

# Application of equilibrium binding model for analysis of conformational transitions in poly(rA)poly(rU) complexes with metal ions

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**Aim.** The study is aimed at generalization of the previous experimental results on the metal ion ( $Mg^{2+}$ ,  $Ni^{2+}$ ,  $Cd^{2+}$ ) effects on conformation transitions in poly(rA)poly(rU). The objective was to find out how the type of a metal ion-polynucleotide complex influences the phase transitions and to estimate the constants ( $K$ ) of ions binding to polymers of different structures. **Methods.** The  $K$  values were obtained upon theoretical and experimental transition temperature fitting by the least-square method with the root mean square deviation minimized through the procedure of the gradient descent in the multidimensional space. **Results.** Calculations of diagrams with  $Mg^{2+}$  are shown to permit obtaining satisfactory results if concentration-independent, mean values of constants are used. For  $Ni^{2+}$  and  $Cd^{2+}$  the concentration dependence of  $K$  must be taken into account, especially for high ion contents at which compaction of single-stranded poly(rA) emerges. It was revealed that the main factor responsible for the differences in diagrams with  $Ni^{2+}$  and  $Cd^{2+}$  is a significant distinction of their constants of binding to poly(rA) and poly(rU). **Conclusions.** The model theory of equilibrium binding is capable to describe adequately conformation transitions in polynucleotides in the presence of metal ions.

**Keywords:** phase diagrams, conformational transitions, metal ions, poly(rA), poly(rU), poly(rA)poly(rU).

**Introduction.** By the present time, a great number of studies on conformational transitions in double and triple chains formed by homopolynucleotides poly(rA) and poly(rU) in solutions with different metal ions ( $Mg^{2+}$ ,  $Ni^{2+}$ ,  $Cd^{2+}$  and others) has been carried out, using a number of experimental methods (UV-, IR- and VCD-spectroscopy) [1–10]. Dependences of duplex-coil ( $AU \rightleftharpoons A + U$ , or  $2 \rightarrow 1$ ), duplex-triplex ( $2AU \rightleftharpoons A2U + A$ , or  $2 \rightarrow 3$ ) and triplex-coil ( $A2U \rightleftharpoons A + 2U$ , or  $3 \rightarrow 1$ ) transitions, characterized by the melting temperature ( $T_m$ ) on ion concentrations, have been ascer-

tained. Therefore, it is of interest to consider possibilities of applying existing model theories of helix-coil transitions for describing the results obtained. This permits to predict the transition character at different ion concentrations and to determine the constants of ion-polymer binding.

In our previous works [1, 2], the equilibrium binding model [11] was successfully used for describing  $2 \rightarrow 1$  transitions («melting») of heterogeneous native DNA in the presence of biologically active substances. With respect to the binding of monomeric substances, in this case both DNA strands are equivalent after their separation. Nevertheless, upon the

polymer binding to ions, mutual repulsion of the cations bound and possible compaction of single-stranded molecules have to be taken into account in the presence of the ions mentioned. The problem is more complicated in the case of synthetic homopolynucleotides forming three-stranded helices consisting of purine and pyrimidine strands. In this case several transition types (2 → 1, 3 → 2, 3 → 1) are possible. The constants of ions binding to purine and pyrimidine strands and dependences of these constants on the binding degree and temperature differ significantly. Besides, the transition of polynucleotides into a compact form is observed, followed with significant changes of the binding constants and the precipitation of compact particles. In particular, this process can provide an explanation for irreversibility of 2 → 3 transition in solution with Ni<sup>2+</sup> [9]. All the stated require additional experimental and theoretical data on ion-polynucleotide complexes in order to avoid ambiguity in calculation results.

In the previous papers related to the Mg<sup>2+</sup>, Ni<sup>2+</sup> and Cd<sup>2+</sup> complexes with AU and A2U the dependence of binding constants on ions concentration was not taken into account [1–8], the averaged values of constants *K* were used, and the description was of the qualitative character.

In the present work an attempt is made for more rigorous describing the experimental data for transitions in polynucleotide structures formed by poly(rA) and poly(rU) with Mg<sup>2+</sup>, Ni<sup>2+</sup> and Cd<sup>2+</sup>. All the above factors have been taken into consideration.

**Methods.** Experimental dependences of melting temperatures of polynucleotides in solutions with metal cations can be described theoretically by formulas based on the model of the most probable distribution [11–14]. At the same time the constants of ion binding to polynucleotides of the appropriate structures can be determined. More generally, formulas for transitions are the following [3, 4]:

for 2 → 1 transition

$$T_{m2 \rightarrow 1} = T_{0^2 \rightarrow 1} / \left( \left( 1 - \frac{1}{2} R T_{0^2 \rightarrow 1} / H_{2 \rightarrow 1} \right) \ln \left( (1 - K_{AU} A_f)^2 / \left( (1 - K_A A_f) (1 - K_U A_f) \right) \right) \right), \quad (1)$$

for 3 → 1 transition

$$T_{m3 \rightarrow 1} = T_{0^3 \rightarrow 1} / \left( \left( 1 - \frac{1}{3} R T_{0^3 \rightarrow 1} / H_{3 \rightarrow 1} \right) \ln \left( (1 - K_{A2U} A_f)^3 / \left( (1 - K_A A_f) (1 - K_U A_f)^2 \right) \right) \right), \quad (2)$$

for 2 → 3 transition

$$T_{m2 \rightarrow 3} = T_{0^2 \rightarrow 3} / \left( \left( 1 - \frac{1}{2} R T_{0^2 \rightarrow 3} / H_{2 \rightarrow 3} \right) \ln \left( (1 - K_{AU} A_f)^2 / \left( (1 - K_{A2U} A_f) (1 - K_A A_f) \right) \right) \right). \quad (3)$$

