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Effect of Cd^{2+} ions on conformational equilibrium of three-stranded polyU·polyA·polyU polynucleotide at near-physiological conditions

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Cd^{2+} ions effect on the conformational equilibrium of three-stranded polyU·polyA·polyU polynucleotide (0.1 M Na^+, pH 7.0) has been studied by the method of differential UV spectroscopy. It has been revealed that Cd^{2+} ions do not bind to heteroatoms of nitrogen bases of polyA and polyU being in triple (A2U) and double (AU) helices and do not change their conformation. The heating of A2U results in two subsequent processes: the first one represents separation of one strand of polyU from A2U i.e. A2U AU+U transition (3 2 transition) is realised, and the second one corresponds to more cooperative melting of AU, i.e. 2 1 (U+A U+A+U) transition. The concentration dependences of melting temperatures for these processes have some intersection point (T_m = 60°C; $[Cd^{2+}]$ ~ 3.5·10–4 M) at which thermal stability of A2U and AU becomes identical. The theoretical calculations of concentration dependences $(T_m)_{3>2}$ revealed that the best agreement between experiment and theory is observed at the enthalpy of transition $H_{3\ \ 2}$ = 5 kcal/mol·triplet.

Keywords: metal ions, three-stranded polynucleotides, conformational transitions

Introduction. Pollution of environment with heavy metal salts [1–3] from chemical and metallurgical works – the main source of pollution – in developing countries (India, China) nowadays presents a serious ecological problem because of ecological control of the latter. Cadmium (Cd) belongs to the group of the most toxic metals [1, 4]. According to [1], the rate of Cd entering the environment started increasing significantly since 1950s, and in 1980s Cd concentration in rain water in the areas near nonferrous metallurgy works exceeded that in the Earth hydrosphere 2-fold. Pollution of waters in cases of incidents is of special attention danger-wise. For example, one incident in South-East China in 2005 resulted in multifold excess in Cd concentration in river waters. The flow of heavy metal salts into the waters of World Ocean is of specific danger in the light of constantly growing human consumption of marine organisms, which accumulate heavy metal salts in the course of their vital activity.

Metal ions with d-electrons on external atomic orbitals (d-ions) are known to form specific complexes with heteroatoms of nitrogen bases of nucleic acids [5], which results in errors during synthesis of polynucleotide sequences [6]. The latter may lead to disorders in normal functioning of genetic and regulatory apparatuses of cellular and sub-cellular organisms, particularly manifested by the occurrence of mutagenic and carcinogenic activity in cadmium and nickel salts [1, 6]. Maximal carcinogenic indices are specific to chrome and nickel [7], and cadmium was assigned to Group 1 carcinogens by International Agency for Research on Cancer (IARC) [4].

In connection with the above mentioned, the investigation of d-ion complexes formed with biologically functional macromolecules of different levels of structural organization is of current importance. Recently, three-stranded oligo- and polynucleotides, the structure of which remains stable at physiological conditions (0.1 M Mt$^+$, pH 7), have been demonstrated to reveal this functionality [8]. Close attention to these structures is determined by the possibility of their being used in treatment of viral (including AIDS [9]) and cancer diseases [9, 10].

There are hardly any experimental data on complexes of triplexes with d-ions. At the moment, there have been obtained diagrams of conformational equilibrium for complexes of Ni^{2+} ions with three-stranded polyI·polyA·polyI [11] and polyU·polyA·polyU (A2U) polynucleotides [12]. However, in the latter case [12] the effect of Ni^{2+} ions on temperatures of conformational transitions has been examined in low ionic strength solutions ([Na^{2+}] 0.03 M). No similar information for Cd^{2+} ions has been obtained.

Taking into account the above mentioned facts, the aim of current work was to collect the data on the effect of temperature and Cd^{2+} concentration on structure and stability of three-stranded A2U in high ionic strength solutions (0.1 M Na^{2+}), which is similar in the content of Mt$^+$ ions in the cell and, thus, is of great interest.

