

Biophysics

Influence of K^+ Ions on Thermodynamic Stability of DNA G-Quadruplex

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ABSTRACT. It is supposed that presence of monovalent cations is necessary for in vitro formation of G-quadruplex, and the most effective cation is K^+ . We demonstrated that 15-mer sequences like GGG(TGGG)3 (abbreviated name - G3T) are capable to form stable quadruplexes even in double-distilled water with heat transition parameters: $UH^{VH} = 2.15$ kcal/mol, $T_m = 34.0^\circ$ C and $T_m = 34.5^\circ$ C. In the range of K^+ ion concentrations from 12 μ M to 3 mM inclusive, an intensive increase in quadruplex melting temperature T_m , moderate decrease in melting width UT_m and intensive increase in melting enthalpy UH^{VH} to 6.6 kcal/mol are observed. On the basis of CD melting curves we have calculated UH^{VH} ; dependence curve $UH^{VH} = f(T_m)$ was built; and heat capacity increment $UC_m = (1.56 \pm 0.17)$ kcal/K.mol was calculated. The extremely high value of heat capacity increment is in a good agreement with theoretical suggestions, according to which DNA G-quadruplexes are highly flexible. © 2017 Bull. Georg. Natl. Acad. Sci.

Keywords: G-quadruplex, G3T, GGG(TGGG)3, UV CD Melting Curve

It is determined that G-quadruplex structures formed in promoter regions and the telomeric DNA ends play an important role in regulation of gene expression [1-5].

It is considered that, besides specific sequences, presence of monovalent cations is necessary for formation of those four-strand G-quadruplex structures of various conformations. K^+ and Na^+ ions have the strongest stabilizing influence on G-quadruplex structures.

However, K^+ ions incorporate between the G-tetrads and stabilize G-quadruplexes better than Na^+ ions that are placed on planes formed by guanines [6, 7].

In the given work, we presented some new data about the dynamic structure of 15-mer G-quadruplex forming sequences, in particular, the possibility of G-quadruplex (G3T) formation in absence of K^+ ions, and we have demonstrated the extremely high flexibility of G3T.

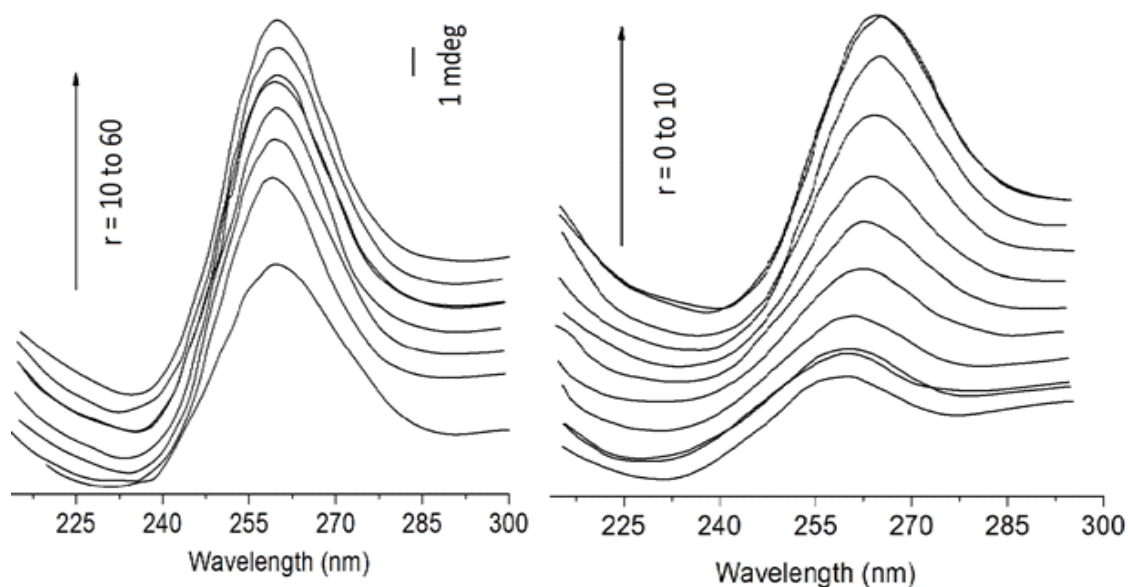


Fig. 1. CD UV spectra of G3T sequence at different $r = K^+/G3T$ molar mixing ratios: 0, 0.5, 0.5, 1, 2, 3, 4, 6, 8, 10, 10, 10, 15, 20, 25, 30, 30, 35, 60; G3T concentration is $3 \mu M$

Materials and Methods. CD spectra were recorded with spectropolarimeter JASCO 500 A at various temperatures. UV CD melting curves were recorded in the temperature range from 10° to 100° C using 0.5 cm quartz cells. GGG(TGGG)₃ was purchased from Integrated DNA Technologies, BE.

Results

CD Studies of K^+ -Induced G-Quadruplex formations

Figs. 1,2 show the UV CD spectra of G3T in the absence and presence of different KCl concentrations in double-distilled water at 45° C. As it is seen from Fig. 1, at absence of K^+ ions, the band intensity at 264 nm is 1.6 mdeg, the increase in K^+ ion concentration within the region 0-90 μM causes the increase in bend intensity at 264 nm, and further increase in K^+ concentration does not influence the bend intensity that keeps 10.1 mdeg unchanged. The curve profiles are characteristic of parallel structures with double chain reversal loops bound to G-quadruplex [8]. Dependence of intensity of those bands on K^+ concentration is presented in Fig. 2. The presented data show that G3T quadruplex is completely formed in double-

distilled water at $90 \mu M K^+$, i.e approximately 30 molecules of K^+ are bound to G3T quadruplex.

