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IR-SPECTROSCOPIC STUDY ON DNA INTERACTION WITH Cd^{2+} IONS

V.V. Onyshchenko, K.V. Kutko, S.V. Kornilova, Yu.P. Blagoi

B.I. Verkin Institute for Low Temperature Physics and Engineering of National Academy of Sciences of Ukraine, 47 Lenin Ave., Kharkov 61103, Ukraine

e-mail: onyshchenko@ilt.kharkov.ua

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The IR-spectroscopy method was used to study a DNA interaction with Cd^{2+} ions in aqueous solution. While interacting with metal ions, DNA with the high concentration transits into the compact structure. Association constants are determined, cooperativity of the Cd^{2+} ion binding is demonstrated, and nonmonotonous dependence of the binding degree (r) on the concentration of free metal ions (C_f) is ascertained. Binding isotherms are of the S-like shape similar to Van-der-Waals isotherms for the phase transition liquid-vapour, and the process of the Cd^{2+} ion binding to DNA molecules is highly cooperative.

KEY WORDS: DNA, Cd^{2+} ions, IR-spectroscopy.

Divalent metal ions are biologically active and they have cancerogeneous and mutageneous properties as well as a possibility to induce the formation of DNA compact forms [1,2]. Earlier results on the IR-spectroscopic study of DNA interactions with divalent metal ions permitted to determine and to compare binding constants for different divalent metals, to evaluate their influence on the compaction process [3]. One of conditions for the formation of DNA compact forms is decreasing the density of charges on DNA. This is realized due to DNA interactions with cations. Besides, according to the available data [4], the main factor is metal ion affinity for DNA bases. Therefore, in addition to the previous studies on effects of calcium, manganese and copper ions [4-8], it was interesting to investigate an effect of cadmium ions which have high binding constants to nucleic acid bases. As well these ions are known for their toxic action on genome of animals. The present work compares effects of some ions on the process of compaction of both native and denatured DNAs. This process is described thermodynamically.

MATERIALS AND METHODS

The work used calf thymus DNA, the molecular weight and protein content being $1.9 \cdot 10^{7}$ and no more than 0.5%, respectively. DNA was extracted in D.Yu. Lando's Laboratory (Minsk, Belarus). The DNA concentration in solution was determined spectrophotometrically at λ=260 nm and it was 20±2 mg/ml. Cadmium chloride (chemically pure) was used in the experiment. The Cd^{2+} ion concentration was in the $6.7 \cdot 10^{-3}$ - $5.36 \cdot 10^{-2}$M range that corresponds to 0.1 - 0.8M $[Cd^{2+}]/[P]$. The Na^{+} ion concentration was $7 \cdot 10^{-2}$M. Differential infrared spectra of DNA complexes with cadmium ions were registered with an infrared spectrophotometer UR-20 (Carl Zeiss, Jena, Germany) in the range of wave numbers 1000-1400 cm^{-1}. The procedure of IR-spectroscopic studies is similar to those applied in works [5,6]. The rate of spectrum registration was 10 cm^{-1}/min and NaCl prism was used. The spectral registration step was 0.5 cm^{-1}. Water absorption was realized when the cuvette of variable thickness with the solvent was placed into the comparative channel of the spectrophotometer. In this case the thickness of the solvent layer should correspond to the solvent amount in the run of measuring ray. The data obtained were recorded by a personal computer and processed by the Origin 6.1 Program.

RESULTS AND DISCUSSION

IR spectra of DNA solutions and DNA-Cd^{2+} ion complexes were obtained in the range of wave numbers 1000-1400 cm^{-1}. This range corresponds to absorption of phosphate groups and desoxyribose. IR spectra of DNA at different concentrations of Cd^{2+} ions are given in Fig. 1.

Cd^{2+} ions studied modify significantly the spectrum that is seen from a sharp increase of intensities of absorption bands of phosphate groups. The absolute intensity of IR absorption bands is proportional to the square of the derivative of the dipole moment with respect to the normal coordinate. Thus, increasing intensity is related to the rise of the value of this derivative upon the Cd^{2+} ion binding to the above groups of the DNA molecule, at its compaction. [5,6].

