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P h y s i c s

THE INFLUENCE OF UREA ON G-QUADRUPLEX AND i-MOTIF STRUCTURES IN COMPLEMENTARY DNA SEQUENCES

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In the present study, the methods of circular dichroism and UV/Vis spectrophotometry were used to study the influence of urea on the structural transitions i-motif $\leftrightarrows$ unfolded single strand in cytosine-rich d[3′-$(CCCAAT)_3CCC$-5′] region of telomeric DNA (Tel22C) and G-quadruplex $\leftrightarrows$ unfolded single strand in complementary guanine-rich strand d[5′-$A(GGGTTA)_3GGG$-3′] (Tel22G) at pH 5.5 and 400 *mM* Na^+. Under these conditions, Tel22C and Tel22G were found to form stable i-motif and G-quadruplex structures. It has been shown that urea ($0-8$ *M*) destabilizes the i-motif and G-quadruplex structures, but unlike thermal denaturation, it does not destroy the structures completely. The melting processes of G-quadruplex and i-motif are separated in the temperature scale (at any concentration of urea, the melting of the G-quadruplex starts at temperatures where the melting of the i-motif has already been completed).

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***Keywords*:** telomeric DNA, G-quadruplex, i-motif, urea, circular dichroism, UV/Vis spectrophotometry.

Introduction. With the development of new antitumor therapy concerning the inhibition of telomere synthesis or oncogene expression *in vivo*, the investigation of noncanonical structures as G-quadruplex and i-motif in various regular DNA sequences is still of interest. The conditions for formation of G-quadruplexes in various G-rich sequences of telomeric regions and oncogene promoters are being purposefully studied. A significant amount of data has been collected on the influence of the consistency and length of sequence, metallic ions, and various low molecular weight compounds on the transition G-quadruplex $\leftrightarrows$ single-stranded structure [1–7]. Unlike G-quadruplexes, the formation of i-motifs in complementary regions of DNA has been much less studied [8–12]. The conditions for the transitions i-motif $\leftrightarrows$ single-stranded structure have been poorly studied, and the transitions i-motif $\leftrightarrows$

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duplex and G-quadruplex $\leftrightarrows$ duplex have not been characterized. There is no data on the effect of various organic cosolvents on the mentioned transitions. Such studies are very important, since the knowledge gained can lead to an understanding of the biological role of genomic switches based on G-quadruplexes and i-motifs *in vivo*.

i-motifs and G-quadruplexes are unusual nucleic acid structures that have features characteristic of globular proteins. They are of globular form, less charged than other nucleic acids. In addition, G-quadruplexes are compressible due to the presence of a central cavity. In this regard, there can be essential differences when comparing the influence of urea on G-quadruplexes and i-motifs, as well as on the canonical structures of DNA and RNA. In the paper [13], we have thoroughly carried out a systematic study of the influence of urea (in dependence of the concentration of Na^+ ions and temperature) on G-quadruplex $\leftrightarrows$ single-stranded structure transition in d[5′-A(GGGTTA)$_3$GGG-3′] sequence of human telomeric DNA. An important conclusion has been made in [13, 14] that the preferable interaction of urea with the G-quadruplex-forming DNA sequences can be used for the thermodynamic characterization of the transition G-quadruplex $\leftrightarrows$ disordered single-stranded structure as it is used in studying the structure of proteins. The logical continuation of this conclusion is the study of the effect of urea on the i-motif structure in the complementary C-rich sequence d[3′-(CCCAAT)$_3$CCC-5)′], which we attempted to carry out in this work.

It is known that the sequence d[3′-(CCCAAT)$_3$CCC-5)′] in the range $4.5 <$ pH < 7.0 can form an i-motif structure, which has a semiprotonated C–C^+ pair as a basic element [8–12]. It has been shown by the NMR method that the lifetime of i-motif does not depend on pH or salt conditions, while the lifetime of G-quadruplex is approximately 10 times longer in 100 *mM* NaCl than in 10 *mM* NaCl. It has been exhibited by spectral methods that the thermostability of i-motif strongly depends on pH, but it is not sensitive to the ionic strength of the buffer [11]. In the case of pH 4.5–7.0 and 100 *mM* NaCl, thermal denaturation of i-motif and G-quadruplex leads to the hyperchromicity of the CD absorption bands at 245 *nm* and hypochromicity at 295 *nm* [12].

