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МОЛЕКУЛЯРНА БІОФІЗИКА

INTERACTION OF Ni²⁺ IONS WITH HOMOPOLYNUCLEOTIDES CONTAINING ADENINE AND URACIL AND THEIR MONOMERS

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Interactions of Ni²⁺ ions with AMP, UMP, single-chain poly A, poly U, double-chain poly A-poly U and three-chain poly A-2 poly U have been studied by the method of differential UV spectroscopy. The coil→helix transition observed in polynucleotides is due to the Ni²⁺ ion binding to oxygen atoms of phosphate groups. The nickel coordination to bases takes place only in the case of single AMPs and single-chain poly A. The formation of a chelate N7-Ni²⁺-(PO₄)⁻ induces melting of this polymer. Melted parts form compact particles with the effective radius ~100Å.

KEY WORDS: UV spectroscopy, homopolynucleotides, metal ions.

Among transition metals nickel (along with chrome) being of the maximum cancerogenic index is one of the most toxic [1]. Its possibility to influence functions of biological macromolecules makes interesting studies of the Ni²⁺ interaction not only with natural DNA and nitrogen base derivatives [2,3] but as well with homopolynucleotides forming noncanonical single- and multichain helices being of biological functions [4]. Experimental results available for these systems are not enough. So, interactions of Cu²⁺ ions with four-chain poly G [5] and of Cu²⁺, Mn²⁺, Ca²⁺ and Mg²⁺ ions with double-chain poly A and poly C [6] have been studied.

The present work was aimed at the determination of atoms coordinating Ni²⁺ ions in single-, double- and three-chain polynucleotides monomers of which are AMP and UMP. To ascertain ion-induced conformational transitions in these polynucleotides is another task of the work.

MATERIALS AND METHOD

Research objects were potassium salts of poly A, poly U, poly A-poly U (AU) and poly A-2 poly U (A2U) as well as disodium salt nucleoside-5'-phosphates (AMP and UMP). Poly A, poly U and nucleotides were supplied by Serva (Germany) and AU and A2U - by Sigma Chemical Company (USA). NiCl₂·6H₂O was doubly recrystallized. To increase the binding constant of Ni²⁺ ions, nucleotides were solved in acetate buffer with the low (10⁻³M) concentration of Na⁺ ions. But under these conditions at room temperature AU and A2U are in a partially melted state [7] therefore AU as a more stable polymer [7] was solved in 10⁻²M NaAc and A2U (and poly U) was solved in the same solution added with NaCl. In this case the total Na⁺ ion concentration was 3·10⁻²M. To avoid the formation of nickel hydroxides, acetate buffer with pH6 was used. But at 10⁻²M Na⁺ and pH6 poly A are able to form double helices [8]. To eliminate this process, poly A was dissolved in cacodilate buffer 10⁻²M Na⁺ with pH7.

Concentrations of nucleotide and polynucleotide phosphorus (P) were determined by values of extinction molar coefficients in UV absorption maxima [7, 9, 10] with an error not exceeding 0,5%. In all the cases the value of P was (1±0,2)·10⁻⁴M. The Ni²⁺ ion concentration ([Ni²⁺]) was evaluated by weight and controlled by the complexometric method. An error in determining [Ni²⁺] was not more than 0,5%.

Ni²⁺ ion-induced differential UV spectra (Δε_a-DUVS) were registered at T=(20±2)⁰C with a Specord M40 spectrophotometer (Carl Zeiss Jena, Germany), using the four-cuvette measuring scheme. All the DUV spectra obtained correspond to the equilibrium situation that follows from their form and intensity retained for (30-40) min. In the case of single-chain poly A at [Ni²⁺] > 10⁻³M kinetics of spectral changes induced with the formation of light scattering particles was observed.