Fig. 2 presents dependences of the relative change in the optical density (R) of absorption bands of symmetric and asymmetric vibrations of DNA phosphate groups on the total Cd^{2+} ion concentration in solution. As Fig.2 shows, the increase of the intensity of absorption bands 1090 and 1225 cm^{-1} begins at the Cd^{2+} ion

concentration ~0.02M. To ascertain Cd^{2+} ion effects on DNA structural transitions, the experimental data obtained were compared with experimental results for Tb^{3+}, Cu^{2+}, Mn^{2+} and Ca^{2+} ions [5,6]. As it follows from Fig. 3, to induce DNA compaction, different concentrations of the studied ions are required: $Tb^{3+} << Cu^{2+} < Cd^{2+} << Mn^{2+} < Ca^{2+}$.

On the metal ion interaction with DNA and the polymer aggregation induced by this interaction strong cooperative processes similar to condensation of a gaseous substance to the liquid or solid phase could manifest themselves [5,6]. To describe thermodynamically the binding process, it is appropriate to present the ion interaction with a polyelectrolyte represented as a chain of freely-conjugated segments carrying the negative charge [5]. At the first stage one or some metal ions bind to two chain-remoted segments, followed with the loop formation. Some entropy loss would take place on the formation of such a complex.

The constant of the first bond formation can be written as:

$$K_0 = \exp\left(\frac{-\Delta G_0}{RT}\right) \tag{1}$$

where $\Delta G_0 = \Delta H_0 - T\Delta S_0$ is a change in Gibb's free energy. ΔH_0, ΔS_0 are enthalpy and entropy changes on the bond formation. The low probability of this process is conditioned by a large absolute value of ΔS_0 ($\Delta S_0<0$, $\Delta H_0<0$). In this case $|T\Delta S_0| \approx |\Delta H_0|$ and $|\Delta G_0|$ is small.

On the metal ion induced-formation of the second bond between segments entropy will decrease but a loss will be smaller as on the second bond formation the mobility of only a rather small chain part would be lost.

$$\Delta G_1 = 2\Delta H_0 - (\Delta S_1 + \Delta S_0), \quad |\Delta S_1| << |\Delta S_0| \tag{2}$$

Since the enthalpy gain will be the same on the formation of every bond, ΔG_0 will increase with the rise of the binding degree and, as a result, the association constant will increase, providing cooperativity of the DNA compaction process under the metal ion action.

Upon the formation of an N-th bond

$$\Delta G_N = \Delta H_0 - T\Delta S_0 + (N-1)(\Delta H_0 - T\overline{S_i}) = \Delta G_0 + (N-1)(\Delta H_0 - T\Delta\overline{S_i}) \tag{3}$$

where ΔS_i is a mean entropy change upon the bond formation with the second one to N. In the present case N is a number of the bonds formed between polyelectrolyte segments by a divalent metal. Thus, N is proportional to the number of segments having formed the bonds. Let N_t be the total number of segments in the freely-conjugated chain, the binding of which is required for complete compaction.

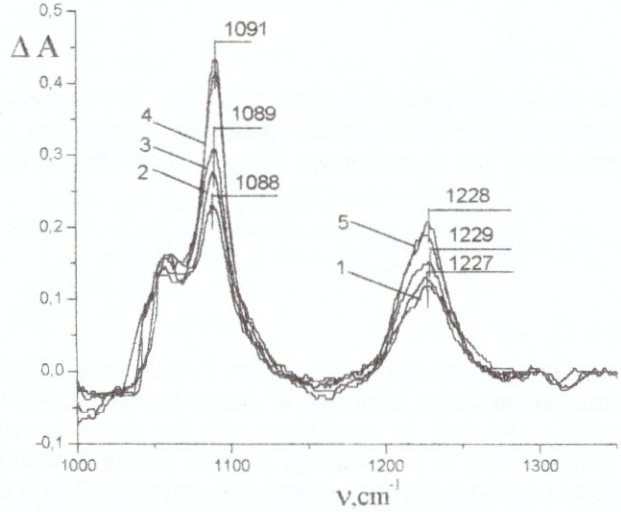

Fig. 1. IR-spectra of DNA and DNA complexes with Cd^{2+} ions. Relationships between concentrations of Cd^{2+} ions and DNA: 1. - 0,0 $[Cd^{2+}]/[P]$; 2 - 0,2 $[Cd^{2+}]/[P]$; 3 - 0,4 $[Cd^{2+}]/[P]$; 4 - 0,6 $[Cd^{2+}]/[P]$; 5 - 0,7 $[Cd^{2+}]/[P]$.