Here *T<sub>m</sub>* is the melting temperature of the appropriate transition, *T<sub>0</sub>* is the melting temperature at zero concentration of divalent cations, *H<sub>ij</sub>* is enthalpy of the corresponding transition, *R* is the gas constant, *A<sub>f</sub>* is the concentration of free cations in solution, and *K<sub>A</sub>*, *K<sub>U</sub>*, *K<sub>AU</sub>*, *K<sub>A2U</sub>* are constants of the cation binding to the polynucleotide of certain structure.

Besides, for simplification of calculations and for greater visualization of the results, the formulas (1)–(3) were rearranged to

for 2 → 1 transition

$$T_{2 \rightarrow 1} = B_{2 \rightarrow 1} \ln \frac{(1 - K_{AU} A_f)^2}{(1 - K_A A_f) (1 - K_U A_f)}; \quad (4)$$

$$B_{2 \rightarrow 1} = \frac{R T_0 T_{m2 \rightarrow 1}}{2 H_{2 \rightarrow 1}};$$

for 3 → 1 transition

$$T_{3 \rightarrow 1} = B_{3 \rightarrow 1} \ln \frac{(1 - K_{A2U} A_f)^3}{(1 - K_A A_f) (1 - K_U A_f)^2}; \quad (5)$$

$$B_{3 \rightarrow 1} = \frac{R T_0 T_{m3 \rightarrow 1}}{2 H_{3 \rightarrow 1}};$$

for 2 → 3 transition

$$T_{2 \rightarrow 3} = B_{2 \rightarrow 3} \ln \frac{(1 - K_{AU} A_f)^2}{(1 - K_{A2U} A_f) (1 - K_A A_f)}; \quad (6)$$

$$B_{2 \rightarrow 3} = \frac{R T_0 T_{m2 \rightarrow 3}}{2 H_{2 \rightarrow 3}}.$$

where *T<sub>ij</sub>* = *T<sub>m</sub>* – *T<sub>0</sub>*.

Table 1  
Parameters used in calculations of transition temperatures in systems of poly(rA)poly(rU) with cations

Transition	H, kcal/mol	B <sub>ij</sub> , grad	T <sub>0</sub> , K
2 1	9	12.1	332 1
3 1	12	9.6	332 1
2 3	4	25	332 1

As  $T_m$ ,  $T_0$ ,  $B_{ij} = R T_0^2 / 2 H_{ij}$  was used in calculations.

As is seen from the formulas (1)–(3) for  $T_m$  calculations, the data are necessary on transition enthalpies  $H_{ij}$  and on ion binding constants being functions of temperature and concentrations of the ions. The above formulas applied for describing experimental phase diagrams permit to determine  $K$  values for polynucleotides of different structures and to appreciate differences in the ion action. However, in a general case the determination of correct values can be ambiguous due to a great number of unknown parameters in the formulas (1)–(3). Therefore, some literature data were used in our calculations using the above formulas.

Enthalpies of transitions  $H_{ij}$  were taken from experimental works of Stevens and Felsenfeld [15], Krakauer [16], Klump [17]. However, if to take into account the supposition that enthalpies of transitions in homopolynucleotides are conditioned with the degree of the chain disordering and, hence, the stacking failure [18], these values are not dependent on the ion kinds.

$H$  values used (Table 1) are within dispersion of the data obtained by different authors at  $T_m \sim (55–60)^\circ\text{C}$ . All initial parameters used in our calculations are presented in Table 1.

To determine the binding constants and their concentration dependences, we used two approaches:

1. In the simplest case (the constants are not dependent on ions concentration) the least-squares method and the method of the gradient descent in 4-dimensional space [19] were used for calculations. At that constants varied up to the moment when the mean square deviation of theoretical  $T_m$  values (1)–(3) from the experimental ones

$$2. \quad S(K_A, K_U, K_{AU}, K_{A2U}) \min_k (T_{m_{theor}}(A_{jk}) - T_{m_{exp}}(A_{jk}))^2 \quad (7)$$

took on the minimum value.  $k$  is index of the preset experimental points.

Unfortunately, the method gave satisfactory results only for  $\text{Mg}^{2+}$  ions for which concentration dependences of binding constants were not be taken into account. The reasons will be discussed below. For more precise calculations in the case of  $\text{Ni}^{2+}$  and  $\text{Cd}^{2+}$  ions the dependence of ion binding constants on concentration must be taken into account.