In this case DUV spectra induced with changes in the poly A absorption (Δε_a(ν)) were determined by the extrapolation method using the formula [11]:

$$\Delta \varepsilon_a(\nu) = [\Delta A(\nu) - \Delta A_{\nu_0}(\nu / \nu_0)^t] / p \quad (1)$$

where $\Delta A(\nu)$ is a change in the optical density of the poly A solution immediately after the addition of Ni^{2+} ions. $\Delta A_{\nu_0} = \Delta A$ at $\nu = \nu_0 = 32000 \text{ cm}^{-1}$. The value of the wave exponent i in Angström formula was determined by the $\Delta A(\nu)$ dependence at $\nu < \nu_0$ where the light absorption is absent.

As well, differential spectra conditioned with melting of polynucleotide helical links ($\Delta \epsilon_h$) were registered with a Specord UV VIS spectrophotometer. In this case the standard cuvette was thermostatted at $T = T_0$ while the working one was heated up to 96°C and then the spectrum $\Delta \epsilon_h$ was taken.

EXPERIMENTAL RESULTS

Monomers

AMP. In the range of $(10^{-4} - 5 \cdot 10^{-2})\text{M}$ Ni^{2+} ions induce AMP DUV spectra the shape of which coincides with that of DUV spectra of Ni^{2+} +dAMP complexes [2]. This evidences the ion interaction with nitrogen atoms of the adenine ring (Figs 1, 2). In aqueous solutions of Ado atoms coordinating Ni^{2+} ions are N1 and N7. In the range of neutral pH values the interaction with N1 is preferential [13]. But in AMP the main atom binding M^{2+} ions is N7 [3, 9, 14]. This is, in particular, promoted with the Ado anti-conformation that is suitable for the formation of a chelate including the simultaneous ion binding to N7 and the phosphate group oxygen (O(P)) [14]. The very strong difference between the shape of AMP (and dAMP [2]) DUV spectra induced by Ni^{2+} ions and that of AMP DUV spectra resulted from N1 protonation (Fig. 1) evidences these data.

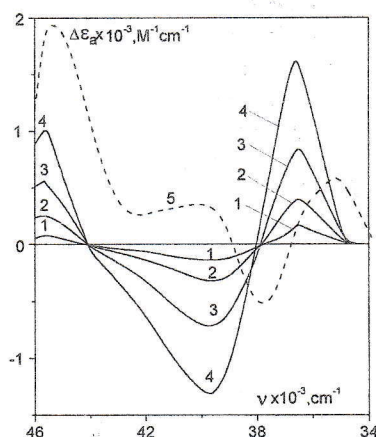


Fig. 1. Differential UV spectra of AMP, induced with Ni^{2+} ions. 1 - $1,25 \cdot 10^{-4}\text{M}$ Ni^{2+} ; 2 - $7 \cdot 10^{-4}\text{M}$; 3 - $2,5 \cdot 10^{-3}\text{M}$; 4 - $3,5 \cdot 10^{-2}\text{M}$; 5 - AMP DUV spectrum induced with N1 protonation (calculated by data from [12]).

UMP. As in the case of dTMP [2], Ni^{2+} ions induce no changes in UMP absorption spectra up to $[\text{Ni}^{2+}] = 2,5 \cdot 10^{-2}\text{M}$ that evidences the absence of ion interactions with heteroatoms of the uracil ring as well as with oxygen of the sugar ring because deprotonation of hydroxyl groups of the pyrimidine ribose induces significant changes in absorption spectra of bases [12]. Besides (and it is essential), for ribose hydroxyl groups of UMP $\rho K_a = 13 - 13,7$ [12] that makes improbable the substitution of their protons with Ni^{2+} ions. Thus, in accordance with the data from [3], the only UMP atom binding nickel is O(P). But the interaction with this atom has no influence on absorption spectra of nucleotides [12].

