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QUARTERLY

DNA interaction with biologically active metal ions. Cooperativity of metal ion binding at compacting of DNA*°

Svetlana Kornilova✉, Elene Hackl, Larisa Kapinos, Valery Andrushchenko and Yuri Blagoi

B.I. Verkin Institute for Low Temperature Physics and Engineering, National Academy of Sciences of Ukraine, 47 W. Lenin Ave., 310164, Kharkov, Ukraine

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The interaction of Cu^{2+}, Mn^{2+} and Ca^{2+} ions with DNA in aqueous and water-ethanol solutions at different metal ion concentrations was studied by IR-spectroscopy. At sufficiently high DNA concentrations, DNA interaction with Cu^{2+}, Ca^{2+} and Mn^{2+} ions results in compacting of DNA in the aqueous solutions. This process shows a very high cooperativity. In the presence of alcohol, DNA condensation takes place at much lower concentrations of metal ions used than in pure aqueous solution. Binding constants and cooperativity of the metal ion binding rise, and the non-monotonous dependencies of the binding degree, r, on the concentration of free metal ions, C_f, become pronounced. Binding isotherms take the S-like form similar to van der Waals isotherms for phase transitions of the liquid-vapour type. Cu^{2+} and Ca^{2+} ion binding to DNA in water-ethanol solutions also results in compacting of DNA macromolecule. The process is characterised by a high positive cooperativity and has a phase transition character.

Metal ions play a leading role in DNA functioning *in vivo*. The investigation of the nature of DNA interaction with biologically active divalent metal ions could help to explain ion effects on DNA biosynthesis and determine Me^{2+} ions role in mutagenesis and carcinogenesis.

It is known that many antibiotics, carcinogens, etc. act mainly through their direct interaction with DNA [1–3]. When binding to DNA, these substances are able to disrupt matrix synthesis, resulting in changes in cell functioning [4]. The effect of many drugs on DNA is similar to the influence of ions. In par-

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✉To whom correspondence should be addressed.

Abbreviations and symbols: DUVS, differential ultraviolet spectroscopy; A_0 and A_i, absorbance without and with metal ions respectively; C_f, concentration of free metal ions; D, total concentration of ions; Me^{2+}, divalent metal ions; P, molar DNA concentration; r, binding degree.

ticular, our previous work showed that the usual B- to A-form transition resulting from decreased humidity could be prevented by Me^{2+} ion interaction [5, 6]. DNA-binding drugs also can have a similar effect, "freezing" DNA in its B-form [7]. Thus, divalent metal ions can simulate the action of many drugs present as mono- (for example, distamycin A) or divalent (netropsin) cations in solutions. Phillips & Carlyle [8] reported on the existence of a daunomycin-Cu^{2+}-DNA complex, showing that Me^{2+} ions are able to influence DNA interaction with drugs.

IR spectroscopy of DNA complexes with Me^{2+} ions at the high DNA concentration used in the present work (all DNA in cells and viruses exists in a highly condensed state), permits us to study the macromolecule transition into the compact state upon interaction with divalent metal ions. Aggregation and condensation of DNA in the presence of different substances, such as metal complexes, polyamines, divalent and multivalent metal ions, have been known for some time [9-12]. Multivalent cations have also been shown to play an essential role in the condensation of DNA incorporated into chromatin [13, 14]. However, despite considerable research concerning DNA condensation, so far the process of DNA condensation in the presence of metal ions has not been studied thoroughly enough. In particular, the question of DNA condensation in the presence of divalent metal ions in aqueous solutions remains unresolved.

MATERIALS AND METHODS

Materials

Calf thymus DNA (molecular mass 1.9×10^7 Da, protein content 0.5%) was obtained from the laboratory of Professor D. Lando (Institute of Bioorganic Chemistry of the Academy of Sciences of the Belarus Republic, Minsk). The hypochromic effect was 36% at $\lambda = 260$ nm. The amount of Na^+ and K^+ in relation to DNA dry weight, determined with an FPL-1 flame photometer, was 7.0 ± 0.2% and 0.6 ± 0.2%, respectively.

