES buffer (pH 7.8) at 2÷4°C. Experiments were conducted at 1.2°C in low-temperature room thermostated at 4°C. Such temperature was chosen because of two reasons. In the first place, if the temperature of measurements is higher than the temperature of the dialysis process, the solution should be degassed. This would lead to differences in concentrations of the buffer in the solution and the solvent, and the latter causes errors in the A and Φ_v values. In the second place, the temperature 1.2°C is considerably smaller than the melting temperature of the double helix of investigated nucleic acids at the ionic strength that has been used in the experiments.[28] In order to reduce the viscosity of the DNA solution, it was sonicated by an ultrasonic disintegrator UZDN-2 (USSR) with 22 KHz prior to dialysis. The sonication was made in buffer with 0.1 M NaCl and 0.01 M EDTA, which contained 1 mg/cm^3 of DNA and was saturated with nitrogen. After the sonication, the DNA solution was filtered through a 0.45 μ millipore filter. It was confirmed by the UV absorption melting curves that DNA did not denature either before or after sonication. Molecular weights of biopolymers were determined by the use of agarose gel electrophoresis, and the values were 1.2×10^5-3×10^6 dalton for DNA, 1.2×10^5-1.5×10^6 for poly(A)·poly(U), 3×10^6 for poly(dA)·poly(dT), and 10^5 for poly[d(A-T)]·poly[d(A-T)]. The sample of poly[d(A-T)]·poly[d(A-T)] contained a small admixture of higher molecular weight component.

Concentrations of these solutions were determined optically. The molar extinction coefficient values at 260 nm, ε, were 6550 for DNA, 6650 for poly[d(A-T)]·poly[d(A-T)], and 6000 $M^{-1}cm^{-1}$ for poly(dA)·poly(dT), respectively. The value of ε, 6980 $M^{-1}cm^{-1}$ for poly(A)·poly(U) was determined by the method of hydrolysis by phosphodiesterase from snake venom (the sample was obtained from the Academy of Sciences of the Estonian SSR). The value obtained was in good agreement with the literature data.[29]

The solution densities were measured with a densimeter DMA-602 (Anton Paar). The values of the apparent molar volume, Φ_v, of the solute were calculated by the relation

$$\Phi_v = M/\rho - (\rho-\rho_o)/(\rho\cdot\rho_o\cdot c)$$

where ρ_o and ρ are the densities of the solvent and solution, respectively.

The changes of ultrasonic velocities were measured by a device RADA-2 developed in the Institute of Biological Physics, USSR Academy of Sciences,[30-33] based on the so-called resonance method[34] and working at 7.0-7.2 MHz frequency. The apparatus consists of the measuring and reference cells (acoustic resonators) with the volume of about 0.8 cm^3 and an electronic unit. Changes in the ultrasonic velocity were determined by the changes in the frequency of the maximum of the resonance peak of the preset resonance harmonic of the cell using the expression:

$$(u-u_o)/u_o = [(f-f_o)/f_o] \cdot (1+\gamma)$$

where f and f_o are frequency values of the maximum of the resonance peak of the preset resonance harmonic of the cells filled by the solution and the solvent, respectively, γ is a small constant value determined by calibration. At polynucleotide concentration of about 1 mg/cm^3 the $(u-u_o)/u_o$ value is $\sim 2.5 \times 10^{-4}$. The relative experimental error was about 5×10^{-7}. The details of the measurement procedure are discussed elsewhere.[11] The increment of ultrasonic velocity of solution, A, was calculated by Eq. (2).

The acoustic titrations of polynucleotide solutions were performed as follows. The concentrated salt solutions were prepared using the polynucleotide buffer solution. The measuring and reference cells were filled with the solution and buffer using a syringe with a special high-precision adapter. The titration with CsCl solution by using a microsyringe with a precision adaptor (Hamilton) continued till the A value became constant. After titration with CsCl, the polynucleotide and reference solutions were taken from the acoustic cells and the Φ_v value was determined (except for poly(dA)·poly(dT)). After this, the titration with $MgCl_2$ solution was performed in the same manner. The polynucleotide concentrations were 2-2.5 mM (per nucleotide) in all measurements. It is necessary to point out that the values of Φ_v, Φ_{ks}, and A are independent of the polynucleotide chain length, since these values are determined by the state of water within the range of 0.3-0.4 nm from the polynucleotide surface.[4]