It has also been shown that an increase in ionic strength stabilizes the formation of G-quadruplexes, while a decrease in pH weakens their formation [11, 12]. The question arises as to what will be the structural preferences of the influence of urea on the almost equally stable i-motif and G-quadruplex structures.

The current work presents the results of a comparative study of the influence of urea on the structural transitions i-motif $\leftrightarrows$ single-stranded structure in regular C-rich regions of telomeric DNA d[3′-(CCCAAT)$_3$CCC-5)′] (Tel22C) and G-quadruplex $\leftrightarrows$ single-stranded structure in its complementary G-rich d[5′-A(GGGTTA)$_3$GGG-3′] (Tel22G) strand at pH 5.5 and 400 *mM* Na^+.

Materials and Methods. The 22-mer d[3′-(CCCAAT)$_3$CCC-5)′] (Tel22C) cytosine-rich and d[5′-A(GGGTTA)$_3$GGG-3′] (Tel22G) guanine-rich regular sequences of human telomeric DNA purchased from Integrated DNA Technologies, Inc., were used in this study. The studies were carried out in 0.4 *M* Na^+ acetate buffer, pH 5.5. The concentration of urea was varied in the range 0–8 *M*, since 8 *M* is the maximal

concentration of urea at which it dissolves in 0.4 M Na^+ acetate buffer at room temperature. The concentrations of Tel22C and Tel22G were determined spectrophotometrically by measuring absorbance at 260 nm at 80°C using the following extinction coefficients: $\varepsilon_{260} = 228500\ M^{-1}cm^{-1}$ for Tel22G and $\varepsilon_{260} = 194600\ M^{-1}cm^{-1}$ for Tel22C. The thermal denaturation studies of Tel22C and Tel22G were carried out by a Lambda-800 (Perkin Elmer) UV/Vis spectrophotometer. The melting curves were obtained at characteristic bands for i-motif (290 nm) and for G-quadruplex (295 nm). The conformational changes of Tel22C and Tel22G were studied by the Circular Dichroism method using a DSM 20 (Olis, USA) CD spectrophotometer.

Results and Discussion.

Obtaining i-Motif Structure. As mentioned, the i-motif structure is formed in the case of slightly acidic pH. Therefore, we firstly defined the range of pH values at which i-motif is formed in Tel22C. The oligonucleotide was dissolved and stored in a solution of 10 mM NaCl + 0.1 mM EDTA + 0.1 mM NaN_3, pH 7.0. By adding appropriate amounts of HCl (for acidic pH range) or NaOH (for alkaline pH), the pH of the solution was adjusted to the desired value. The formation of the i-motif structure in Tel22C at different pH was controlled by CD spectra. The presence of a positive CD band at 290 nm indicated the formation of the i-motif structure in Tel22C in the pH range $4.7 < \text{pH} < 6.6$ (Fig. 1). Based on the obtained data, further studies of conformational changes in Tel22C and in its complementary guanine-rich strand Tel22G upon addition of urea were carried out in 0.4 M Na^+ acetate buffer, pH 5.5.

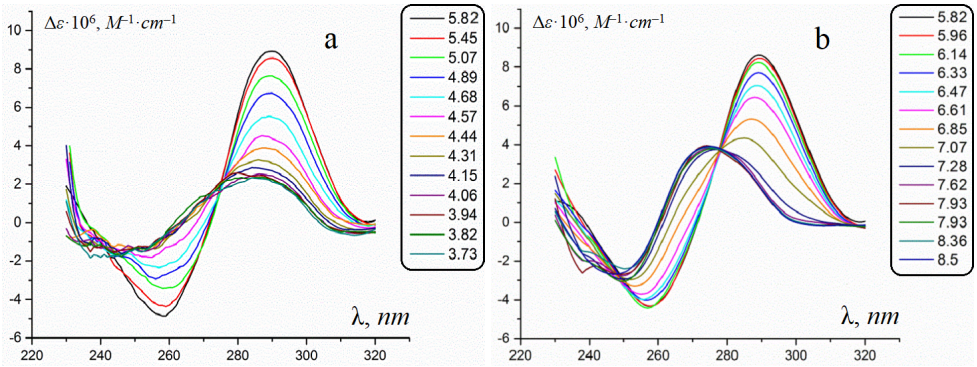

Fig. 1. The CD spectra of Tel22C at different pH values.

The CD Spectra of Tel22C and Tel22G. In Fig. 2 the CD spectra of Tel22C and Tel22G at different concentrations of urea in solution are shown.