Methods

IR spectra of DNA complexes with Ca^{2+}, Mn^{2+} and Cu^{2+} ions in H_2O and D_2O solutions were recorded with an UR-20 IR-spectrophotometer (Carl Zeiss, Jena, Germany) in the regions 1000-1350 cm^{-1} and 1400-1800 cm^{-1}, respectively.

The samples were held in specially designed collapsible CaF_2 cells, developed by Kal'vin and Vel'yaminov [15] with path length of 63.8 μm, accurately thermostated to 29.0 ± 0.2°C.

The DNA and metal ion solutions were prepared in cacodylate buffer (buffer concentration was 5×10^{-3} M). The pH of the solutions was 7 ± 0.1.

Metal ion concentration was varied from 10^{-2} M to 5×10^{-1} M.

The final DNA concentration in the cell was 18 ± 2 mg/ml. DNA samples were dissolved in appropriate volumes of cacodylate buffer and kept in refrigerator at 5°C for 3 days to ensure that the DNA was completely dissolved. Metal ion solutions were added dropwise to DNA solutions directly in the depression of the bottom cell window, with constant stirring of the mixture. The bottom window was then covered with a window cover and the cell was placed in a metal holder.

For measurements in the 1400-1800 cm^{-1} region, all solutions (DNA, buffer and metal ions) were deuterium exchanged. To ensure that H_2O was completely replaced by D_2O, the solutions were dried (using a lyophilization setup) and redissolved in D_2O three times.

RESULTS AND DISCUSSION

Figures 1 and 2 represent IR spectra of non-deuterated and deuterated solutions of DNA and DNA complexed with Cu^{2+}, Ca^{2+} and

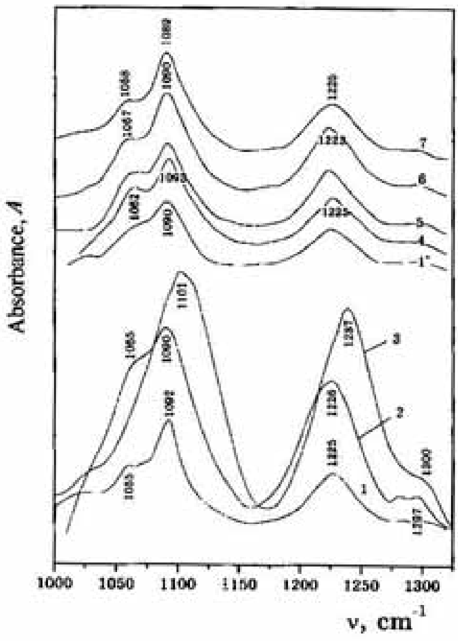

Figure 1. The IR-spectra of phosphate groups of DNA (1, 1´) and DNA complexes with:

Cu^{2+}, 2.54 × 10^{-2} M (2); 3.4 × 10^{-2} M (3); Mn^{2+}, 9.14 × 10^{-2} M (4); 2.16 × 10^{-1} M (5) and Ca^{2+}, 4.32 × 10^{-1} M (6); 8.68 × 10^{-1} M (7).

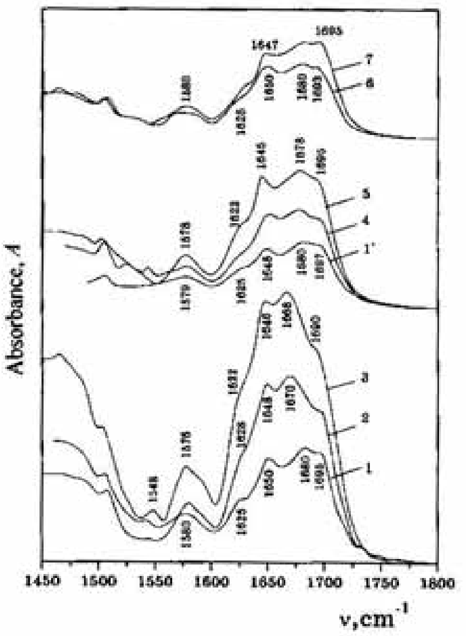

Figure 2. The IR-spectra of nitrogen bases of DNA (1, 1') and DNA complexes with:

Cu^{2+}, 1.7 × 10^{-2} M (2); 5.0 × 10^{-2} M (3); Mn^{2+}, 1.06 × 10^{-2} M (4); 4.86 × 10^{-1} M (5) and Ca^{2+}, 2.58 × 10^{-1} M (6); 1.81 × 10^{-1} M (7). DNA concentration was 5.5 × 10^{-2} M *P* (1) and 3.3 × 10^{-2} M *P* (1´). Top: the IR-spectra of native and denatured DNA (data from [23]).