2. Obtained by a differential UV spectroscopy (DUVS) method, the experimental dependences of binding constants  $K$  on the binding degree  $C$  [7, 8] were re-calculated to the dependence of  $K$  on  $A_f$  with the formula

$$K(A_f) = \frac{C}{(1 - C) A_f}, \quad (8)$$

substituting  $K(A_f)$  in the formulas (4)–(6) and correcting their values up to coincidence of the calculated  $T_m$  with their experimental values. To smooth stochastic fluctuations of experimental points in diagrams, at first, we approximated them by cubic splines (solid curves in Figs 1–3), and then (formulas (1)–(6)) were fitted to points at these spline curves.

After this, the dependences of  $K$  on  $A_{fk}$  were defined more exactly (formulas (1)–(3)) by the least squares method with the minimization of the mean square declination

$$S(K_A(1:N_C), S(K_U(1:N_C)), S(K_{AU}(1:N_C)), S(K_{A2U}(1:(N_C - N_{2-1})))) \min \quad (9)$$

by the method of the gradient descent in  $N$ -dimensional space [19]. Here  $N = 4 \cdot N_C - N_{2-1}$  is the number of varied parameters,  $N_C$  is the total number of  $A_{fk}$  concentrations in the diagram (Figs 1–3),  $N_{2-1}$  is concentrations number for 2 1 transition ( $N = 40$  and  $N = 48$  for AU with  $\text{Cd}^{2+}$  and  $\text{Ni}^{2+}$ , respectively) and  $S(\dots)$  is determined by formula (7). In this case we used exact formulas (1)–(3). Initial values of constants were taken from [3–10]. Obtained in this procedure, dependences of  $K$  on  $A_{fk}$  ( $k = 1, \dots, N_C$  is the index of preset points) are presented in Figs 4–6.

It is seen that calculated  $T_{m_{theor}}(A_{fk})$  values coincide with  $T_{m_{exp}}(A_{fk})$  preset on spline curves with a big accuracy (see Figs 1, 2: black squares on solid spline

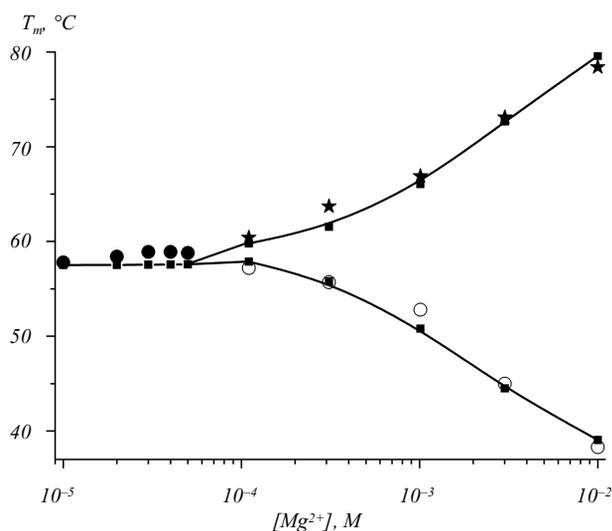


Fig. 1. Phase diagram of poly(rA)poly(rU) with  $Mg^{2+}$ . Experimental  $T_m$  values [4] are shown by signs: filled circle –  $T_{m2,1}$ ; open circle –  $T_{m2,3}$ ; asterisk –  $T_{m3,1}$ . Square are calculation values

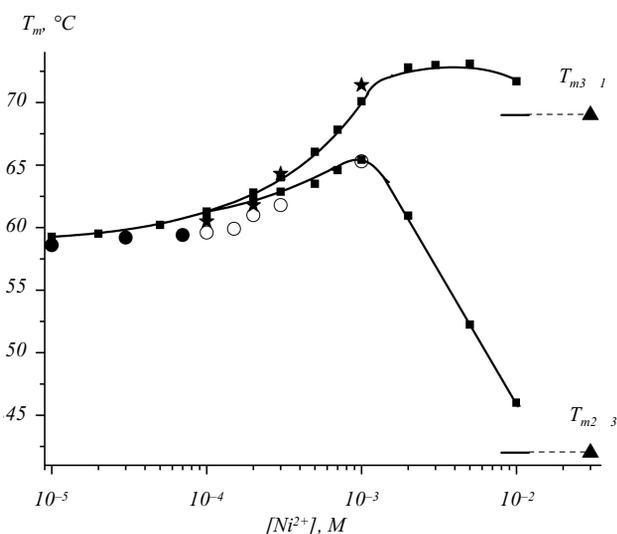


Fig. 2. Phase diagram of poly(rA)poly(rU) with  $Ni^{2+}$ . Experimental  $T_m$  values [7] are shown by signs: filled circle –  $T_{m2,1}$ ; open circle –  $T_{m2,3}$ ; asterisk –  $T_{m3,1}$ . Square are calculation values. Triangle –  $T_{m3,1}$  and  $T_{m2,3}$  are transition temperatures obtained by IR and VCD methods at corresponding total ion concentrations in solution [9]. ---  $T_m$  values corresponding to approximated concentration  $A_f$  of free ions, used in calculations

curves). The deviation of experimental  $T_m$  values from calculated  $T_m$  on spline curves is about 1 K.