From Fig. 2 it can be seen that the CD spectra of both oligomers have a positive band with a maximum at 290–295 nm and a negative band at 260 nm even in the absence of urea. This indicates that under these experimental conditions Tel22C in the absence of urea is in the i-motif conformation, while Tel22G is in the G-quadruplex conformation. The addition of urea leads to a decrease in the absolute value of the amplitude of these bands and a contraction of the spectra to the baseline. The changes in the maximum and minimum wavelengths of CD spectra are insignificant. More

detailed characteristic changes in CD bands with increasing urea concentration are shown in Fig. 3.

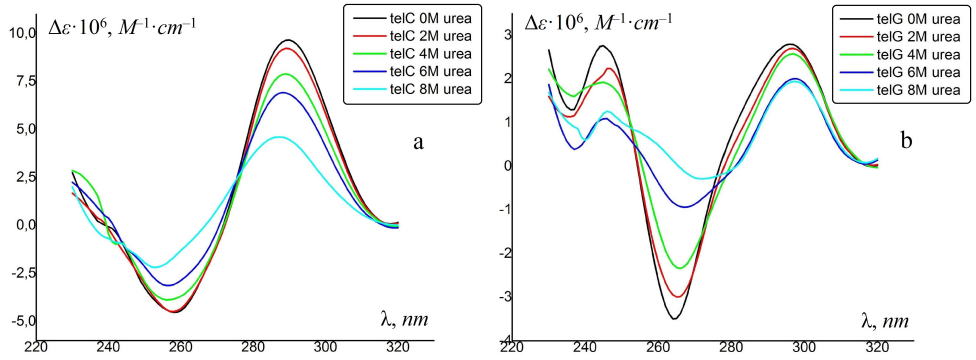

Fig. 2. The CD spectra of Tel22C (a) and Tel22G (b) at different concentrations of urea.

As can be seen in Fig. 3, the band at 260 *nm* changes in a similar way for both Tel22C and Tel22G. With an increase in the concentration of urea, this negative band at the minimum grows rapidly approaching zero, while the band at 295 *nm* decreases faster for Tel22C. The reduction in the anisotropy of CD bands of ordered structures of Tel22C and Tel22G caused by urea indicates the destabilization and destruction of the i-motif and G-quadruplex structures.

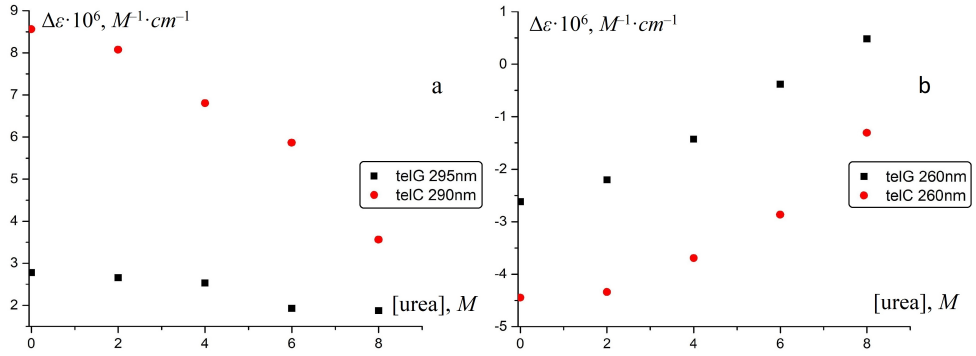

Fig. 3. Changes of positive (a) and negative (b) CD spectra bands of Tel22C and Tel22G at different concentrations of urea.

The comparison of the CD spectra of Tel22C in the presence of urea obtained at room temperature and after thermal denaturation makes it possible to assess the extent of urea-induced rearrangements in the i-motif structure (Fig. 4).

From Fig. 4 it is obvious that thermal denaturation leads to more essential changes in the CD spectra of i-motif than denaturation with urea. Beside a decrease in the amplitude of the CD spectra bands, thermal denaturation of i-motif also leads to changes in the position of extrema. The spectrum of i-motif denatured at 80°C has a maximum at 275 *nm* and a minimum at 255 *nm*, which is characteristic of a

single-stranded molten structure of DNA. Therefore, it can be assumed that 8 M urea destabilizes the i-motif structure of Tel22C but does not destroy its structure completely; some extent of ordering remains in the structure of i-motif.