Materials and Methods. Three-stranded A2U were obtained after long term (not less than a month) keeping at 4°C in buffer solution (0.001 M sodium cacodylate, pH 7; 0.099 M NaCl) of equimolar solution of two-stranded polynucleotide polyA·polyU (AU) and potassium salt of single-stranded polyU. To achieve fuller completion of the process of A2U formation, polynucleotide solutions contained phosphorus in high concentration (0.008 0.01 M). Prior to measuring, the solution was diluted to P (2 3)·10^{-5} M. P value was determined according to the values of molar extinction coefficients of A2U and AU in the absorption maxima [12].

The concentrations of Cd^{2+} ([Cd^{2+}]), introduced to the solution directly before measuring as $CdCl_2 \cdot 0.5H_2O$ salts (*Reachim*, Russia), were determined according to mass and controlled additionally by the complexonometric method. Statistical errors of [Cd^{2+}] and P determination did not exceed 0.5%. Regardless of a relatively high pH value in the investigated Cd^{2+} concentration range (up to 0.001 M), the formation of hydroxides can be neglected [13]. All polynucleotides (*Sigma*, USA) were used without any purification.

A2U melting curves, *i.e.* the dependence of changes in the optical density of solution on temperature (A(T)) at wavenumber ν = 38 500 cm^{-1}, and differential UV spectra, *i.e.* A(ν) (ν – wavenumber) at T_0 = 20±2°C, were measured using spectrophotometers UV-VIS and M40 (*Carl Zeiss Jena*, Germany), respectively. A2U melting curves were recorded as h(T) = A(T)/A_{T0}, where A_{T0} is the optical density of solution at T=T_0, and melting curves of their derivatives dh(T)/dT were recorded using PCs. The melting temperature T_m, corresponding to the transition of approximately half of nucleotide links into new conformation, was determined according to the position of maxima on dh(T)/dT. Melting curves were obtained using the following differential scheme: two cuvettes, located in working and standard channels of the spectrophotometer, contained identical solutions with identical concentrations of polynucleotides and Cd^{2+}. Standard cuvette was thermostated at T=T_0 in the range of ±0.5°C, the working one was heated slowly (heating rate – 0.25°C/min) in order to provide the equilibrated condition of the polymer at each temperature point. DUV-spectra of A2U and AU, induced by Cd^{2+}, were measured using 4-cuvette scheme, which compensates absorption of polynucleotides and ions in both channels of the spectrophotometer.

Results and Discussion. Macrochelates are known to be definitely preferred type of complexes of d-ions with nucleotides (NMP) of purine bases (AMP, GMP, IMP) [14]. Inside these complexes Cd^{2+} ions are innerspherically bound to N7 of purine ring and outerspherically (*i.e.* via one of the molecules of the octahedral hydrate ion shell) bound to the oxygen atom of the phosphate group. The formation of similar structures is promoted by both energy-wise advantageousness of chelation and structure-wise advantageousness, determined by anti-conformation of the sugar ring [15]. Dications of d-ions are capable of binding to oxygen atoms of phosphate groups only, as NMP belong to dianions. The latter type of interaction is preferable for NMP complexes with ions of alkali-earth metals, as well as for d-ions complexes with pyrimidine nucleotides [14, 16]. In the case of uracyl derivatives, d-ions may interact with N3 and/or O4 of nitrogen bases [5, 17]. However, these interactions are rather weak [16].

Innerspheral interaction of d-ions with heteroatoms of nitrogenous bases results in changes in the position and intensity of their absorption bands and, consequently, in the occurrence of DUV-spectra of the certain shape. Detection of these spectra in complexes of single-stranded polyA and polyU with Ni^{2+} and Cd^{2+} [12, 18] testifies to the presence of their interactions with N7 (in the form of macrochelates) and N1 of polyA, as well as O4 and N3 of polyU.

However, it has been revealed that at 0.1 M Na^+ the change in A2U absorption spectrum in the presence of Cd^{2+} does not exceed 80 $M^{-1}\cdot cm^{-1}$ (*i.e.* it is within the measurement error) up to 0.02 M Cd^{2+}. This means that at $T=T_0$ cadmium does not bind to heteroatoms of nitrogen bases of polyA and polyU as a part of three-stranded A2U and does not change its conformation. This result seems to be natural as in the structure of U-A-U triplet (Fig.1) there are potential atoms for binding – N7 and N1 in polyA and N3 in two chains of polyU, included into the system of hydrogen bonds, which makes them unreachable for interaction with Cd^{2+} ions, and apparently with other d-ions as well. Principally possible interaction of cadmium with O4 (Fig.1) is not realised in the current concentration range because of very weak contacts of their ions with polyU uracyl [18].