**Results and discussion.** Analysis of calculated  $T_m$  dependences on ion concentrations and their comparison with the experimental data (Figs 1–3) permit to ascertain principal factors determining forms of phase diagrams for various ion types. First of all, as noted

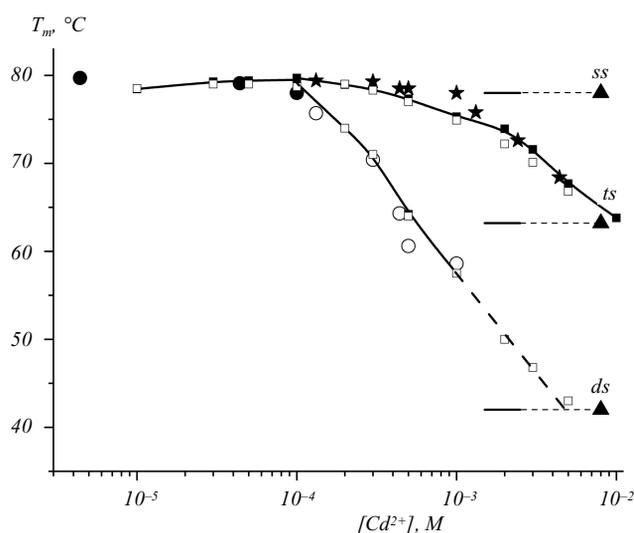


Fig. 3. Phase diagram of poly(rA)poly(rU) with  $Cd^{2+}$ . Experimental  $T_m$  values [10] are shown by signs: filled circle –  $T_{m2,1}$ ; open circle –  $T_{m2,3}$ ; asterisk –  $T_{m3,1}$ . Filled square are preset values, open square are calculation values.  $\blacktriangle_{ss}$ ,  $\blacktriangle_{ts}$ ,  $\blacktriangle_{ds}$  mark existence conditions for molecule in single-, three- and double-stranded states, obtained by IR and VCD methods and corresponding to full ion concentrations,  $A_{tot}$  [10]. --- Regions corresponding to approximated contents of free ions ( $A_f$ ), used in calculations

above in the case of  $Mg^{2+}$  ions it is possible to calculate diagrams by fixed constants independent on ion concentrations (Formulas (1)–(3)). This may be related to the fact that  $Mg^{2+}$  ions (as well as  $Na^+$  and  $K^+$ ) bind only to negative charges of phosphate groups, not inducing compaction of polymeric molecules. In this case the binding constants for all polynucleotide strands (single, double and triple ones) decrease with the rise of ion concentrations, and ratios of these constants in formulas (4)–(6) keep about constant. Therefore, mean values of constants used in calculations (Table 2) describe rather well the phase diagram (Fig. 1).

$T_{2,3}$  value lowers and  $T_{3,1}$  increases with the ion concentration rise. This calculation diagram agrees well with the experimental data and is similar to those for  $Na^+$  and  $K^+$  which also interact only with charges of phosphate groups.

By the value order,  $Mg^{2+}$  binding constants (Table 2) agree with earlier values [4, 20] and describe  $T_m$  dependences on  $A_f$  for this system more precisely.

In contrast to  $Mg^{2+}$ , more complicated concentration dependences of transition temperatures  $T_{3,1}$  and  $T_{2,3}$  are observed for  $Ni^{2+}$  and  $Cd^{2+}$ . Besides, at  $Ni^{2+}$  concentration up to  $10^{-3}$  M values of both  $T_{3,1}$  and  $T_{2,3}$

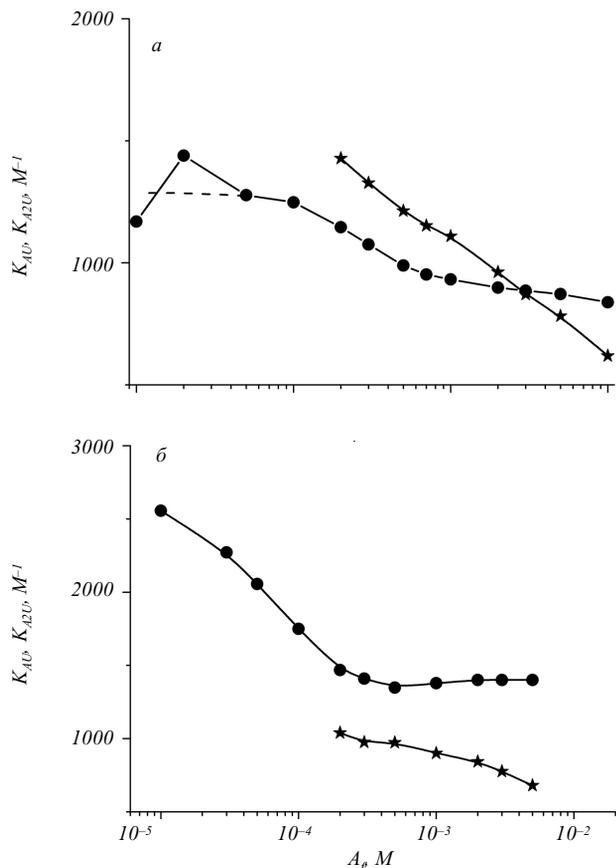


Fig. 4.  $K_{AU}$  and  $K_{A2U}$  dependences on  $Ni^{2+}$  (a) and  $Cd^{2+}$  (b) concentrations, used in calculations. Circle –  $K_{AU}$ ; asterisk –  $K_{A2U}$

