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EFFECT OF Mg^{2+} ON DOUBLE AND TRIPLE HELIX FORMATIONS BETWEEN POLY(dA) AND POLY(dT)

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The Mg^{2+} ion effect on conformational transitions in double- and triple-helical structures formed by poly(dA) with poly(dT) has been studied in 10 mM cacodylate buffer, pH 7, containing 0.1 M Na^+. The investigations were carried out by the method of thermal denaturation using UV absorbance and Rayleigh light scattering detections of melting transitions. Temperatures of helix-to-coil transition of poly(dA)·poly(dT) and triplex-to-duplex of poly(dA)·2poly(dT) increase linearly with log[Mg^{2+}] and more quickly in the last case that results in superposition of these transitions at [Mg^{2+}]≥15 mM. At [Mg^{2+}] ≥ 20 mM during the melting of the both structures aggregation was observed, which arised, according to Bloomfield and co-workers, due to formation of cross-links by Mg^{2+} ions bridges between partially unplaited polymer strands and disappeared after the end of melting. It is established that at the presence of 0.1 M NaCl magnesium ions do not induce the partial formation of the triplex structure in poly(dA)·poly(dT) (disproportionation) as is postulated by other authors in some papers.

KEY WORDS: polynucleotides, double and triple helices, magnesium ions, helix-to-coil transitions, Rayleigh scattering

Up to now the effect of Mg^{2+} ions on the stability of duplex and triplex structures and conformational transitions of nucleic acids has not been completely studied. It has been shown [1] that Mg^{2+} ions stabilize the DNA duplex but at increased concentration they induce also the polymer aggregation during the process of helix-to-coil transition. Because of the completeness of the phenomenon, it is worth-while to study similarly more simple systems with synthetic polynucleotides. In one of such works [2] using calorimetric measurements it was studied the Mg^{2+} ion effect on the helix-to-coil transition of poly(dA)·poly(dT) but gave no attention to the possible occurrence of polymer aggregation. The other authors [3] studied by absorption spectroscopy the Mg^{2+} ion effect on the transition of poly(dA)·poly(dT) duplex as well as of poly(dA)·2poly(dT) triplex, but the scanty information was obtained. The aim of the present work was detailed study of the Mg^{2+} ion effect on conformational transitions of duplex and triplex structures formed by poly(dA) with poly(dT) at the physiological Na^+ ion concentration.

MATERIALS AND METHODS

Sodium salts of poly(dA)·poly(dT) and poly(dT) were purchased from Sigma Chemical Co. and other chemicals used were of analytical grade. For all the experiments 10 mM sodium cacodylate buffer, pH 7, containing 0.1 M NaCl was used as a solvent. The required concentration of Mg^{2+} ions was created by addition of $MgSO_4$. Polynucleotide concentrations were determined by UV absorbance, using extinction coefficients of 12000 $M^{-1}cm^{-1}$ for poly(dA)·poly(dT) and 8460 $M^{-1}cm^{-1}$ for poly(dT) at 260 nm and the temperature of 25 °C.

Thermal denaturation profiles for the polynucleotides were obtained by registration of the absorbance change at 260 nm as a function of temperature. To investigate aggregation processes, the temperature dependence of Rayleigh scattering of light was measured at a right angle to incident beam, at 540 nm. Absorption melting experiments were carried out on SPECORD UV/VIS spectrophotometer (VEB Carl Zeiss, Jena). Rayleigh scattering was measured by the method of photon counting on a laboratory spectrofluorimeter constructed on the basis of a double monochromator DFS-12 (Russia). Absorbance and light scattering were measured in the same 1.0 x 0.2 cm quartz cuvette. The cuvette was inserted into a copper holder which was heated by a computer-operated Peltier element. Moreover, a personal computer collected the melting data. Melting experiments were carried out at a heating rate of 1 °C/min.

RESULTS AND DISCUSSION

Figs. 1 and 2 show melting curves for poly(dA)·poly(dT) and poly(dA)·2poly(dT) in buffer solution with 0.1M NaCl without Mg^{2+} ions and at different Mg^{2+} concentrations. It is seen from Fig. 1 that for poly(dA)·poly(dT) the only sharp double-helix-to-coil transition (2→1) is realized, shifting to high temperatures with the increase