Remarkable is the fact that although at 0.1 M Na^+ and $T=T_0$ Cd^{2+} ions do not change the absorption spectra of two-stranded polyA·polyU up to 0.003 M Cd^{2+}, however in this case N7 atoms of polyA most active for the binding to Cd^{2+} ions seem to be open for interaction. This result is in good qualitative correlation with results earlier obtained in [12], which state that Ni^{2+} ions do not interact with N7 of AU.

The reason of low sensitivity of AU-pairs of polyA·polyU towards the presence of d-ions in the solution is as follows. According to the data in [20–22], the formation of macrochelates is performed due to the change in conformation of single-stranded polynucleotides, resulting in the formation of short loops, 4–5 nucleotides long [20]. This is connected with the fact that the lengths of statistical segment ($\bar{a}$) and lateral dimension (D) in single-stranded helices are commensurable (D $\bar{a}$ 2 nm) [23]. In double-stranded polynucleotides D = 2 nm and $\bar{a}$ 100 nm [23]. Significant rigidity of molecules makes the formation of mentioned loops impossible, which apparently impedes the formation of macrochelates. Interaction of d-ions with N7 atoms only, reconstructed by their interaction with adenosine (Ado) N7, has got to be very weak. Indeed, binding constants of Ni^{2+}, Cu^{2+}, and Cd^{2+} with AMP N7 in the forms of macrochelates are 310, 1380, and 480 M^{-1}, respectively [14], whereas binding constants of Ni^{2+} and Cu^{2+} ions to Ado are 8 and 20 M^{-1} [24], *i.e.* 40 and 70 times lower.

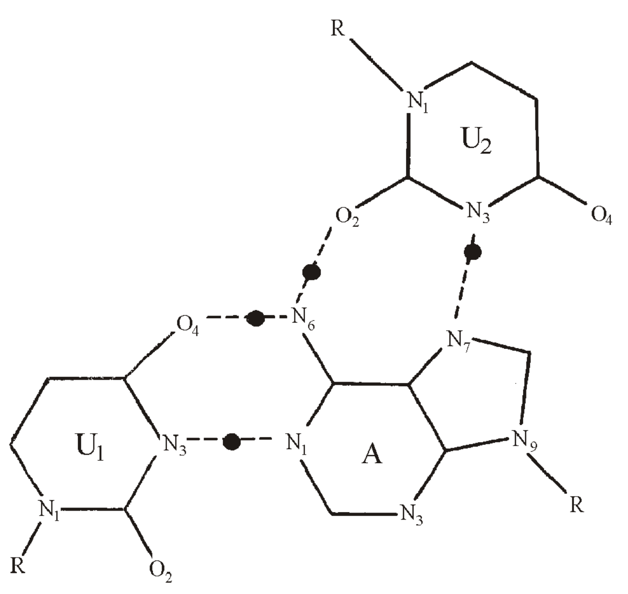

Fig.1 Structure of U-A-U triplet [19]; U_1-A – Watson-Crick pair, (A-U_2) – Hoogsteen reverse pair

Phase diagrams. According to [25–27], heating of A2U in the absence of Mt^{2+} induces two consecutive processes (Fig.2). The first intensive maximum on the differential melting curve corresponds to the separation of one polyU strand (U_2) from A2U (Fig.1), that is to realisation of A2U   AU+U transition (3   2 transition), the second intensive maximum