Mn^{2+} ions in the ranges of 1000–1300 cm^{-1} and 1400–1800 cm^{-1}, respectively. The frequency shifts and intensity increases of absorption bands of different DNA groups show that the divalent metal ions significantly modified the DNA spectra. These changes could be due to binding of the Me^{2+} ions to functional groups of the biopolymer, conformational transitions within the macromolecules resulting from this interaction, or a combination of both.

An absorption band at 1055 cm^{-1} corresponding to vibrations of the C–O–P sugar-phosphate backbone, and an absorption band at 1223 cm^{-1} arising from antisymmetric vibrations of phosphate groups [5, 6, 16] were observed for DNA and DNA complexes with Cu^{2+}, Ca^{2+} and Mn^{2+} at all ion concentrations. Both these absorption bands point to a B-form DNA structure [5, 6, 16]. Furthermore, one of the marker bands of the DNA B-form at 1223 cm^{-1} in the spectra of complexes was not shifted to values characteristic of the A-form (1240 cm^{-1}), or to those characteristic of the Z-form of DNA (1215 cm^{-1}). The observed lack of the DNA structural transition upon divalent metal ion binding is in agreement with the results of NMR studies [17, 18].

It should be mentioned that some competition between Na^+ and Me^{2+} ions was observed when Na^+-DNA samples were used. Na^+ ions concentration is determined mostly by DNA concentration and is about 5 × 10^{-2} M (concentration of Na^+ ions added with buffer is about 10 times less). At such low concentrations Na^+ ions stabilize DNA B-form by neutralizing phosphate negative charges. Because of their much higher binding constants [18] Mn^{2+} and Cu^{2+} ions can easily replace Na^+ ions bound to phosphate groups without considerable competition even at low divalent metal ion concentrations. Much stronger competition takes place between Ca^{2+} and Na^+ ions [18–20], but at higher Ca^{2+} ions concentration it also becomes much less significant because of the large difference between Ca^{2+} and Na^+ ion concentrations. These conclusions can also be confirmed by the comparison of processes of Ca^{2+}, Mn^{2+} and Cu^{2+} ion binding to DNA phosphate groups (Fig. 3). It is clearly seen that binding of Ca^{2+} ions begins at their concentration higher than that required for binding of Mn^{2+} and Cu^{2+} ions, which means that competition between Ca^{2+} and Na^{+} ions is stronger than that between Mn^{2+} or Cu^{2+} and Na^{+} ions.

The most significant changes in the DNA spectrum occur during DNA interaction with copper ions. Cu^{2+} ions at $[Cu^{2+}]/[P] > 0.35-0.5$ induce partial disordering of the macromolecule (disordering of different segments of a macromolecule) within the B-form resulting in significant increases in intensities, and shift and extension of absorption bands of the phosphate groups (1090 → 1100 cm^{-1}, 1223 → 1237 cm^{-1}) and the bases. Shifts of the bands 1055 → 1060–1070 cm^{-1}, 1290 → 1296–1300 cm^{-1} are also observed (Fig. 1). All these changes in the DNA spectrum are observed, albeit to a lesser extent, in the presence of Ca^{2+} and Mn^{2+} ions.

The absorption bands at 1055 cm^{-1} and 1223 cm^{-1} are very sensitive to even minor structural changes of DNA macromolecules. Therefore, it may be possible to identify DNA structural changes using these bands. Moreover, these changes and particularly changes in the other absorption bands corresponding to the phosphate groups can be induced by strong electrostatic interaction of metal ions with phosphate groups and point to intense metal ion binding to phosphates. Hence, partial conformational changes in the DNA macromolecule in the range of the B-form accompanying the intense metal ion binding to phosphate groups can be suggested. Data about minor changes of DNA structure in the frame of B-conformation upon metal ion binding were also obtained by other authors from NMR experiments [17, 18, 21].