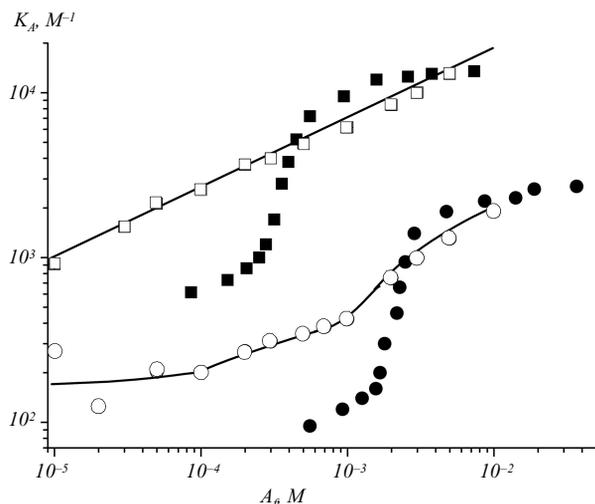


Fig. 5.  $K_A$  dependences on  $A_f$  for solutions with  $Ni^{2+}$  and  $Cd^{2+}$ . Filled circle; filled square – values for  $Ni^{2+}$  and  $Cd^{2+}$ , respectively, obtained by DUVS method and recalculated from linear dependence  $K_A$  on occupation degree ( $C$ ) of binding sites of poly(rA) bases [7, 8]. Open circle, open square – total values of  $K_A$  for  $Ni^{2+}$  and  $Cd^{2+}$ , respectively, used in calculations and taking into account ion binding to phosphates groups of polynucleotides

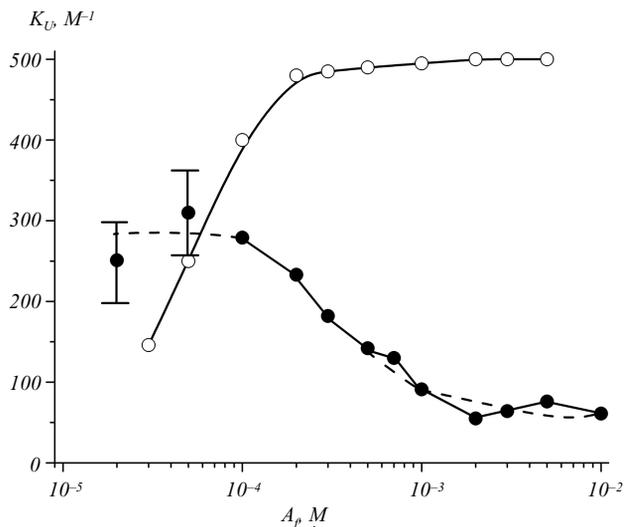


Fig. 6.  $K_U$  dependences on  $Ni^{2+}$  (filled circle) and  $Cd^{2+}$  (open circle) concentrations, obtained upon calculations of diagrams

increase that is both diagram branches are up-directed. At higher  $Ni^{2+}$  concentrations light scattering is observed, evidencing the initiation of polymer compaction (presumably, of poly(rA)), and this prevents from measurements by UV spectroscopy [3]. IR and VCD spectroscopy applied [9] permitted to determine  $T_{2-3}$  and  $T_{3-1}$  values at  $A_f$  corresponding to  $\sim 10^{-2}$  M  $Ni^{2+}$ . At this concentration  $K_A$  is about  $2 \cdot 10^3$   $M^{-1}$  because of the ion binding to bases in compact particles. In this case  $T_{2-3}$   $-17^\circ C$  and  $T_{3-1}$   $+10^\circ C$  are close to values for  $Mg^{2+}$  (Fig. 2). It should be noted too that a higher concentration of poly(rA)poly(rU) ( $\sim 10^{-2}$  M) shifts the beginning of disproportion 2-3 transitions into the region of higher concentrations. Unfortunately, in this case correct  $A_f$  calculations for  $Ni^{2+}$  are impossible because of lack of the data on constants of the ion binding to polynucleotides, especially taking into account a possible compaction of poly(rA). Therefore, Fig. 2 presents points  $\blacktriangle$  of 2-1 ( $T_{m2-1}$ ) and 3-1 ( $T_{m3-1}$ ) transitions at the total ion concentration  $3 \cdot 10^{-2}$  M and  $A_{tot}/P = 0.4$ , where  $P$  and  $A_{tot}$  are a polymer content and total  $Ni^{2+}$  concentration, respectively. The regions of approximated  $A_f$  values for corresponding transitions are marked with horizontal lines.