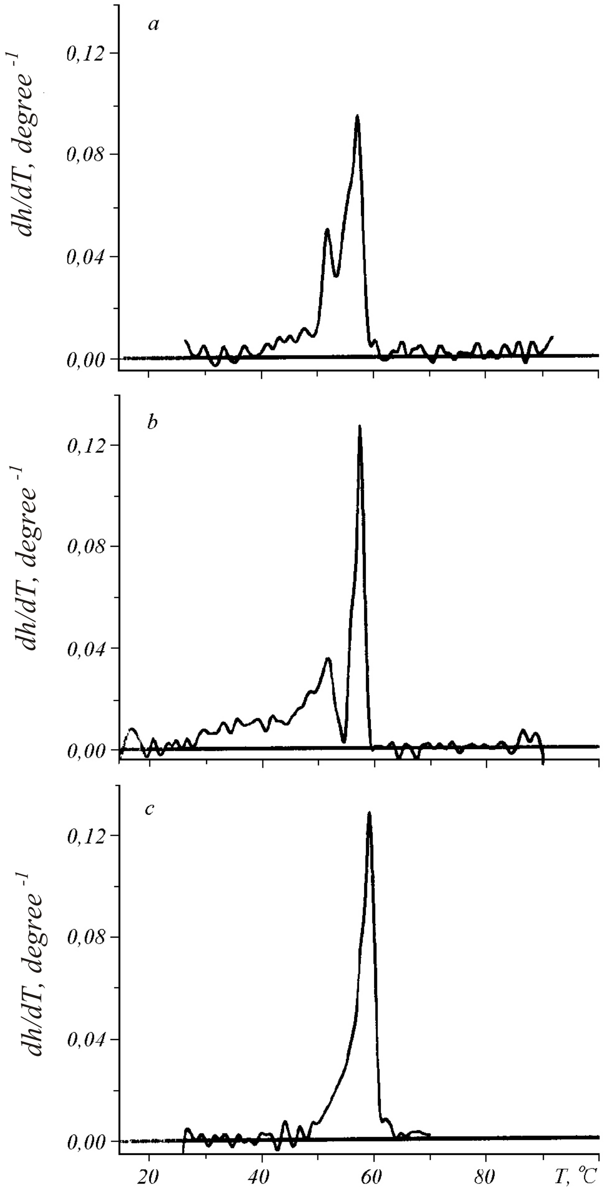

Fig.2 Differential melting curves of A2U (0.1 M Na$^+$, pH 7.0): *a* – [Cd^{2+}] = 0; *b* – 3.5·10^{-5} M Cd^{2+}; *c* –3.5·10^{-4} M Cd^{2+}

corresponds to melting of AU, that is to more cooperative transition 2 1 (U+UA U+A+U).

Though in the cases of different preparations of solutions, corresponding T_{mo} values differ significantly – $(T_{m0})_{3\ \ 2}$ = 50.3 ± 2°C; $(T_{m0})_{2\ \ 1}$ = 56.6± 1.5°C, they are in tolerable correlation with the data of other authors, obtained with 0.1 M Na$^+$ (Table 1).

Table 1
A2U Melting temperatures (0.1 M Na$^+$, pH 7.0) in the absence of Mt^{2+} ions

Transition	$T_{mo,}$°C		
	Current work	[26]	[27]
3 2	50,3±2	50,8	55,5
2 1	56,6±1,5	56	57,7

Dependences of $(T_m)_{3\ \ 2}$ and $(T_m)_{2\ \ 1}$ on Cd^{2+} concentration (Fig.3) are seen to cross in the point corresponding to the critical concentration ([Cd^{2+}]$_{cr}$ 3.5·10^{-4} M) and temperature $(T_m)_{cr}$ = 60°C. Thus, at [Mt^{2+}]=[Mt^{2+}]$_{cr}$ the values of thermostability of A2U and AU are identical (Fig.3) and during heating of A2U only one transition takes place A2U U+A=U (3 1 transition) (Fig.2, *c*). We were not able to obtain the dependence of $(T_m)_{3\ \ 1}$ on [Cd^{2+}] because of high deformation of melting curves, conditioned by partial precipitation of metal-complexes of single-stranded polyA in the region of the conformational transition.

Figure 3 shows that the change in $(T_m)_{2\ \ 1}$ of A2U at the increase of Cd^{2+} concentration to 3·10^{-4} M is in the range of 2°C, whereas in the same concentration range $(T_m)_{3\ \ 2}$ is increased in 8°C. These special forms of the phase diagram obtained may be explained by comparison of experimental concentration dependencies of T_m to those calculated using equilibrated binding theory [11, 12, 28].