At the same time, Cu^{2+}, Ca^{2+} and Mn^{2+} ions induce significant changes in the DNA spectrum in the absorption region of nitrogen bases (Fig. 2). In the spectra of DNA complexes with Cu^{2+} ions one sees frequency shifts as well as intensity increases, of absorption bands at 1580 cm^{-1} (mostly $C{=}N_{(7)}$ of guanine, adenine), 1650 cm^{-1} (mostly $C_{(2)}{=}O$ of cytosine) and 1680 cm^{-1} ($C_{(6)}{=}O$ of guanine, $C_4{=}O$ of thymine) [5, 6, 16]. A weak band appearing at 1547 cm^{-1} (C=N of cytosine), suggesting copper ion binding to $N_{(7)}$ and $O_{(6)}$ of guanine and $O_{(2)}$ and $N_{(3)}$ of cytosine, may support the realising of Zimmer's model [22]. A comparison of the spectra of DNA complexed with copper ions with the spectrum of denatured DNA [23] (see Fig. 2, top) shows that even at the highest Cu^{2+} ion concentration no denaturation of the DNA molecule does occur. Ca^{2+} and Mn^{2+} ions increase the intensity and shift the absorption bands at 1675 and 1575 cm^{-1} (binding to $N_{(7)}$ and $O_{(6)}$ of guanine).

Me^{2+} ion binding to $O_{(6)}$ and especially to $N_{(7)}$ of guanine was observed by many authors [17–19, 21, 24–26]. Atom $N_{(7)}$ of guanine was clearly indicated as the main binding site on DNA bases by chemical shifts in NMR spectra [17, 18, 24]. It was shown that Me^{2+} ion binding affinity to $N_{(7)}$ of adenine is approximately by one order of magnitude smaller than that of guanine [18]. Therefore, all changes in the absorption band at 1580 cm^{-1} can be assigned to metal ion binding to $N_{(7)}$ of guanine. According to theoretical calculations [25, 26], $N_{(7)}$ of guanine has the highest negative electrostatic potential and is one of the main binding sites on DNA nitrogen bases. Hence, formation of the chelate between $N(_7)$ and $O_{(6)}$ of guanine can be considered as a possible binding mode. Our data confirm both theoretical [26] and experimental [19] predictions of such binding.

Thus, Cu^{2+}, Ca^{2+} and Mn^{2+} ions must bind actively to both phosphate groups and nitrogen bases of DNA.

To study the concentration dependence of Cu^{2+}, Ca^{2+} and Mn^{2+} ion binding to DNA, dependencies of the change in the absorbance in relative units R ($R = A_i/A_0$, where A_0 – is absorbance of the band of DNA without divalent metal ions, A_i – is absorbance of the band of DNA complex with the i-th metal ion concentration) in relation to the molar concentration of ions (C), were obtained for absorption bands of the following groups: 1090 cm^{-1} (symmetric vibrations of phosphate groups), 1223 cm^{-1} (antisymmetric vibrations of phosphate groups), 1575 cm^{-1} (C=N vibration of guanine), 1675 cm^{-1} (mostly $C_{(6)}$=O vibration of guanine) (Fig. 3, curves 1, 3 and 5). In each case, a sharp increase of intensity occurring over a relatively narrow concentration interval was observed. Such a character of the R dependencies on C cannot be due to the ordinary coordination of divalent metal ions with the nearest binding centres on the macromolecule, because, in this case, the process should have been more monotonous over the wide range of concentration used. The observed effect points to the high positive cooperativity of the binding process. With the increase of manganese ion concentration up to 5×10^{-2} M, and the copper ion concentration up to 2×10^{-2} M, the $R = f(C)$ dependencies for absorption bands of bases begin to show saturation, indicating the occupation of all binding sites on nitrogen bases. Similar patterns exist for the absorption bands of phosphate groups, but the saturation occuring only at $[Mn^{2+}] > 2 \times 10^{-1}$ M ($[Cu^{2+}] > 3 \times 10^{-2}$ M) (Fig. 3), indicates that Mn^{2+} and Cu^{2+} ion binding to phosphate groups ends later. From the above results the conclusion may be reached that the ions in question bind to DNA bases and DNA phosphate groups independently. This was also confirmed by NMR data [18, 24]. Taking into account that Na^+-DNA was used in the experiment, it may be assumed that upon total occupation of binding sites on the nitrogen bases, Na^+ ions are displaced from phosphate groups. Unlike Mn^{2+} and Cu^{2+} ions, the Ca^{2+} binding to DNA phosphate groups and DNA bases finishes practically at the same concentration (about 5×10^{-1} M). This can be explained by the fact that calcium ions form an external spherical complex with phosphate groups, and $N_{(7)}$ and $O_{(6)}$ of guanine, simultaneously. Our conclusions confirm the results of the X-ray diffraction study of the Ca^{2+}-dGMP complex [19]. In that study, it was shown that Ca^{2+} ions, having 8 coordination bonds, interact with $N_{(7)}$ of guanine, a PO_3^{2-} group, and 6 water molecules; Sr^{2+} with 8 coordination bonds is bound to a PO_3^{2-} group and 7 water molecules, while H_2O molecules are hydrogen-bonded to $N_{(7)}$ and $O_{(6)}$ of guanine [19]. Thus, Ca^{2+} ions in the crystalline state bind directly to phosphate groups (internal spherical complex) and nitro-