In contrast to  $Ni^{2+}$  ions,  $Cd^{2+}$  reduces temperatures  $T_{2-3}$  and  $T_{3-1}$  (Fig. 3), at that  $T_{2-3}$  decreases especially sharply [10]. Unlike data for  $Ni^{2+}$ , those for  $Cd^{2+}$  obtained in [10] (Fig. 3) characterize not the transition temperatures but structural states of polynucleotides,

Table 2

Values of cation-polyribonucleotide binding constants, obtained by least-squares method for experimental phase diagram with  $Mg^{2+}$  (concentration dependences of constants were not taken into account)

$K_A$	$K_U$	$K_{AU}$	$K_{A2U}$
264	170	282	774

namely 1) only single-stranded molecules –  $\blacktriangle_{ss}$ , 2) triple-stranded ones + single-stranded poly(rA) –  $\blacktriangle_{ts}$ , 3) double-stranded molecules –  $\blacktriangle_{ds}$ . These states are realized in appropriate concentration and temperature regions. As seen from Figure, UV spectroscopic data on existence regions of double-, triple- and single-stranded structures correlate qualitatively with IR and VCD spectroscopic findings. As in the case of  $Ni^{2+}$ , it was impossible to perform exact quantitative comparison of  $A_f$  values obtained by UV and IR spectroscopy because of high polynucleotide concentrations and molecule compaction in the last method. In this case in theoretical calculations the concentration of free ions ( $A_f$ ) is approximate. The transition of poly(rA) molecules into the compact state results in irreversibility of the disproportional transition 2 → 3 [3, 10]. At least, in the experiment period under cooling up to  $T_m \sim 20$  °C the equilibrium in the system practically does not change. To clear up the reasons of such an essential difference between diagrams with  $Ni^{2+}$  and  $Cd^{2+}$ , it is necessary to consider concentration dependences of binding constants of these ions to all three structures of polynucleotides ( $K_A$ ,  $K_U$ ,  $K_{AU}$  and  $K_{A2U}$ ).

The least distinction between binding constants of these ions is observed for the triple chain of polynucleotides ( $K_{A2U}$ ). In this case only Coulomb interactions of ions with negative charges of phosphate groups and mutual ion repulsion are possible which decreases binding constants upon the rise of the ion concentration in solution. Therefore, in the case of equal ion charges, their  $K_{A2U}$  constants are of identical concentration dependences within the limit of the calculation accuracy (Fig. 4).

Unlike the above, the constants of ion binding to the double chain ( $K_{AU}$ ) differ highly essentially (Fig. 4). The difference is conditioned with the possibility of the ion binding to bases, mainly to N7 and, to a somewhat lesser extent, to N1 of adenine, resulting in the forma-

tion of macrochelate with phosphate. In comparison with  $Ni^{2+}$  ions,  $Cd^{2+}$  has higher affinity to these atoms. As a result,  $K_{AU}$  is greater than  $K_{A2U}$  (Fig. 4) in spite of the fact that the density of negative charges on the triple chain is higher. In this case, with concentrations from  $10^{-5}$  M to  $5 \cdot 10^{-4}$  M  $Cd^{2+}$  interaction with phosphates AU prevails and the binding is of the anticooperative character. In  $5 \cdot 10^{-4}$ – $10^{-2}$  M  $Cd^{2+}$  interaction with nitrogen bases in compact poly(rA) dominates, and the binding is of the cooperative character. As a result, the dependence of  $K_{AU}$  on  $A_f$  goes through the minimum at about  $A_f = 5 \cdot 10^{-4}$  M. This peculiarity of  $Cd^{2+}$  interaction has an influence on the form of poly(rA)poly(rU) diagram and especially on the 2 → 3 transition character, as follows from the formula (6).

The main factor determining the differences of diagrams for  $Ni^{2+}$  and  $Cd^{2+}$  is binding of these ions to single-stranded polynucleotides poly(rA) and poly(rU), especially to the polyadenylic chain ordered at the given temperature.

As noted earlier, in the case of  $Cd^{2+}$  the formation of macrochelate complexes and subsequent compaction of the polymer take place at a significantly lower concentration of ions than that for  $Ni^{2+}$ . Besides,  $Cd^{2+}$  binding constant  $K_A$  is noticeably higher than that for  $Ni^{2+}$  (Fig. 5) and reaches  $1.5 \cdot 10^4 M^{-1}$  in the region of the molecule compaction. This value is significantly higher than  $K_{AU}$  and  $K_{A2U}$ . Such a high constant used in calculations agrees well with  $K_A$  value [8] obtained earlier at the high degree of the binding site occupation ( $C \sim 1$ ), using DUVS method. Distinctions between  $K_A$  calculated and obtained by UV-spectroscopy method at low ion concentrations are caused by the fact that calculations provide the total value of constants, evaluating the sum of all the binding types (to phosphates and bases) while DUVS method records only binding to polymer bases. It follows from the formulae (5) and (6) that upon such a significant increase of  $K_A$  the values  $T_{3 \rightarrow 1}$  and  $T_{2 \rightarrow 3}$  have to lower sharply (Fig. 3).

An additional contribution into forms of calculated diagrams are made by differences in the constants of  $Ni^{2+}$  and  $Cd^{2+}$  binding to poly(rU) (Fig. 6). As shown earlier [7],  $Ni^{2+}$  induces no changes in DUV spectra of poly(rU), and this is an evidence of its binding only to negative charges on oxygen atoms of phosphate groups. Because of Coulomb ion repulsion upon the

binding site occupation,  $K_U$  value decreases. As shown in [10], unlike  $Ni^{2+}$  ions,  $Cd^{2+}$  binds additionally to poly(rU) bases, forming chelate complexes and ordering poly(rU) molecule.  $K_U$  used in calculations (Fig. 6) agrees with values obtained in our experiments.