Polynucleotides

Poly A. In the range of their low concentrations Ni^{2+} ions induce DUV spectra the shape of which is "reverse" to that of DUV spectra resulted from the helix-coil transition of the single-chain poly A (Fig. 3a). Thus, at $[\text{Ni}^{2+}] \leq 4 \cdot 10^{-4}\text{M}$ DUV spectra of metal complexes are mainly conditioned with the rise of the helicity degree of the single-chain poly A, induced by the Ni^{2+} ion interaction with O(P) that is characteristic of poly A complexes with all the M^{2+} (Cu, Mn, Co, Mg, Sr, Ba, Be) ions [9]. At $[\text{Ni}^{2+}] > 4 \cdot 10^{-4}\text{M}$ the ion binding to adenine of poly A becomes pronounced and is followed with melting of helical parts. Indeed, at these ion

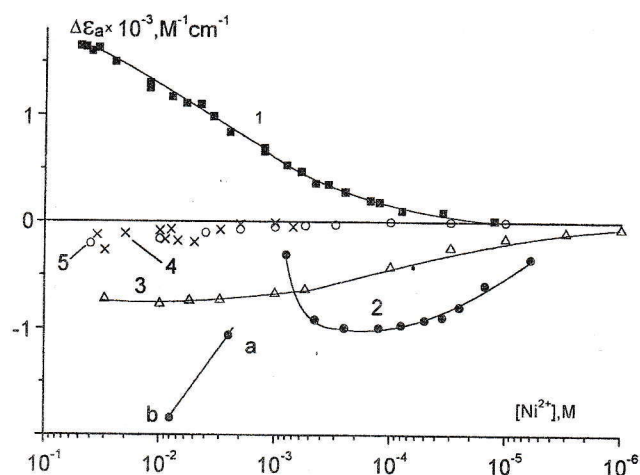
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Fig. 2. Concentrational dependences of intensities of DUVS extrema of AMP and polynucleotides, induced with Ni^{2+} ions. 1 - $\nu=36500 \text{ cm}^{-1}$ (AMP); 2 - $\nu=39500 \text{ cm}^{-1}$ (single-chain polyA); 3 - $\nu=39000 \text{ cm}^{-1}$ (AU); 4 - $\nu=39000 \text{ cm}^{-1}$ (single-chain polyU); 5 - $\nu=37000 \text{ cm}^{-1}$ (A2U). a and b - $\Delta\epsilon_a$ values calculated by formula (1) ($i=4$).

concentrations the longwave isobestic point on DUV spectra of poly A shifts to the shortwave side (Fig. 3b) and with the rise of the nickel content the derivative $[\partial(\Delta\epsilon_a)/\partial([\text{Ni}^{2+}])]$ goes through zero and changes its sign (Fig. 2). This result is in a qualitative agreement with other experimental data [9], according to which, melting of helical parts of the single-chain poly A is observed in the presence of all the transition metal ions. It is essential that at $2,5 \cdot 10^{-3} \text{ M Ni}^{2+}$ the shape of poly A DUV spectra is similar to that of DUV spectra of Ni^{2+} complexes with AMP that evidences the ion coordination with N7 (Fig. 3b). Accordingly to the NMR spectroscopic data obtained for poly A complexes with Mn^{2+} and Cu^{2+} ions [15, 16], melting of helical parts is conditioned with the formation of chelates $\text{N7-Mt}^{2+}\text{-O(P)}$. As Fig. 2 shows, the melting process is cooperative that is, perhaps, conditioned with positive cooperativity of the Mt^{2+} ion binding to bases of single-chain polynucleotides [9]. Light scattering observed at $[\text{Ni}^{2+}] > 10^{-3} \text{ M}$ meets the Angström formula [11] with the exponent index $i \approx 4$. Such a value of i evidences the formation of particles with the effective radius $r_e \leq 100 \text{ \AA}$ [17]. The inertial radius (R_i) of the single-chain statistical coil is an order of magnitude higher [18]. Such a difference between r_e and R_i evidences compaction of the single-chain poly A in the range of concentrations