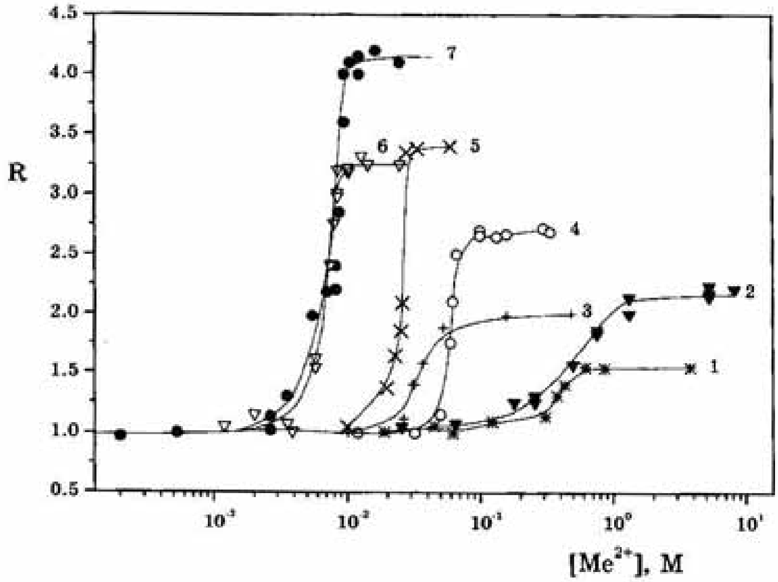

Figure 3. Dependencies of relative intensity $R = A_i/A_0$ on total metal ion concentration C for absorption band at $\lambda = 1090$ cm^{-1} for DNA complexes with Me^{2+} ions (*, Ca^{2+} ions; +, Mn^{2+} ions and ×, Cu^{2+} ions in aqueous solutions; ▼ and ○, Ca^{2+} ions in 5% and 15% ethanol solutions, respectively; ∇ and ●, Cu^{2+} ions in 10% and 15% ethanol solutions, respectively).

gen bases. In aqueous solutions an equilibrium can exist:

$$\text{base...}H_2O\text{-}Me^{2+}\text{-}PO_3 \leftrightarrow$$
$$\leftrightarrow \text{base-}Me^{2+}\text{-}H_2O\text{...}PO_3.$$

Our data also confirm the results of earlier NMR studies of Ca^{2+}-DNA complexes [20].

The increase of the absorption band intensity for different DNA groups upon their interaction with metal ions was presented in [27], but at metal concentrations used in that work, $R = f(C)$ dependencies did not reach saturation and were not of a cooperative character.

On the basis of the results obtained, we suggest that compacting and aggregation of macromolecules occur upon the binding of Cu^{2+}, Ca^{2+}, and Mn^{2+} ions to DNA. The processes of compacting and aggregation of DNA can even be observed visually at high copper concentration.