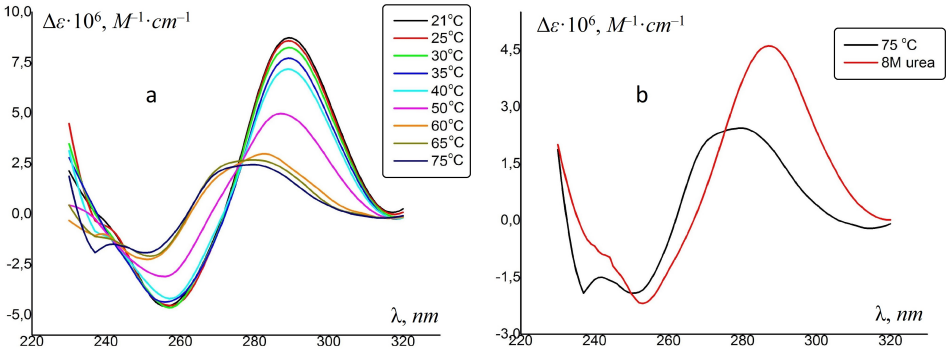

Fig. 4. Tel22C CD spectra at different temperatures without urea (a), and at thermal denaturation (black line) and denaturation with 8 M urea (red line) (b).

The Melting Curves of Tel22C and Tel22G. In the next step, we investigated the influence of urea on the melting curves of Tel22C and Tel22G (Fig. 5). The melting curves were recorded at the characteristic wavelengths for i-motifs $(290\ nm)$ and G-quadruplexes $(295\ nm)$ and were normalized according to Marky and Breslauer [15].

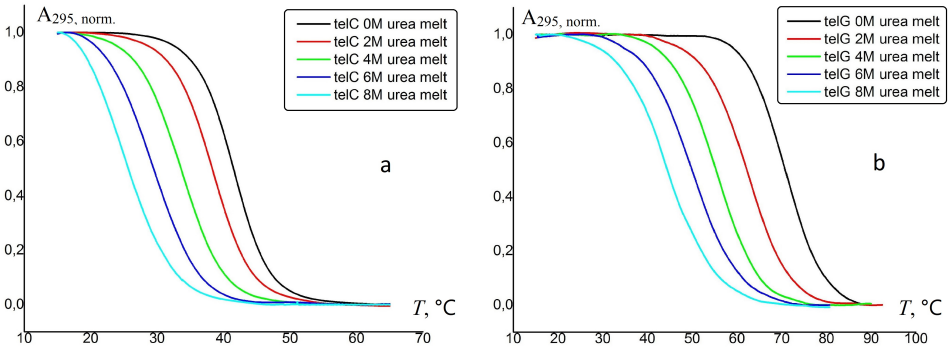

Fig. 5. The melting curves of the i-motif (Tel22C) and G-quadruplex (Tel22G) structures at different concentrations of urea.

As seen from Fig. 5, with an increase in the concentration of urea, the slope of the melting curves for Tel22C and Tel22G practically does not change, but the curves shift to the low temperature region. For each concentration of urea, calculating the slope $(\partial\alpha/\partial T)_{T=T_m}$ of the melting curves at the melting point and taking into account that in our case the number of DNA strands is equal to unity $(n=1)$, it is possible to calculate the Van't Hoff melting enthalpies ΔH_{VH} by the following formula [15]:

$$\Delta H_{\mathrm{VH}} = (2+2n)RT_m^2\left(\frac{\partial\alpha}{\partial T}\right)_{T=T_m}.$$

The results are presented in the table.

The dependence of melting temperature and enthalpy of i-motif and G-qudruplex on the concentration of urea

[urea]	Tel22G		Tel22C	
M	T_m, °C	ΔH_{VH}, $kcal \cdot mol^{-1}$	T_m, °C	ΔH_{VH}, $kcal \cdot mol^{-1}$
0	70.73	55.63	41.47	71.20
2	62.17	46.63	38.27	64.46
4	55.14	45.98	33.58	58.30
6	49.93	39.63	29.47	52.73
8	44.43	41.44	25.65	49.50

As it follows from Fig. 5 and Table, the melting processes of G-quadruplex (Tel22G) and i-motif (Tel22C) are strongly separated in the temperature scale (the melting temperature difference (ΔT_m) between G-quadruplex and i-motif in the case of the absence of urea is approximately 30°C). With an increase in the concentration of urea, the melting temperatures decrease linearly, but T_m of G-quadruplex decreases faster than that of i-motif. The difference ΔT_m at the maximum soluble concentration of urea $(8\ M)$ is less than 20°C.