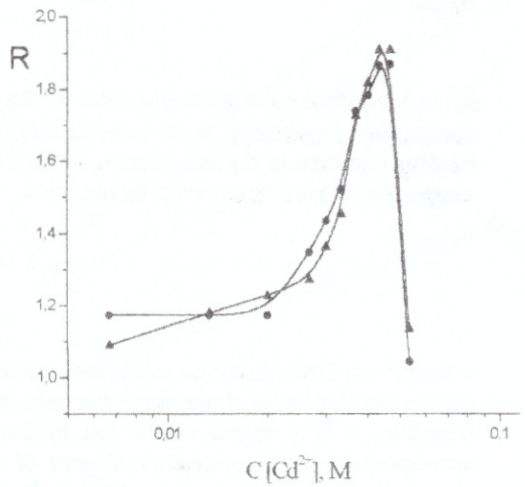

Fig. 2. Dependence of R on C $[Cd^{2+}]$ of maximum of absorption bands at 1090 (•) and 1225 (▲) cm^{-1} for DNA complexes with Cd^{2+} ions; R is ratio of optical density of absorption bands of DNA complexes to their values without ions.

Then N/N_t will be proportional to the compaction degree of the polyelectrolyte r. With $(N-1) \approx N$ for large N, ΔG_N can be written as:

$$\Delta G_N \approx \Delta H_0 - T\Delta S_0 + \frac{N}{N_t} N_t (\Delta H_0 - T\Delta\overline{S_i}) \approx \Delta G_0 - r \cdot W \tag{4}.$$

here W is a coefficient equal to $-N_i(\Delta H_0 - T\Delta S_i)$. Thus, an addition to the free energy varies with the compaction degree.

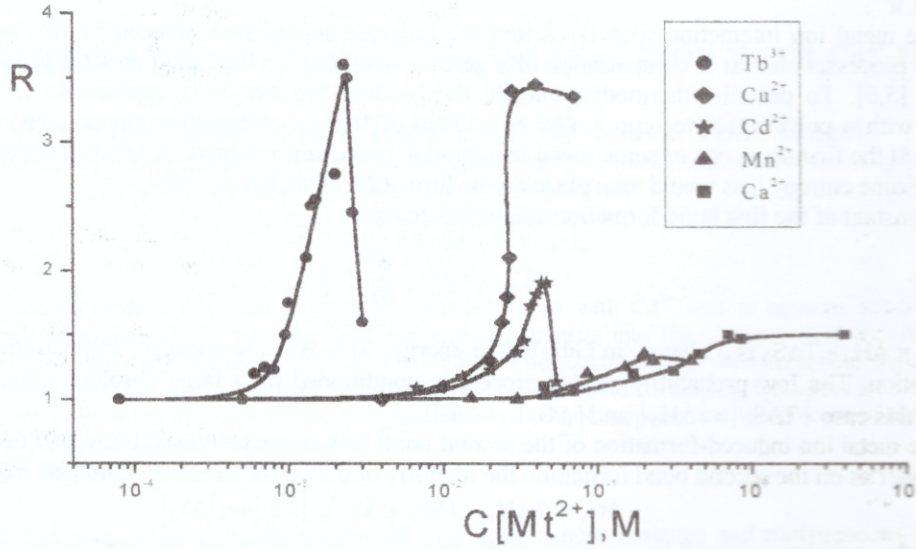

Fig. 3. Dependences of relative change in optical density (R) on ion concentration in solution for maximum of absorption band 1090 cm^{-1} (symmetrical vibrations of phosphate groups) for DNA complexes with Cu^{2+} [6], Cd^{2+}, Mn^{2+} [5], Ca^{2+} [5] and Tb^{3+} [6] ions.