For two independent types of binding of ions to the polynucleotide (by phosphates (P) and by nitrogen bases (N)) the dependence is presented as follows [28]:

$$T_m([Mt^2\ ])\quad T_{m_0}\quad T_m([Mt^2\ ])\quad T_{m_o}\quad \frac{RT_{m_0}T_m}{H}\ \ln(\ \ _P\quad _N),\ (1)$$

where T_{m0} = T_m at [Mt^{2+}] = 0; H is enthalpy of the conformational transition.

For 3 2 transition the dependence is presented as follows:

$$_P\quad _N\quad ^{P,N}[\frac{(1\ \ K_3\ \ A_f)^{2\,n_3}}{(1\ \ K_2\ \ A_f)^{n_2}\ (1\ \ K_U\ \ A_f)^{n_U}}].\qquad (2)$$

where *K* is binding constants; *n* is stoichiometry of the complex (the number of ions for one binding site). Indices 3 and 2 mean binding of ions to A2U and AU, re-

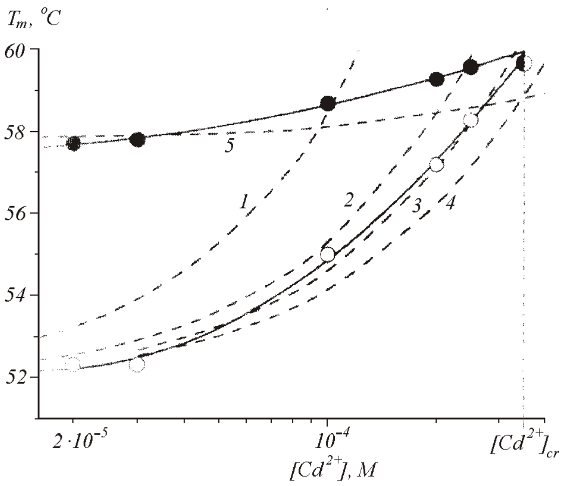

Fig.3 Phase diagram of A2U complex with Cd^{2+} ions (0.1 M Na^+, pH 7.0); T_m is temperature of conformational transition; white and black circles – 3>2 and 2>1; regular lines – experimental dependences of T_m ([Cd^{2+}]), calculated according to the method of the least squares; sectioned lines – dependences of $T_{m3>2}$ on [Mt^{2+}] (1?4) and ($T_{m2>1}$) on [Mt^{2+}] (5), calculated according to (1)–(6)

spectively; U is single-stranded polyU; A_f is the concentration of free ions,

$$A_f \quad [Mt^2 \quad] \quad A_b,(3)$$

where

$$A_b \quad \overset{P,N}{} \quad [P \ (C_3 \ n_3 \quad {}_3 \quad C_2 \ n_2 \quad {}_2 \quad C_U \ n_U \quad {}_U)]. \quad (4)$$

where   is a part of nucleotide links in the corresponding conformation at $T=T_m$ (Table 2); C is the occupation degree of binding sites, which can be calculated according to the formula $C = K\,A_f/1+KCA_f$.

According to the data obtained in the current work, Cd^{2+} are bound to A2U and AU phosphates only, and their interaction with polyU uracyl is very weak (K_N < 100 M^{-1}) [18]. $_N$ in (1) is set to be 1 for the 3 2 transition. Binding constants for Cd^{2+} binding to A2U and AU phosphates and components of their single-stranded polynucleotides remain unknown. However, the interaction of cations with negatively charged oxygen atoms of phosphates is purely Coulomb interaction and can be determined by the values of interacting charges only. Stating on the abovementioned, when calculating the dependence of $(T_m)_{3 \quad 2}$ on [Cd^{2+}] we used K values obtained for Mg^{2+} (Table 2). At the conditions of compensation of charges on nucleotide phosphates by Mt^{2+} ions we supposed that $n_3 = n_2 = n_U = 0.5$ [29]. The calculation showed that due to low P and K values, A_b concentration did not exceed 2% of [Cd^{2+}], *i.e.* A_f = [Cd^{2+}] within this range.