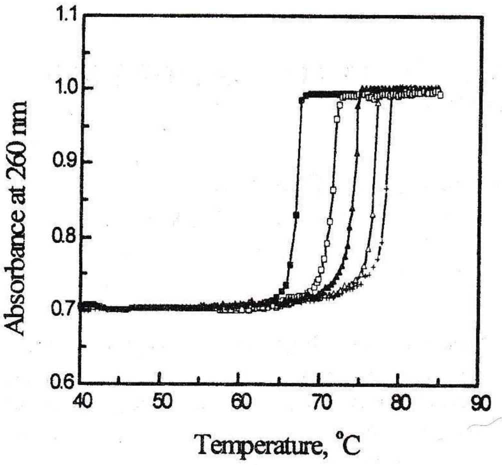

Fig.1. Thermal denaturation profiles of poly(dA)·poly(dT) in 10 mM cacodylate buffer, pH 7, at 0.1 M NaCl alone (■) and with different concentration of added Mg^{2+} ions: (□) 1mM, (▲) 2mM, (Δ) 5mM, (+) 10mM. The polymer concentration was 55 μM in base pairs.

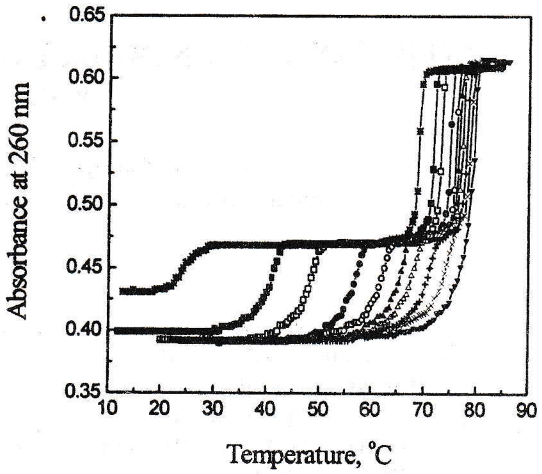

Fig.2.Thermal denaturation profiles of poly(dA)·2poly(dT) in 10mM cacodylate buffer, pH 7, at 0.1 M NaCl alone (*) and with different concentration of added $MgSO_4$: (■) 0:5mM, (□) 1 mM, (•) 2 mM, (o) 3 mM, (▲) 4 mM, (Δ) 5mM, (+) 7 mM, (x) 10 mM, (▼) 15 mM. The polymer concentration was 110 μM in base triplets.

in Mg^{2+} concentration. For the triplex system poly(dA)·2poly(dT) two transitions are observed (Fig. 2). The low temperature transition characterizes the separation of poly(dT) strand bound with poly(dA)·poly(dT) by Hoogsteen hydrogen bonds (transition 3→2) while the high temperature transition corresponds to melting of poly(dA)·poly(dT) itself (2→1). Hyperchromicity following the triplex melting in the absence of Mg^{2+} ions, lower than that in the presence of the ions, evidences the incompleteness of the triplex structure formation at 0.1M NaCl. However, Mg^{2+} ions added, beginning with 1 mM concentration, promote the complete attachment of poly(dT) to poly(dA)·poly(dT). As Figs. 1 and 2 show, Mg^{2+} ions increase temperatures of the both transitions but for the transition 3→2 this effect is essentially higher than for the transition 2→1. This results in the fact that at $[Mg^{2+}] \approx 15$ mM the both transitions merge into one continuous transition from the triplex structure to disordered strands.

At $[Mg^{2+}] \geq 20$ mM a strong increase of Rayleigh light scattering was observed during the melting process both for the double- and triple-helical polymers that was not yet observed at 15 mM Mg^{2+} (Fig. 3). In this case absorption melting curves become two-phase and the temperature interval of the transition broadened. This is due to arising of turbidity in the solution (that was well observed visually), which contributes into the absorption increase. It is remarkable that the scattering intensity increased with the temperature rise to a value approximately corresponding to the temperature of the transition midpoint (T_m) after which it decreased sharply at the transition end. Some "steps" on absorption melting curves induced with the absorption increase due to the medium turbidity are observed in the region of this point (Fig. 3). On cooling down to the room temperature the systems studied returned to the starting structure that was evidenced by the absorption data. But at $[Mg^{2+}] \geq 30$ mM for the poly(dA)·2poly(dT) system and at $[Mg^{2+}] \geq 50$ mM for poly(dA)·poly(dT) system ordinary aggregation was observed already at the room temperature, resulting in the precipitation of the polymers.