The nature of binding cooperativity of divalent metal ions and the cooperative influence on macromolecule condensation can be described using the example of ion binding to chains of freely-conjugated polyelectrolytes [28]. In such interactions, the ion is able to bind to two (or more) molecules (in the case of intermolecular aggregation), or to remote parts of one single chain (intramolecular compacting). In this case, the ion binding degree is determined by the value of the binding constants. These constants can be determined by the expression:

$$K = \exp\left(\frac{-\Delta G}{R \times T}\right), \qquad (1)$$

where ΔG is the usual Gibbs free energy change on binding,

$$\Delta G = \Delta H - T\Delta S$$

where ΔG, ΔH and ΔS are changes in thermodynamic parameters (free energy, enthalpy and entropy) upon ion binding to the macromolecule. The low ion binding degree is characterised by a high loss in entropy that is greater than the absolute value of ΔS. Because $\Delta S < 0$ and $\Delta H < 0$, $|T\Delta S| \sim |\Delta H|$. As a result, $|\Delta G|$ and K are low. With the increase of the binding degree and the relative ordering of the complex structure, the loss in entropy will decrease upon the attachment of the next ion. Then ΔG increases and K rises exponentially.

The theory of ligand adsorption on DNA attempts to take into account the long range interaction between the ligands adsorbed. One way to factor long range interactions into the adsorption theory was proposed by Scatchard [29]. This method considers the mutual influence of all the adsorbed ligands (not only neighbouring ones) on the DNA. Scatchard determined the free energy of the adsorbed ligand in terms of the linear dependence on the relative concentration (c) of ligands bound to the biopolymer (occupation degree), $G = G_0 - wc$, where G_0 is the free energy of the ligand adsorbed on DNA at the occupation degree approaching zero, and w is the proportionality coefficient. The principal supposition is that the free energy between the adsorbed ligands is determined by the degree of DNA occupation by ligands, and not ligand position along the polymer chain.

Using Scatchard's approach, let us assume that the increase in free energy is proportional to the binding degree r (that is, to the relative concentration of ions bound to the macromolecule) :

$$\Delta G = G_0 - W \times r, \qquad (2)$$

where W is the interaction energy between ions attached to the macromolecule at low r ($r << 1$). The energy W may also be considered as the surface energy for units of the ions attached the bounds of which are formed by the sites remaining nonoccupied after the ion attachment to the macromolecule.

Substituting equation (2) into equation (1) leads to

$$K = \exp\left(\frac{-G_0 + W \times r}{R \times T}\right) = K_0 \times \exp(\omega \times r), \quad (3)$$

where $K_0 = \exp\left(\frac{-G_0}{R \times T}\right)$, $\omega = \frac{W}{R \times T}$, and ω the parameter of cooperativity.

Using this expression for K and the simple equation of Scatchard, one can obtain the dependence of r on the concentration of free ions in solution, C_f, at different binding constants and cooperativity parameters:

$$\frac{r}{(1-r)} = K_0 \times \exp(\omega \times r) \times C_f, \quad (4)$$

$$D = C_f + P \times r, \quad (5)$$

where P is the molar concentration of DNA and D is the total ion concentration in solution.

Assuming that the change in absorbance at a given frequency is proportional to the degree of ion binding to the given group, it is possible to calculate the dependency of the binding degree r ($r = [C_b]/[P]$, C_b the concentration of bound ions) on the concentration of free ions in solution (C_f). For the transition from the absorbance in relative units R to the binding degree r, we assumed that, when the dependency of R on D approaches saturation (when all the binding sites are occupied), the binding degree r is at its maximum and equals 1. The maximum value of r can be equal to 0.5, taking into account that one divalent metal ion can bind to two different groups of DNA. In this case, the denominator index in equation (4) has to be taken as 0.5 as well. The point where the concentration of divalent metal ions added is zero, corresponds to $r = 0$. When calculating C_f, based on the total concentration of ions added, D, we apply the empirical formula:

$$C_f = D - (k_P \times r_P \times P + k_N \times r_N \times P) \quad (6)$$

where r_P and r_N are binding degrees for the divalent metal ion interaction with DNA phosphate groups and nitrogen bases, respectively, k_P and k_N are numerical coefficients proportional to the number of binding sites for one ion bound to DNA, and P is the molar DNA concentration. One divalent metal ion is able to bind to two DNA phosphate groups at a time. Thus, the number of binding sites for one ion bound to a DNA phosphate group (k_P) is equal to 0.5. Taking into account that upon the interaction with nitrogen bases, divalent metal ions bind preferentially to G–C base pairs [19], and that G–C pairs represent 40% of the total number of nucleotides in the DNA we used, one may assume $k_N = 0.4$. Thus, equation (6) takes the form of

$$C_f = D - (0.5 \times r_p \times P + 0.4 \times r_N \times P) \quad (7)$$

In our previous work we applied similar reasoning to calculate binding constants for ions interacting with DNA and to determine cooperativity parameters [30]. However, when evaluating the degree of occupation of binding sites, Na^+ ions bound to DNA phosphate groups were not taken into consideration. On the basis of [31], at DNA concentration about 5×10^{-2} M the binding degree for Na^+ is 0.6–0.7, i.e. 60–70% of phosphate groups are occupied by Na^+. Therefore, to calculate r and C_f it is necessary to use Scatchard's equation for the concurrent binding of two ion types [29].

It is also necessary to take into account that ionic strength of DNA solutions should be constant in order to exclude the influence of its changes on the registered spectral changes.

However, for the conditions of our experiment this was not possible because Na^+ ion concentration was relatively low while Me^{2+} ion concentration was relatively high, and different each time. Hence, we can only speak about constant Na^+ ion concentration and not about constant ionic strength. Besides, the ionic strength was relatively low in the experiment (about 1.5 for the highest divalent metal ion concentration) while according to [32] changes in the DNA structure can occur rather at much higher ionic strength.

Figure 4 shows dependencies $r = f(C_f)$ for Cu^{2+}, Ca^{2+} and Mn^{2+} ions, obtained from equation (7) for the absorption band at 1090 cm^{-1} (dots 1, 2, 3). Theoretical dependencies, $r = f(C_f)$, (calculated with the help of equations (4) and (5) for different values of parameters K_0 and ω) are shown as solid lines (curves 1, 2, 3, 5). Parameters K_0 and ω were selected so as to minimize the square sum of deviations of the experimental points from the theoretical curve. As may be seen from Fig. 4, binding isotherms are of a monotonous character corresponding to the continuous increase of r with the increase of C_f. The isotherm with $\omega = 8$, and having the intersection point with the vertical tangent, is critical. The dependence $r = f(C_f)$ for Cu^{2+} ions is of non-monotonous character. Nonstable parts with the reverse dependence r on C_f are present.

The data obtained using the differential ultraviolet spectroscopy (DUVS) method, illustrates the high binding cooperativity of divalent metal ions with thermally denatured DNA ($[P] < 10^{-4}$ M) [19, 33]. Unlike the IR spectroscopic data, the cooperativity of the binding to single-chained DNA is characterised by significantly higher absolute values for binding constants ($K = 10^2 \dots 10^4$ M) and appears at lower ion concentrations. Under these conditions, the helix-coil transition shows a second type phase transition character and is accompanied by DNA aggregation [19, 33].

With respect to the energetic barriers involved in polymer condensation, those most significant are due to electrostatic repulsion and hydration structure [11]. One way of reducing the electrostatic barrier is to decrease