RESULTS

The values of A, Φ_v, Φ_{ks}, $(\Phi_v - V_M)$ and ΔA for polynucleotides solutions are presented in Table 1. The values of Φ_{ks} were

TABLE 1. Concentration increment of ultrasonic velocity (A), apparent molar volume (ϕ_v), apparent molar adiabatic compressibility (ϕ_{ks}), difference ($\phi_v - \bar{V}_M$) (where $\bar{V}_M$ is the intrinsic molar volume of the nucleotide within the double helix) and the change of the concentration increment of ultrasonic velocity upon exchange of Mg^{2+} ions for Cs^+ ions in ionic atmosphere of nucleic acids (ΔA) of the compound studied in dilute solutions at 1.2°C

Polymer	Mol.wt.	A (cm^3/mol)	ϕ_v (cm^3/mol)	$\phi_{ks} \cdot 10^4$ (cm^3/(mol·bar))	$(\phi_v - \bar{V}_M)$ (cm^3/mol)	ΔA (cm^3/mol)
Poly(dA)·poly(dT)	331.7	92.4±0.6	140.6±1.5	-112.7±2.1	-21.1	-2.1±0.2
Poly[d(A-T)]·poly[d(A-T)]	331.7	87.9±0.5	147.5±1.5	-101.8±2.0	-14.2	-3.8±0.2
DNA	331.9	80.1±0.4	148.2±1.0	-93.7±1.3	-12.2	-4.7±0.3
Poly(A)·poly(U)	340.7	72.4±0.4	146.2±1.5	-92.5±1.9	-15.6	-5.6±0.3

All measurements were carried out at solution concentrations varying from 2 to 3 mM (per nucleotide). The A, ϕ_v and ϕ_{ks} values were obtained in the buffer containing 2 mM NaCl and 2 mM Hepes, pH 7.8. The ΔA values were obtained by titration with $MgCl_2$ in the buffer containing 2 mM Hepes, 2 mM NaCl, 20-30 mM CsCl. The errors of the A, ϕ_v and ϕ_{ks} values given in the table do not include systematic errors (as all the changes were done under the same conditions using the same increments) and the errors in determining the extinction coefficient. The $\bar{V}_M$ values are calculated from the data of Pavlov and Fedorov[23] for intrinsic volumes of the AT and GC pairs within the double helix.

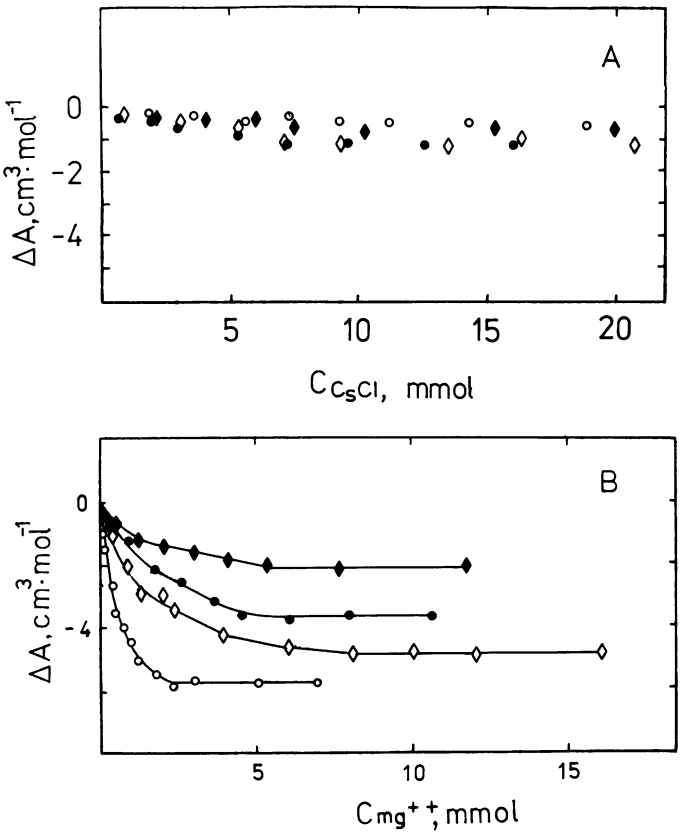

Fig. 1. Curves of acoustic titration of poly(A)·poly(U) - o, salmon DNA - ◇ , poly[d(A-T)]·poly[d(A-T)] - o, poly(dA)·poly(dT) - ◆ with CsCl (a) and $MgCl_2$ (b). Temperature is 1.2°C, concentrations of preparations are as follows: DNA - 3.0 mM, poly(dA)·poly(dT) - 2.1 mM, poly[d(A-T)]·poly[d(A-T)] -2.1 mM, poly(A)·poly(U) - 2.4 mM. CsCl titration was carried out in the buffer containing 2 mM NaCl and 2 mM Hepes, pH 7.8. $MgCl_2$ titration was carried out for solutions obtained after termination of CsCl titration. The change of the poly- nucleotide concentrations as a result of two sequential titrations is about 3%.