Arising of the turbidity in the melting process was observed in the work [1] in the case of thymus DNA in the presence of some divalent metal ions. The authors explained this phenomenon with "net aggregation" caused by ionic bridge cross-linking between partially unwound different DNA strands. However, the bridges break after the complete melting of the double-strand polymer structure. Such an interpretation based on theoretical calculations [4, 5] suits completely our case. The work [4] shown that the divalent cation-induced decrease of the free energy on cross-linking is significant and is about 35% of the free energy change on stacking of nucleotide bases. The work [2] studied by calorimetry interactions of poly(dA)·poly(dT) with Mg^{2+} ions, and in the concentration range of 30-50 mM the bands in d.s.c. curves in the premelting region were registered. The bands being assigned to the formation of the triple-helical structure (disproportionation) as intermediates in the melting process. But, in our opinion, these bands evidence the occurrence of the "net

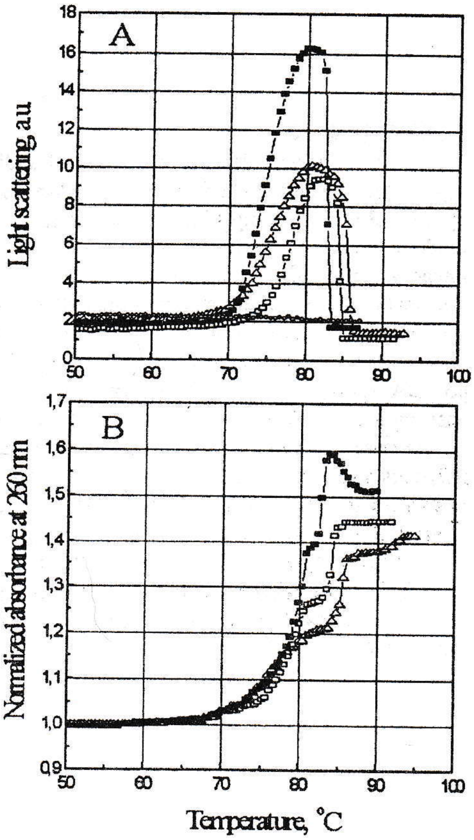

Fig.3. Temperature dependence of the light scattering at 540 nm (A) and absorbance at 260 nm (B) measured in the buffer containing 0.1M NaCl for poly(dA)·poly(dT) with 20mM (□) or 30mM (Δ) Mg^{2+} and for poly(dA)·2poly(dT) at 15mM (o) or 20mM (■) Mg^{2+}. Concentrations of the polymers were as in Figs. 1 and 2.

aggregation" that was revealed in our experiments under the same conditions. This conclusion is confirmed by the fact that, for example, at 30 mM Mg^{2+} the temperature value at the maximum of the pretransition band on d.s.c. curve, 75 °C, established in this work coincides with the maximum of derivative for an ascending branch of light scattering intensity curve obtained in our work.

Obtained in our work T_m values for the 2→1 transition of poly(dA)·poly(dT) at the presence of 2 mM and 5 mM of Mg^{2+} are in well accordance with data obtained by absorption method in [3]. However, in contrast to our work, the authors in [3] have also observed the 3→2 transition in addition to the 2→1 one (Fig.1). They have postulated that at the indicated concentrations Mg^{2+} ions induce a disproportionation of stochiometric poly(dA)·poly(dT). However, our attention has been drawn to the fact that the authors in [3] have used lower molar extinction coefficient E_{257}=8600 $M^{-1}cm^{-1}$ in the measurements of the poly(dA) concentration on the mixing poly(dA) with poly(dT) at the sample preparation. Because of this the molar ratio 1:1 was not ensured, which probably lead to the partially formed triplex structure.

Fig. 4 demonstrates the variation of the temperature of the transition midpoint for 2→1 and 3→2 with magnesium ion concentration in the range 0-15 mM, where the aggregation did not arise. As can be seen, it is fitted well by linear dependence on lg[Mg^{2+}] for the both transitions.

At [Mg^{2+}] ≈ 15 mM the both dependencies are crossing. This means that at high magnesium ion concentration the duplex and triplex formations melt simultaneously. Fig.4. shows data from the work [2] about the Mg^{2+} effect on the transition 2→1, obtained from calorimetric measurements, but at low Na^+ ion concentration in solutions (PIPES buffer). In this case the T_m versus lg[Mg^{2+}] plot is steeper than that in our case, that evidences the enhansment of Mg^{2+} effect on T_m with decreasing in sodium ion concentration.

The data obtained for Mg^{2+} effect can be explained by model theory of Crothers [6] and Frank-Kamenetskii [7], where the change in T_m of helix-to-coil transition is expresses by the equation

$$\Delta T_m = (RT_0T_m/\Delta H)\ln\left[(1+K_2c)^{B_2}/(1+K_1c)^{B_1}\right] \qquad (1)$$

where ΔH is the transition enthalpy, T_0 and T_m are the melting temperatures of the transition in the absence and presence of a ligand respectively, K_2 and K_1 are binding constants of the ligand with native and melted polimer, B_2 and B_1 are the number of phosphate groups per one bound ion, c is the ligand concentration.