**Conclusions.** 1. Calculated concentration dependences of  $Mg^{2+}$ ,  $Ni^{2+}$  and  $Cd^{2+}$  binding constants with polynucleotides permitted to explain the behavior of temperatures of 3 1 and 2 3 conformational transitions in phase diagrams of poly(rA)poly(rU) complexes with these ions.

2.  $Mg^{2+}$  ion effect on conformational transitions may be described by mean values of constants not depending on the concentration. In the case of  $Ni^{2+}$  and  $Cd^{2+}$  the concentration dependence of constants has to be taken into account.

3. Differences in ion effects on conformational transitions are mainly determined by the values of constants for the ion binding to single-stranded poly(rA) and poly(rU).

4. Induced by  $Ni^{2+}$  and  $Cd^{2+}$  ions, compaction of single-stranded structures has a significant influence on the phase equilibrium in the systems studied. This effect may condition irreversibility of some transitions upon the temperature changes.

5. The values of binding constants are conditioned by interactions with both phosphate groups and adenine N7 and N1, and uracil N3 atoms.

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Застосування моделі рівноважного зв'язування до аналізу конформаційних переходів у комплексах poly(rA)poly(rU) з іонами металів

Резюме

**Мета.** Роботу присвячено узагальненню отриманих раніше експериментальних результатів щодо впливу іонів металів ( $Mg^{2+}$ ,  $Ni^{2+}$  і  $Cd^{2+}$ ) на конформаційні переходи в poly(rA)poly(rU). Мета роботи полягала у з'ясуванні впливу типу комплексу іона металу з полінуклеотидом на вид фазового переходу і визначенні констант їхнього зв'язування ( $K$ ) з полімерами різної структури. **Методи.** Величини  $K$  визначали підгонкою теоретично розрахованих значень температур переходів до експериментальних за методом найменших квадратів з мінімізацією середньоквадратичного відхилення методом градієнтного спуску в багатовимірному просторі. **Результати.** Розрахунки фазових діаграм за присутності іонів  $Mg^{2+}$  показали можливість одержання задовільних результатів при використанні постійних, незалежних від концентрації середніх значень констант. У разі  $Ni^{2+}$  і  $Cd^{2+}$  такі залежності мають бути враховані, особливо в межах великих концентрацій іонів, де

відбувається компактизація однитчатої poly(rA). Встановлено, що відмінність діаграм з  $Ni^{2+}$  і  $Cd^{2+}$  обумовлена значною різницею у їхніх константах зв'язування з poly(rA) та poly(rU). **Висновки.** Отримані результати свідчать про можливість успішного опису конформаційних переходів у полінуклеотидах за присутності іонів металів при використанні модельної теорії рівноважного зв'язування.

Ключові слова: фазові діаграми, конформаційні переходи, іони металів, poly(rA), poly(rU), poly(rA)poly(rU).

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Применение модели равновесного связывания для анализа конформационных переходов в комплексах poly(rA)poly(rU) с ионами металлов

Резюме

**Цель.** Работа посвящена обобщению полученных ранее экспериментальных результатов по влиянию ионов металлов ( $Mg^{2+}$ ,  $Ni^{2+}$ ,  $Cd^{2+}$ ) на конформационные переходы в poly(rA)poly(rU). Цель работы – выяснить, как тип комплекса иона металла с полинуклеотидом влияет на вид фазового перехода, и определить константы связывания ( $K$ ) ионов с полимерами различной структуры. **Методы.** Значения  $K$  определяли подгонкой теоретически вычисленных значений температур переходов к экспериментальным по методу наименьших квадратов с минимизацией среднеквадратичного отклонения методом градиентного спуска в многомерном пространстве. **Результаты.** Расчеты фазовых диаграмм в присутствии  $Mg^{2+}$  показали возможность получения удовлетворительных результатов при использовании постоянных, не зависящих от концентрации средних значений констант. В случае  $Ni^{2+}$  и  $Cd^{2+}$  эту зависимость нужно учитывать, особенно в области больших концентраций ионов, где происходит компактизация однитчатой poly(rA). Установлено, что отличие в диаграммах с  $Ni^{2+}$  и  $Cd^{2+}$  обусловлено существенным различием их констант связывания с poly(rA) и poly(rU). **Выводы.** Полученные результаты свидетельствуют о возможности успешного описания конформационных переходов в полинуклеотидах в присутствии ионов металлов с помощью модельной теории равновесного связывания.

Ключевые слова: фазовые диаграммы, конформационные переходы, ионы металлов, poly(rA), poly(rU), poly(rA)poly(rU).