calculated from Eq. (1). The value of β_o in Eq. (1) was calculated from Del Grosso's data[35] on the ultrasonic velocity in pure water. We used the value of ρ_o reported by Kell.[36]

The values given in Table 1 can be referred to infinite dilution, since the solutions were very dilute.[11,37] For example, the values of A and ϕ_v in the DNA solution did not show any concentration dependence within experimental error in the concentration range 0.3-4 mM.[38]

As mentioned above, the values of V_M were taken from the work of Pavlov and Fedorov.[22] The conformation of poly(dA)·poly(dT) differs slightly from that of the normal B-form. But even in the B→A form transformation, the change of V_M per AT pair is only 1 $cm^3 \cdot mol^{-1}$, and this value is smaller than the experimental error in ϕ_v. The conformational difference between poly(dA)·poly(dT) and the normal B-form is much smaller than that between the A- and B-forms. Thus, for for poly(dA)·poly(dT) we used the same value of V_M as that for the AT pair of B-DNA.

The acoustic titration curves of polynucleotide with Cs^+ and Mg^{2+} ions are given in Fig. 1. We selected the Cs^+ ion as a monovalent cation, because the contribution of CsCl to the ultrasonic velocity is small, and it is possible to titrate in a wide range of ionic strength. Before and after titration with CsCl, the values of ϕ_v were the same (for poly(dA)·poly(dT) they were not measured after titration with CsCl).

DISCUSSION

Hydration of Poly(dA)·poly(dT) and Poly(A-T)·poly(A-T)

As can be seen from Table 1, the values of ϕ_{ks} and $(\phi_v - V_M)$ for poly(dA)·poly(dT) are smaller than those for poly[d(A-T)]·poly[d(A-T)]. According to rules 1 and 2, these results show that the first is hydrated more strongly. Moreover, the values of ϕ_{ks} and $(\phi_v - V_M)$ for this polymer are the smallest among those for all the polynucleotides, and this means that the hydration of poly(dA)·poly(dT) is abnomalously large. Recently, the hypothesis of abnomalously large hydration of poly(dA)·poly(dT) was suggested by Chuprina,[39] who analysed the structure of the water spine discovered by Drew and Dickerson[5] within the minor groove of DNA, and Breslauer and his coworkers,[40] who investigated the interaction between ligands and polynucleotides. Our result is the direct experimental verification of their suggestion.

It can be assumed that the peculiarity of hydration of poly(dA)·poly(dT) is connected with the anomalous properties:

its conformation differs from that of the classical B-DNA;[41] it does not transform to the A-form unlike other double helices, when the humidity decreases,[42] it has unusual values of enthalpy and entropy of interaction with small ligands.[40] Also histone octamers do not bind to DNA molecules with tracts of poly(dA)·poly(dT).[43-45]

Effect of Base Composition on the Hydration of the Double Helix

It is possible to reveal the effect of the base composition on the hydration of the double helix if we compare the ϕ_{ks} and $(\phi_v - V_M)$ values of poly[d(A-T)]·poly[d(A-T)] with those of salmon DNA with 50% composition of the GC pair. The value of $(\phi_v - V_M)$ for DNA is very close to that of poly[d(A-T)]·poly[d(A-T)], and the value of ϕ_{ks} for the first is larger than that for the second. According to rule 1, this result means that the AT pair is hydrated more strongly than the GC one. The dependence of the DNA hydration on the base composition was investigated experimentally by Mrevlishvili[8] and Tunis and Hearst[9] and theoretically by Goldblum et al.[46] They showed that the hydration of the AT pair is larger than that of the GC pair. From X-ray scattering in the B-form crystal, Drew and Dickerson[5] found that the regular water spine within the minor groove of the double helix is disrupted at the GC pairs. This result suggests that the hydration of the GC pair is weaker. The results reported in ref. 5, 8 and 9 coincide qualitatively with each other, but were obtained under experimental conditions differing greatly from ours. Our result shows that the hydration of the AT pair is stronger in dilute solution.