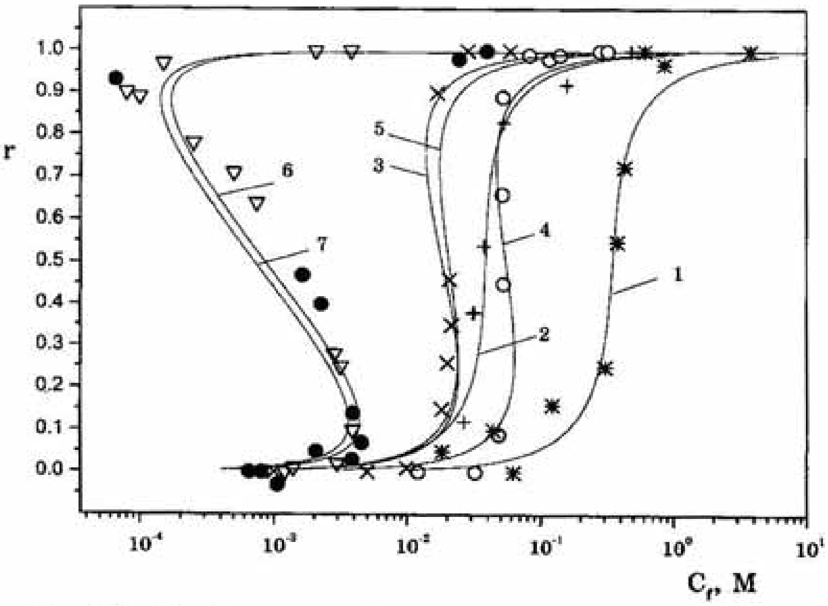

Figure 4. Dependencies of binding degree r on free metal ion concentration C_f for absorption band at $\nu = 1090\ cm^{-1}$ for DNA complexes with Me^{2+} ions (dots: ∗, Ca^{2+} ions, +, Mn^{2+} ions and ×, Cu^{2+} ions in aqueous solutions, and ○, Ca^{2+} ions in 15% ethanol solutions and ∇ and ●, Cu^{2+} ions in 10% and 15% ethanol solutions, respectively) and binding isotherms (lines), calculated using formulas (4) and (5), with different values of parameters k_0 and ω (1, $k_0 = 0.5, \omega = 7$; 2, $k_0 = 4, \omega = 7$; 3, $k_0 = 3.5, \omega = 11$; 4, $k_0 = 1.5, \omega = 10$; 5, $k_0 = 4, \omega = 10$; 6, $k_0 = 10, \omega = 19$; 7, $k_0 = 12, \omega = 19$).

the charge density on DNA through counterion binding. Using an extension of Manning's counterion condensation theory, Wilson and Bloomfield showed that DNA condenses when about 90% of its charges are neutralised by counterion binding [34, 35]. Their theory considers only electrostatic interactions, and does not account for non-electrostatic interactions between divalent cations and DNA. For divalent cations, the necessary degree of neutralisation occurred only in the presence of alcohol, presumably through a lowering of the dielectric constant [34]. Monovalent Na^+ and Cs^+ ions do not induce DNA condensation even in the presence of methanol [36]. In the present work we show that, at sufficiently high DNA concentrations, DNA interaction with Cu^{2+}, Ca^{2+} and Mn^{2+} ions results in compacting of DNA in aqueous solution. It is logical to suppose that with a decrease of the dielectric constant of solution through alcohol addition, this compacting will be pronounced. We studied DNA interaction with Cu^{2+} and Ca^{2+} ions in aqueous-alcohol solutions containing 5, 10 or 15 vol.% of ethanol. The dependencies of the absorbance in relative units R on the molar concentration of ions C for such systems are given in Fig. 3 (curves 2, 4, 6, 7). As can be seen, the Cu^{2+} and Ca^{2+} binding to DNA in aqueous-alcohol solution results in a stronger increase of the intensity of absorption bands of phosphate groups than in pure aqueous solutions. At the same time, the concentration range of Me^{2+} ions at which this intensity increase takes place, narrows sharply (especially for Ca^{2+} ions). In this case, DNA condensation takes place at much lower concentration of Me^{2+} ions than that in aqueous solution.

Figure 4 presents r dependencies on C_f for DNA-Cu^{2+} (Ca^{2+}) complexes in aqueous-ethanol solutions (curves 4, 6, 7). In the presence of alcohol, binding constants and cooperativity of the metal ion binding process rise, and in this case the non-monotonous dependence $r(C_f)$ becomes more pronounced. Binding isotherms take the S-like form similar to van der Waals isotherms for phase transition of the liquid-vapour type. Thus, Cu^{2+} and Ca^{2+} ion binding to DNA in aqueous-ethanol solutions results in compacting of DNA macromolecule. The process is characterised by high positive cooperativity, and exhibits a phase transition character.

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