REFERENCES

1. Blagoj Yu. P., Sorokin V. A., Bozhko G. Kh., Valeev V. A., Khomenko S. A. Effect of sodium ions and polycation impurities on DNA heat denaturation // Stud. Biophys. 1977.–65, N 1.–P. 55–67.
2. Blagoj Yu. P., Galkin V. L., Gladchenko G. O., Kornilova S. V., Sorokin V. A., Shkorbatov A. G. Metal complexes of nucleic acids in solutions.–Kiev: Naukova dumka, 1991.–272 p.
3. Sorokin V. A., Valeev V. A., Gladchenko G. O., Degtyar M. V., Blagoj Yu. P.  $Ni^{2+}$  ion effect on conformations of single-, double- and three-stranded homopolynucleotides containing adenine and uracil // Macromol. Biosci.–2001.–1, N 5.–P. 191–203.
4. Sorokin V. A., Valeev V. A., Gladchenko G. O., Degtyar M. V., Karachevtsev V. A., Blagoj Yu. P.  $Mg^{2+}$  ion effect on the conformational equilibrium of polyUpolyApolyU and po-

- lyApolyU in aqueous solutions // *Int. J. Biol. Macromol.*—2003.—**31**, N 4—5.—P. 223–233.
5. Sorokin V. A., Valeev V. A., Gladchenko G. O., Degtyar M. V., Andrus E. A., Karachevisev V. A., Blagoi Yu. P. Mg<sup>2+</sup> and Ni<sup>2+</sup> ion effect on stability and structure of triple polyIpolyApolyU helix // *Int. J. Biol. Macromol.*—2005.—**35**, N 4.—P. 201–210.
  6. Sorokin V. A., Valeev V. A., Usenko E. L., Blagoi Yu. P. Effect of Cd<sup>2+</sup> ions on conformational equilibrium of three-stranded polyUpolyApolyU polynucleotide under near-physiological conditions // *Biopolym. Cell.*—2007.—**23**, N 5.—P. 433–440.
  7. Sorokin V. A., Valeev V. A., Usenko E. L. Ni<sup>2+</sup> ion effect on conformational equilibrium of polynucleotides: polyA, polyU, polyA and polyU under conditions close to physiological ones // *Biopolym. Cell.*—2008.—**24**, N 2.—P. 158–170.
  8. Sorokin V. A., Valeev V. A., Usenko E. L., Andrus E. A., Blagoi Yu. P. The nature of different influence of Cd<sup>2+</sup> ions on the conformational equilibrium of triple-stranded polyribonucleotides PolyUPolyAPolyU and PolyIPolyAPolyI in aqueous solutions // *Biophysics.*—2007.—**52**, N 6.—P. 545–556.
  9. Andrushchenko V., Blagoi Yu. P., van de Sande J. H., Wieser H. Poly(rA)poly(rU) with Ni<sup>2+</sup> ions at different temperatures: infrared absorption and vibrational circular dichroism spectroscopy // *J. Biomol. Struct. Dynam.*—2002.—**19**, N 5—P. 889–906.
  10. Blagoi Yu. P., Gladchenko G. O., Nafie L. A., Freedman T. B., Sorokin V. A., He Ya. Phase equilibrium in poly(rA)poly(rU) complexes with Cd<sup>2+</sup> and Mg<sup>2+</sup> ions, studied by ultraviolet, infrared, and vibrational circular dichroism spectroscopy // *Biopolymers.*—2005.—**78**, N 5—P. 275–286.
  11. Frank-Kamenetskii M. D. Consideration of helix-coil transition in homopolymers by the most probable distribution method // *Mol. Biol.*—1968.—**2**, N 2.—P. 408–419.
  12. Frank-Kamenetskii M. D., Karapetyan A. T. To theory of melting of DNA complexes with low-molecular ligands // *Mol. Biol.*—1972.—**6**, N 4.—P. 621–627.
  13. Lazurkin Yu. S., Frank-Kamenetskii M. D., Trifonov E. N. Melting of DNA: Its study and application as a research method // *Biopolymers.*—1970.—**9**, N 11.—P. 1253–1306.
  14. Cantor C. R., Schimmel P. R. *Biophysical Chemistry.*—San Francisco: W. H. Freeman and Company, 1980.—Part III.—536 p.
  15. Stevens Ch. L., Fensenfeld G. The conversion of two-stranded poly(A+U) to three-stranded poly(A+2U) and polyA by heat // *Biopolymers.*—1964.—**2**, N 1.—P. 293–314.
  16. Krakauer H. A thermodynamic analysis of the influence of simple mono- and divalent cations on the conformational transitions of polynucleotide complexes // *Biochemistry.*—1974.—**13**, N 12.—P. 2579–2589.
  17. Klump H. H. Energetics of order/order transition in nucleic acids // *Can. J. Chem.*—1988.—**66**, N 4.—P. 804–811.
  18. Vesnaver G., Breslauer K. J. The contribution of DNA single-stranded order to the thermodynamics of duplex formation // *Proc. Nat. Acad. Sci. USA.*—1991.—**88**, N 9.—P. 3569–3573.
  19. Wild D. D. *Methods of searching for extremum (in Russian).*—Moscow: Nauka, 1967.—268 p.
  20. Blagoi Yu. P., Egupov S. A., Usenko E. L., Sorokin V. A. Effects of different metal cations on conformational transitions in PolyAPolyrU under concentration and temperature changes // Los Alamos National Laboratory eprint ArXiv: 0904.4100.—New Mexico, 2009.

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