Therefore, as it is seen from formulae (1, 2), the change value $(T_m)_{3 \quad 2}$, except K and n, depends significantly on (H)$_{3 \quad 2}$, the values of which were determined by the methods of differential scanning and isothermal titrating calorimetry to be in the range of 2?4.2 kcal/mol [27, 30], *i.e.* they are 2 times different on the edges of value scattering (H)$_{3 \quad 2}$. This fact may be connected with insufficient equilibrium of 3 2 transiiton during fast heating of the solution (this process is slower that that of 2 1 [30]), with effect of aggregation, complexity of registering the zero line effect on the calculation of heat effect value, using the method of differential scanning calorimetry with different conformations of single-stranded polynucleotides at low and high temperatures (the latter is related to polyA and polyC, which are 50–60% spiralized at $T=T_0$ [31]). Differences in (H)$_{3 \quad 2}$ values, discovered from temperature dependencies of equilibrium constants (Vant-Hoff method: d ln$K$/d(1/T) = – H/R) are even higher and located in the range of 2–8 kcal/mol [30].

Phase diagrams allow obtaining more precise value of (H)$_{3 \quad 2}$ for A2U, using formulae (1)–(4). The concentration dependence $(T_m)_{3 \quad 2}$ was calculated for 4 values $H_{3 \quad 2}$ to be 2, 4, 5, and 6 kcal·mol^{-1}·triplet^{-1}. It is evident that the best correlation between the experiment and the theory is observed in the case of $H_{3 \quad 2}$ = 5 kcal·mol^{-1}·triplet^{-1}. This value is in good correlation with $H_{3 \quad 2}$ = 5.3 kcal·mol^{-1}·triplet^{-1}, obtained in analysis of the phase diagram (T_m = f([Na^+])) of polyA·polyU [32] and values of $H_{3 \quad 2}$ = 4.6 and 4.9 kcal·mol^{-1}·triplet^{-1}, revealed for T·AT triplet using the method of isothermal titrating calorimetry [33] and for oligonucleotide dA_{15}–$2dT_{10}$ (calculated using the “staggering zipper” model, corresponding to the highest values of nucleation constant [34]), respectively.

In the case of 2 1 transition formulae (2) and (4) are presented as follows:

$${}_P \quad {}_N \quad [\frac{(1 \quad K_2 \quad A_f)^{2\,n_3}}{(1 \quad K_U \quad A_f)^{n_U} \ (1 \quad K_A \quad A_f)^{n_A}}]_P \ [\frac{1}{(1 \quad K_A \quad A_f)^{n_A}}]_N; \qquad (5)$$

and

$$A_b \quad \overset{P,N}{} [P \ (C_2 \ n_2 \quad {}_2 \quad C_A \ n_A \quad {}_A \quad C_U \ n_U \quad {}_U)], \qquad (6)$$

Table 2
Parameters of equations (1)–(6) [29]

Transition	Part of links				M^{-1}				kcal mol^{-1} $triplets^{-1}$	kcal mol^{-1} $pairs^{-1}$
	A2U 3	AU 2	A	U	K_{A2U} K_3	K_{AU} K_2	K_A	K_U	$H_{3\ \ 2}$	$H_{2\ \ 1}$
A2U AU+U (3 2)	1/2	1/3	0	1/6	1000	345	200	250	2,1 [27]; 4; 5; 6	–
AU+U A+U+U (2 1)	0	1/3	1/6	1/2	–	–	–	–	–	8,2

– part of polynucleotide links in the corresponding conformation at $T=T_m$; 3 and 2 are A2U and AU respectively; *A* and *U* are single-stranded polyA and polyU; *K* is Mg^{2+} binding constants. *H* is enthalpy of corresponding transitions; *P* = $3•10^{-5}$ M, $(n_A)_P = 0.5$ [29].

where K_A and n_A are binding parameters of Mt^{2+} with single-stranded polyA.