But even in case of such changes of T_m, the melting temperature of G-quadruplex, destroyed in the presence of maximal soluble concentration of urea $(8\ M)$, is 3°C higher than the melting temperature of i-motif without urea. This means that at all concentrations of urea (including its absence) the melting of G-quadruplex starts at temperatures at which the i-motif melting has already ended. The melting enthalpy decreases with increasing urea concentration for both G-quadruplex and i-motif. This result also indicates the destabilization of the ordered G-quadruplex and i-motif structures by urea. It can be seen from the data obtained that at all concentrations of urea the melting enthalpy of i-motif is significantly higher than in the case of G-quadruplex. In the absence of urea, the melting enthalpy of i-motif is 71.2 $kcal \cdot mol^{-1}$, while for the more thermostabile G-quadruplex it is significantly smaller, namely 55.63 $kcal \cdot mol^{-1}$.

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ՄԻԶԱՆՅՈՒԹԻ ԱԶԴԵՑՈՒԹՅՈՒՆԸ i-ՄՈՏԻՖ և G-ՔՈՒԱԴՐՈՒՊԼԵՔՍ ԿԱՌՈՒՑՎԱԾՔՆԵՐԻ ՎՐԱ ԴՆԹ-Ի ԿՈՄՊԼԵՄԵՆՏԱՐ ՀԱՋՈՐԴԱԿԱՆՈՒԹՅՈՒՆՆԵՐՈՒՄ

Տվյալ աշխատանքում շրջանային դիքրոիզմի և ՈւՄ/տեսանելի սպեկտրոֆոտոմետրիայի եղանակներով ուսումնասիրվել է միզանյութի ազդեցությունը թելոմերային ԴՆԹ-ի (Tel22C) ցիտոզինով հարուստ d[3′-$(CCCAAT)_3CCC$-5′] փեղամասում i-մոտիֆ ⇆ բացված միաշղթա և դրան կոմպլեմենտար գուանինով հարուստ d[5′-$A(GGGTTA)_3GGG$-3′] (Tel22G) շղթայում G-քուադրուպլեքս ⇆ բացված միաշղթա կառուցվածքային անցումները pH 5.5 և 400 *մմոլ*/*լ* Na^+ պայմանների դեպքում: Պարզվել է, որ նշված պայմաններում Tel22C և Tel22G ձևավորում են կայուն i-մոտիֆ և G-քուադրուպլեքս կառուցվածքներ: Ցույց է տրվել, որ միզանյութը (0–8 *մոլ*) ապակայունացնում է i-մոտիֆ և G-քուադրուպլեքս կառուցվածքները, սակայն ի տարբերություն ջերմային դենատուրացիայի չի քանդում դրանք ամբողջությամբ: i-մոտիֆի և G-քուադրուպլեքսի հալման պրոցեսներն առանձնացած են ջերմաստիճանային սանդղակում. միզանյութի ցանկացած կոնցենտրացիայի դեպքում G-քուադրուպլեքսի հալումը սկսվում է այն ջերմաստիճանում, որում i-մոտիֆի հալումն արդեն ավարտվել է:

Е. Б. ДАЛЯН, Л. Г. АСЛАНЯН, И. В. ВАРДАНЯН

ВЛИЯНИЕ МОЧЕВИНЫ НА G-КВАДРУПЛЕКС И i-МОТИВ СТРУКТУРЫ В КОМПЛЕМЕНТАРНЫХ ПОСЛЕДОВАТЕЛЬНОСТЯХ ДНК

В работе методами кругового дихроизма и УФ-/видимой спектрофотометрии исследовалось влияние мочевины на структурные переходы i-мотив ⇆ развернутая одиночная нить в d[3′-$(CCCAAT)_3CCC$-5′] цитозин-богатом участке теломерной ДНК (Tel22C) и G-квадруплекс ⇆ развернутая одиночная нить в комплементарной ей d[5′-$A(GGGTTA)_3GGG$-3′] гуанин-богатой нити (Tel22G) при pH 5.5 и 400 *мМ* Na^+. Обнаружено, что в этих условиях в Tel22C и Tel22G образуются устойчивые i-мотив и G-квадруплекс структуры. Показано, что мочевина (0–8 *M*) дестабилизирует i-мотив и G-квадруплекс структуры, но в отличие от термической денатурации не разрушает их полностью. Процессы плавления G-квадруплекса и i-мотива сильно разведены по температурной шкале (при любой концентрации мочевины плавление G-квадруплекса начинается при температурах, когда плавление i-мотива уже закончено).