The Difference in the Hydration of the A- and B-forms

Among all polynucleotides, only poly(A)·poly(U) has an A-form and is also different from poly[d(A-T)]·poly[d(A-T)] and poly(dA)·poly(dT) in having an 2'-OH group, and no thymine CH_3 group. Therefore, the smallest hydration of poly(A)·poly(U), as compared with poly(dA)·poly(dT), may be ascribed to two effects: (1) the difference of the A and B conformations of the double helix, and (2) the difference of the chemical structure. It is necessary to know the contribution of the CH_3 and -OH groups to ϕ_{ks} to distinguish these effects.

Recently, we measured ϕ_{ks} at 25°C and its temperature dependence for the nucleic acid bases and nucleosides.[11,27] According to these results, the exchange of thymine CH_3 group

for an H group at 1°C should increase Φ_{ks} approximately by 3×10^{-4} cm^3/mol·bar. For the 2'-OH group, this value is about 9×10^{-4} cm^3/mol·bar. This means that the second effect decreases the Φ_{ks} value of poly(A)·poly(U), as compared with poly(dA)·poly(dT). The experiments give us a reverse relation between the Φ_{ks} values of these polynucleotdies. So, we can conclude that in this case the B-form of the double helix is more hydrated than the A-form.

The Effect of Ionic Strength on the Hydration of Polynucleotides

As can be seen from Fig. 1, the values of A for polynucleotide solutions decrease with increasing the concentration of CsCl ($\Delta A < 0$). It means that the dehydration of the polynucleotide-plus-counterion system takes place. The dehydration is very small because the ΔA value is only 0.5-1.0 cm^3/mol. Indeed, a complete dehydration of the NH_2 and O plus H groups of nucleic bases should give a decrease of A at 3.2 and 4.9 cm^3/mol. The dehydration of the Cs^+ cation gives much larger values.

The decrease of the A value obtained after titration with CsCl was the same as that obtained earlier after titration of Na DNA solution with NaCl.[37] It is, therefore, thought that the substitution of Na^+ for Cs^+ ion does not occur only as

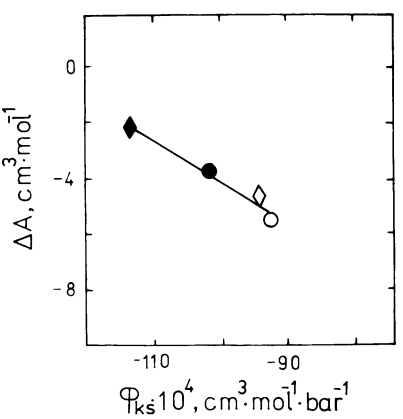

Fig. 2. Dependence of the change of the concentration increment of ultrasonic velocity upon exchange of Mg^{2+} for Cs^+ in ionic atmosphere of the studied polynucleotides ($MgCl_2$ titration), ΔA, on the value of the apparent molar adiabatic compresssibility Φ_{ks}. Experimental conditions are the same as in Fig. 1.

a result of the exchange of the cations in the ionic atmosphere of the double helix, and the other cause for the change of ΔA may be an increase of the ionic strength in the solution.

Does the Hydration Effect of Cs^+ to Mg^{2+} Exchange in Ionic Atmosphere of the Double Helix Depend on the Nucleotide Sequence?

Contrary to CsCl, the values of A decrease markedly with an addition of $MgCl_2$. This means that the dehydration of the Mg^{2+} plus polynucleotide system occurs as a result of the complex formation. The possibility of such dehydration has been confirmed earlier by CD and apparent molar volume measurements.[47-49] Since the equilibrium constants of binding of Mg^{2+} to DNA_2 are much larger than for Cs^+, a complete exchange of Mg^{2+} for Cs^+ takes place in ionic atmosphere of polynucleotides at a concentration larger than 5 mM. The values of ΔA differ largely for different polynucleotides. This means that the hydration effect of binding of Mg^{2+} to Cs DNA depends on the nucleotide sequence of the double helix. The relation between ΔA and ϕ_{ks} for the polynucleotides in $MgCl_2$ solutions is shown in Fig. 2. Taking into account the above discussions, we conclude that the lesser is a polynucleotide hydration, the larger the dehydration at the exchange of Mg^{2+} for Cs^+ in ionic atmosphere.

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