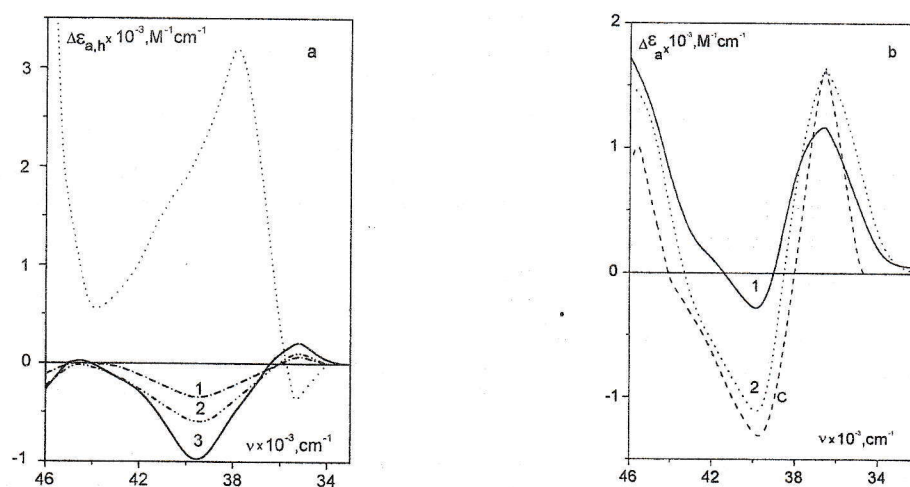


Fig. 3. Differential UV spectra of single-chain poly A, induced with Ni^{2+} ions. a: region of preferential binding to phosphates - 1 - $6 \cdot 10^{-6} \text{ M Ni}^{2+}$; 2 - $1,5 \cdot 10^{-5} \text{ M}$; 3 - $1,25 \cdot 10^{-4} \text{ M}$; 4 - spectrum $\Delta\epsilon_h$ induced with helix-coil transition of single-chain poly A. b: region of ion binding to adenine - 1 - $8 \cdot 10^{-4} \text{ M Ni}^{2+}$; 2 - $2,5 \cdot 10^{-3} \text{ M}$ (calculated by formula 1, $i=4$). C - AMP DUV spectrum induced with $0,035 \text{ M Ni}^{2+}$ (See Fig. 1).

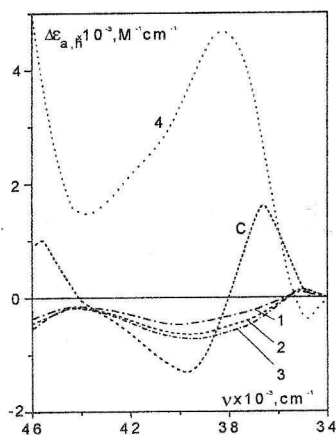


Fig. 4. Differential UV spectra of double-chain polyA-polyU, induced with Ni^{2+} ions. 1 - 10^{-4}M Ni^{2+} ; 2 - $5.5 \cdot 10^{-4}\text{M}$; 3 - 10^{-2}M ; 4 - spectrum $\Delta\epsilon_h$ induced by helix-coil transition of AU. C - the same as in Fig. 3.

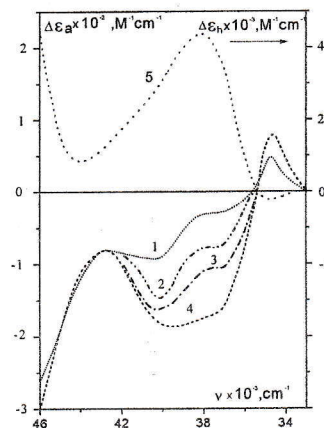


Fig. 5. Differential UV spectra of three-chain poly A-2 polyU, induced by Ni^{2+} ions. 1 - $5.5 \cdot 10^{-5}\text{M}$ Ni^{2+} ; 2 - $2 \cdot 10^{-3}\text{M}$; 3 - $4 \cdot 10^{-3}\text{M}$; 4 - 10^{-2}M (spectra refer to left ordinate); 5 - spectrum $\Delta\epsilon_h$ induced by helix-coil transition of A2U (refers to right ordinate).

corresponding to the Ni^{2+} ion binding to the adenine ring. The formation of compact particles with $r_e \sim 100\text{\AA}$ was observed by us earlier in microphotographies of poly A in the presence of the high concentration of Ca^{2+} ions [19]. Compaction of poly A is a cause of new hypochromicity of its absorption at $[\text{Ni}^{2+}] > 10^{-3}\text{M}$ (Fig. 2).