Taking (4) into account, relation (1) could be transformed into

$$K = \exp\left(\frac{-\Delta G_0 + Wr}{RT}\right) = K_0 \times \exp(\omega \times r), \quad (5).$$

where

$$\omega = \frac{W}{R \cdot T} \quad (6).$$

K_0 is a constant conditioning a value of the threshold concentration of divalent metal ions, corresponding to the compaction beginning; ω is a cooperativity parameter. With this equation applied for K and with a simple binding equation in the Scatchard's form, a dependence of r on C_f at different values of the binding constant and cooperativity parameter could be obtained:

$$\frac{r}{(0,5 - r)} = K_0 \times \exp(\omega \times r) \times C_f, \quad (7).$$

$$D = C_f + 0.5 \times P \times r, \quad (8).$$

where P is a DNA molar concentration, D is the total Me^{2+} concentration in solution, C_f is a concentration of free Me^{2+} ions that is of those ions that take no participation in the formation of bonds between polyelectrolyte segments, r is a degree of the ion binding to DNA. As well, in accordance with the above description, r corresponds to the compaction degree of the polymer (that is to a fraction of parts having formed ordered structures under the present conditions). Fig. 4 shows binding isotherms calculated by formulae (7-8) at different values of K_0 and ω. At large ω ($\omega > 8$) these binding isotherms have a S-like form (similar to Van-der-Waals isotherms for phase transitions liquid-vapor). Such binding isotherms include metastable parts and absolutely nonstable ones (with the reverse r dependence on C_f) and in the case of stable complexes have to be replaced with dependences with a jump of r that is equivalent to the first type phase transition. Binding constants in equations (1) and (5) are dimensionless values. Magnitudes of these constants describe the equilibrium in solution the concentration of which is conditioned with the ratio between the number of dissolved substance particles and that of solvent molecules. With the value of the C_f concentration expressed in mole/litres, used in the Scatchard's equation (7), values of K_0 should be multiplied by the dimensional coefficient conditioning the ratio between concentrations. Values of K_0 obtained this way are given in Table 1. It is obvious that relationships between constants for different ions as well as between values of cooperativity parameters do not depend on the choice of concentration units.

The results presented evidence significant cooperativity of the considered process for all the ions. It should be noted that, despite of the large difference between values of K_0, those of cooperativity parameters differ slightly. This indicates the unified cooperativity mechanism for all the ions, conditioned with the process of DNA molecule compaction.

It should be noted that there are two types of the compaction process considered. In the first, so-called ψ-condensation can be realized when one DNA molecule winds into the ordered particle of the toroidal form. In the second, this can be aggregation of some molecules forming a partially ordered structure. To identify the possible structure of the particles and to ascertain the compaction type, more detailed studies of IR spectra in wide frequency and temperature ranges are required. During these investigations it is necessary to apply such structure-sensitive methods as circular dichroism of the electron-vibrational spectral range (ECD) or vibrational circular dichroism (VCD) as well. The latter method was successfully applied in a number of works [9, 10]. But these works studied ion effect on a relatively low-molecular DNA (~10^5–10^6 Da) that makes it difficult to compare these results with the data for the high-molecular DNA (~10^7 Da).

Table 1. DNA-metal ion binding constants (K_0) and cooperativity parameters ω.

	K_0, M^{-1}		ω	
DNA	DUVS *, [4] denatured DNA	IR, native DNA	DUVS *, [4] denatured DNA	IR, native DNA
Ca^{2+}	90	0,8	5	6
Mn^{2+}	$8 \cdot 10^3$	5	5–6	8
Cu^{2+}	$9 \cdot 10^3$	11	10	12
Cd^{2+}	–	11	–	9

* DUVS - differential UV spectroscopy.