If K_2 and K_1 are independent on c and $K_2c >> 1$, $K_1c >> 1$ then from (1) one can obtained

$$\partial(\Delta T_m)/\partial(\ln c) = (RT_0T_m/\Delta H)[B_2 - B_1] \qquad (2)$$

Equation (2) explains the linearity of T_m versus lg[Mg^{2+}] plots (Fig.4). Because of ΔH for the 3→2 transition is about threefold less than that for 2→1 [8], the steeper dependence for the first transition in comparison with that for the second one is observed.

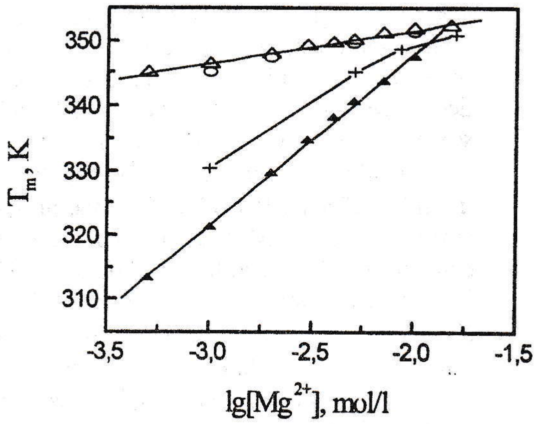

Fig.4. Effect of Mg^{2+} on the temperature at transition midpoints (T_m) for double- and triple-stranded complexes formed between poly(dA) and poly(dT): $T_m^{2\text{-}1}$ determined from Fig.1 (o) and Fig.2 (Δ); $T_m^{3\text{-}2}$ determined from Fig.2; (▲) are the data of Hopkins at al. [2] calorimetrically derived for poly(dA)·poly(dT) in aqueous PIPES buffer (+).

CONCLUSIONS

At the physiological concentration of sodium ions Mg^{2+} stubilizes double- and triple-helix structures formed by poly(dA) with poly(dT). Temperatures of duplex-random coil and triplex-duplex transitions increase linearily with lg[Mg^{2+}] and more quickly in the second case that results in superposition of the both transitions at the determined concentration of Mg^{2+} ions. This was observed in the present work at [Mg2+] ≥ 15 mM for poly(dA)·2poly(dT) with the concentration of 100 μM base triplets. At high molar ratios Mg^{2+}/ polynucleotide (in our case it is more than 200). During melting of the polymers aggregation is occurred due to formation of cross-links by Mg^{2+} bridges between partially unplaited polymer strands. It is established that at the presence of 0.1 M NaCl magnesium ions do not induce the partial formation of the triplex structure in poly(dA)·poly(dT) (disproportionation) as is postulated by other authors in some papers.

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ВПЛИВ ІОНІВ Mg^{2+} НА ДВОХ- ТА ТРЬОХСПІРАЛЬНІ ФОРМУВАННЯ МІЖ POLY(dA) ТА POLY(dT)

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Вивчено вплив іонів Mg^{2+} на конформаційні переходи у двох- та трьохспіральних структурах, сформованих між poly(dA) та poly(dT) у 10 mM какодилатному буфері, pH 7, що містить 0.1 M Na^+. Дослідження проведено методом термічної денатурації з використанням УФ спектроскопії та релеївського розсіювання світла для отримання кривих плавлення. Температури переходів спіраль-клубок для poly(dA)·poly(dT) та триплекс-дуплекс для poly(dA)·2poly(dT) лінійно підвищуються з lg[Mg^{2+}], та більш швидко в останньому випадку, що призводить до злиття переходів при [Mg^{2+}] ≥ 15 mM. При [Mg^{2+}] ≥ 20 mM впродовж плавлення обох структур було виявлено агрегацію, що виникала, згідно Блумфілду та співавторам, із-за поперечних зшивок, що формуються містками іонів Mg^{2+} між частково розплетеними ланцюгами полімеру, та зникала в кінці плавлення. Виявлено, що в присутності 0.1 M NaCl іони магнію не індукують виникнення частково сформованих триплексних структур у poly(dA)·poly(dT) (диспропорційність), як постульвано іншими авторами в деяких роботах.

КЛЮЧОВІ СЛОВА: полінуклеотиди, подвійні та потрійні спіралі, іони магнію, перехід спіраль-клубок, релеївське розсіювання світла