Poly U. Ni^{2+} ions induce no changes in absorption spectra of the coiled poly U up to $2 \cdot 10^{-3}\text{M}$ Ni^{2+} (Fig. 2). In the range of $2 \cdot 10^{-3}\text{M} < [\text{Ni}^{2+}] \leq 3 \cdot 10^{-2}\text{M}$ only a weak hypochromicity of the poly U absorption (Fig. 2) is observed (in the absence of light scattering), conditioned by ordering of the small number of poly U links on the ion interaction with O(P). Thus, in the range from 10^{-5}M to $3 \cdot 10^{-3}\text{M}$ Ni^{2+} ions do not bind to heteroatoms of the uracil ring and their interaction with phosphates induces no compaction of the polymer.

Poly A-poly U. The shape of the AU DUV spectra induced by Ni^{2+} ions is a "mirror reflection" of that of the AU DUV spectra resulted from the disruption of the double helix (Fig. 4). This means that at 0.01M Na^+ AU at 20°C a) has some number of disordered links and b) Ni^{2+} ions eliminate these "defects" and restore the helical structure of the polynucleotide. In accordance with Fig. 2, the nickel-induced coil-helix transition is an essentially noncooperative process that may be induced with negative cooperativity of the Ni^{2+} ion interaction with AU, characteristic, in principle, of the Mn^{2+} ion binding to ordered helices of different structural organization levels [20, 21]. As in the nickel presence poly U retains mainly the nonordered state, the rize of the helicity degree of AU is induced by the Ni^{2+} ion interaction with single-chain parts of poly A. The shape of nickel-induced DUV spectra of AU shows that up to $3 \cdot 10^{-2}\text{M}$ Ni^{2+} ions do not interact with ring heteroatoms of the polynucleotide adenine though the binding to N7A in the polynucleotide is principally possible [22]. The absence of the Ni^{2+} interaction with this atom of the AU double helix is, perhaps, conditioned by the high rigidity of its sugar-phosphate bone, preventing from the formation of $\text{N7-Ni}^{2+}-(\text{PO}_4)^-$ type chelates while Ni^{2+} -N7A complexes are characterized with very low binding constants. For example, for Ni^{2+} +N7 Ado complexes the value of K is 8M^{-1} [13] that is such a binding type is able to manifest itself only at molar nickel concentrations. Besides, the steric accessibility of N7A in helical structures is lower than its value in AMP (by 2.5 times, in accordance with theoretical calculations [23]). Thus, the data obtained permit to conclude that the restoration of the AU helical structure with Ni^{2+} ions results from their interaction with oxygen atoms of phosphate groups.

Poly A-2 poly U. Very low hypochromicity of A2U absorption, induced by Ni^{2+} atoms, evidences that the number of "defects" in its structure is insignificant (Figs 2, 5). The shape of DUV spectra shows that, as in the case of AU, Ni^{2+} ions restore the A2U helical structure on their interaction with phosphate groups of melted parts of the adenine chain while the binding to N7A is absent up to $4 \cdot 10^{-2}\text{M}$ Ni^{2+} . The participation of this atom in the system of hydrogen bonds supporting the triple helix [22] makes this result natural. As well, the data obtained show that the Ni^{2+} ion binding to phosphates of ordered parts of the adenine chain as well as to ordered and nonordered parts of uracil chains has no influence on the conformational state of the polynucleotides studied.

As Fig. 2 shows, quantitative changes in absorption spectra of poly U and A2U are similar. But small values of spectral changes for the both polynucleotides are conditioned with different causes - with the small

number of melted parts in the A2U triple helix and with a low tendency of uracil derivatives to form stacked structures [22].

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