In this case only $[(\ T_m)_{2\ \ 1}]_P$ determined by binding of ions to phosphates (Table 2) can be calculated reliably as $(K_A)_N$ and $(n_A)_N$ for Cd^{2+} are unknown. However, it has been revealed that when taking into account only the phosphate component T_m, calculated and experimental dependences of $(T_m)_{2\ \ 1}$ on $[Mt^{2+}]$ correlate within the range f one degree. The reason for insignificant influence of Cd^{2+} binding to adenine of single-stranded polyA on the decrease in thermostability of A2U is apparently because of $n_{AN} < \frac{1}{2}$. Indeed, as it has already been mentioned, the interaction of Cd^{2+} with single-stranded polyC, the structure of which is similar to that of polyA [31], results in the formation of loops of 4 5 nucleotides long [20], which corresponds to $n = 0.2\ \ 0.5$. Besides, according to [35], for the complex of Ni^{2+} with single-stranded polyA *n* makes up 0.26.

If we suppose that for Cd^{2+} n_{AN} also equals to 0.2 (K_{AN} 1 000 M^{-1} [18]), then $[(\ T_m)_{2\ \ 1}]_N = -1.3$°C, that is to the value within the standard T_m error, which is 2°C.

Higher $H_{2\ \ 1}$ value and low differences between association constants for Cd^{2+} binding to AU and single-stranded polyA and polyU lead to the fact that AU thermostability changes hardly even at $[Cd^{2+}]/P$ 10. Although the difference between K_3 and K_1 is somewhat higher, significant contribution to Cd^{2+} ions – induced increase in A2U thermostability results from the lower $H_{3\ \ 2}$ value in comparison with that for $H_{2\ \ 1}$. Thus, the differences between enthalpies values of these transitions are the constant valuable factor of appearance of "critical" points on phase A2U diagrams in the presence of not only two- but also monovalent metal ions [12, 26, 27, 29].

The data obtained indicate that the structure of rigid multi-stranded polynucleotides formed by both Watson-Crick and Hoogsteen AU-pairs does not allow d-ions to form effective coordinational bonds with adenine and uracyl heteroatoms up to very high ionic concentrations. This results in the conformational and thermal stability of corresponding pairs and triplets towards these ions. Therefore, their mutagenic and carcinogenic activities can hardly be induced by specific interactions of AU- or AT-pairs of double-stranded RNA and DNA of living organisms.

Moreover, we can put forward a supposition that conservatism in the transfer of genetic information by DNA and RNA molecules upon changes in living conditions of organisms is provided by the conformational stability of AT- or AU-pairs towards the effect of not only metal d-ions but also some other biologically active substances, whereas the alternative evolutionary process is conditioned by their interaction with pairs of nitrogen bases containing 6-oxopurines (guanine or hypoxanthine).

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Влияние ионов Cd^{2+} на конформационное равновесие трехцепочечного полинуклеотида полиUполиAполиU в условиях, близких к физиологическим

Резюме

Методом дифференциальной УФ-спектроскопии изучено влияние ионов Cd^{2+} на конформационное равновесие трехцепочечного полинуклеотида полиU полиA полиU (0,1 М

Na^+, pH 7). Показано, что эти ионы не связываются с гетероатомами азотистых оснований полиA и полиU, находящихся в составе тройной (A2U) и двойной (AU) спиралей, и не изменяют их конформации. Нагревание A2U индуцирует два последовательных процесса: первый соответствует отделению от A2U одной нити полиU, то есть реализации перехода 3 2, второй – более кооперативному плавлению AU, т. е. переходу 2 1. Концентрационные зависимости температур плавления, соответствующие этим процессам, имеют точку пересечения (T_m = = 60 ^{o}C; концентрация Cd^{2+} 3,5 10^{-4}M), при которой термостабильности A2U и AU становятся одинаковыми. Теоретические расчеты концентрационных зависимостей $(T_m)_{3\ 2}$ показали, что наилучшее согласие между опытом и теорией наблюдается при энтальпии перехода $H_{3\ 2}$ = 5 ккал моль$^{-1}$ триплет$^{-1}$.

Ключевые слова: ионы металлов, трехцепочечные полинуклеотиды, конформационные переходы.

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