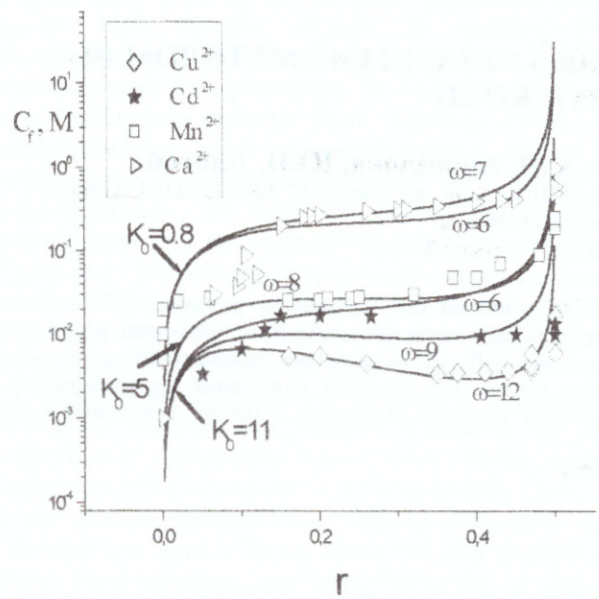

Fig. 4. Dependence of ion binding degree with DNA (r) on free Me^{2+} ions in solution (C_f) for ions Cu^{2+} [6], Cd^{2+}, Mn^{2+} [5], Ca^{2+} [5].

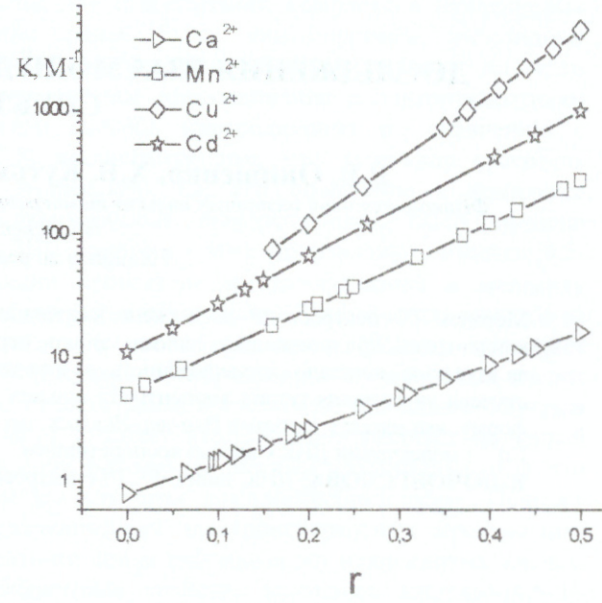

Fig. 5. Dependence of binding constants on DNA-ion binding degree for ions of different divalent metals: Cu^{2+} [6], Cd^{2+}, Mn^{2+} [5], Ca^{2+} [5].

Fig. 5 presents the dependence of the Cd^{2+} ion binding constant (K) on the DNA compaction degree (r), calculated by formula (5) and compares this constant with the data for ions of different divalent metals. The rise of binding constants with the increase of the compaction degree evidences positive cooperativity of the present process.

CONCLUSIONS

Upon binding of Cd^{2+} to native DNA the sharp change in the optical density is observed at frequencies corresponding to vibrations of phosphate groups, as revealed by IR-spectroscopy. This change is supposed to be proportional to the degree of the Cd^{2+} ion binding to DNA phosphate groups. The binding isotherm for DNA complexes with Cd^{2+} ions is calculated. Comparison of the isotherm calculated and the results obtained for other divalent metal ions revealed that the shape of Cd^{2+}-DNA binding isotherms is close to that of near-critical ones. They differ significantly by the value of K_0 that is conditioned with ion affinity for binding centers on DNA.

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ДОСЛІДЖЕННЯ ВЗАЄМОДІЇ ДНК З ІОНАМИ Cd^{2+} МЕТОДОМ ІЧ-СПЕКТРОСКОПІЇ

В.В. Онищенко, Х.В. Кутько, С.В. Корнілова, Ю.П. Благой
Фізико-технічний інститут низьких температур НАН України, пр. Леніна, 47, Харків, 61103, Україна
onyshchenko@ilt.kharkov.ua
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Методом ІЧ-спектроскопії досліджено взаємодію ДНК з іонами Cd^{2+} у водному розчині. ДНК високої концентрації при взаємодії з іонами металів переходить до компактної структури. Визначені константи зв'язування, показано кооперативність зв'язування іонів Cd^{2+} та встановлено немонотонну залежність ступеня зв'язування (r) від концентрації вільних іонів метала (C_f). Ізотерми зв'язування мають S-подібну форму, яка нагадує ізотерми Ван-дер-Ваальса для фазового переходу рідина-пар, а процес зв'язування іонів Cd^{2+} з молекулами ДНК є високо кооперативним.
КЛЮЧОВІ СЛОВА: ДНК, іони Cd^{2+}, ІЧ-спектроскопія.