UV CD Melting Curves of G3T

Fig. 3 presents CD melting curves of G3T quadruplex at various K^+ concentrations in double-distilled water. As is seen, the increase in K^+ concentration causes some shift of melting curves to higher temperatures and narrowing of melting temperature width ΔT . On the basis of the presented data, the

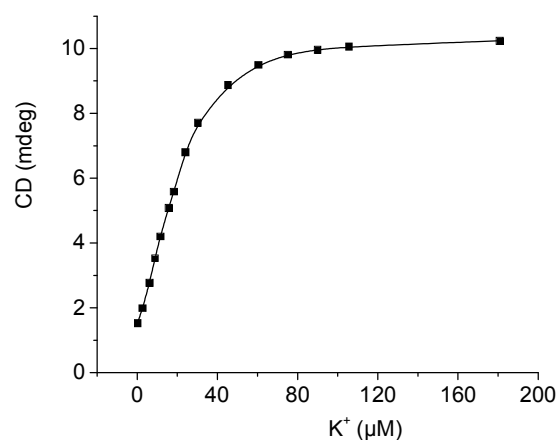


Fig. 2. Dependence of CD band intensity at 263 nm on r calculated from curves from Fig. 1

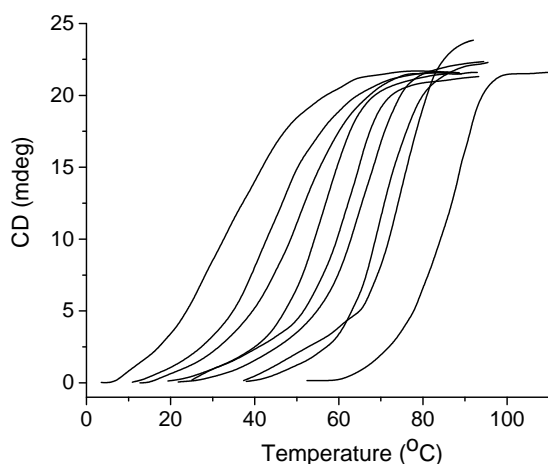


Fig. 3. CD melting curves of G3T quadruplex at 264 nm at different K^+ concentrations – left to right: 0, 1, 12, 50, 100, 200, 400, 800, 3000 μM . Scanning rate = $1^\circ/\text{min}$

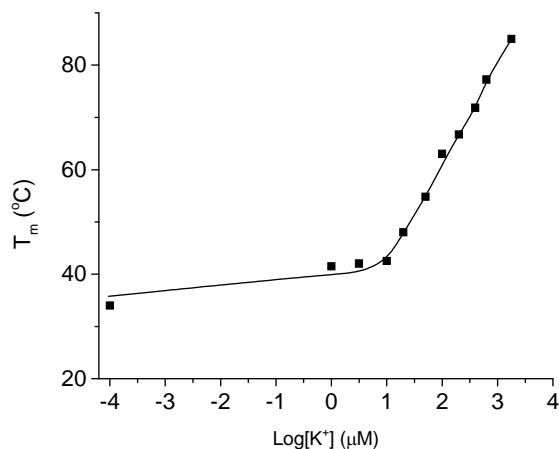


Fig. 4. Dependence of melting temperature T_m of G3T on the Log of potassium ion concentration $\text{Log}[K^+]$

dependence $T_m = \text{Log}[K^+]$ is presented in Fig.4. This dependence shows that there are two K^+ -dependent melting regions. The first region 0-12 μM is weakly dependent on K^+ , and the second one 12 μM to 3.0 mM is strongly dependent on K^+ content. In the first case, T_m increased from 34.0° to 41.5° C, and in the second case T_m increased from 41.5° to 85.0° C.

It is known that melting of G3T quadruplex is a reversible and two-stage process [6]. Hence, the data presented in Fig.3 gave the possibility to calculate Van't Hoff enthalpy with a standard method. The obtained data are presented in Fig.5. As it is seen, the dependence of melting enthalpy on temperature has a linear character, and ΔH^{VH} increases from 2.15 to 6.6 cal/mole when T_m increases from 33.5° to 85.0° C. The linear dependence $\Delta H^{\text{VH}} = f(T_m)$ gives a possibility to calculate the heat capacity increment $d\Delta H^{\text{VH}}/dT_m = \Delta C_m = (1.56 \pm 0.17)$ kcal/K.mol.

Discussion

It is known that human genome contains about 380,000 GC-rich sites which can form non-canonical structures of left-handed duplex Z-DNA and four-stranded structures of G-quadruplex. In both cases, presence of K^+ , Na^+ , Cs^+ , or Li^+ ion is necessary for formation of the abovementioned structures in solutions. However, in case of Z-DNA formation, pres-

ence of 1-2 mole concentrations of relevant salts or non-organic solvents is necessary. As for the G-quadruplex, presence of μM or mM K^+ concentrations is necessary for formation of this structure [6, 7, 9].

The data presented in Figs.1,2 show that G3T quadruplex is already formed in double-distilled water at 45° C, and the UV CD intensity at 264 nm is 1.6 mdeg. Addition of K^+ ions to this solution initiates further formation of G-quadruplexes; and all G-quadruplexes are formed in solution at presence of 90 μM K^+ and 3 μM oligomer i.e not more than 30 molecules of K^+ ions are bound with G3T quadruplex.

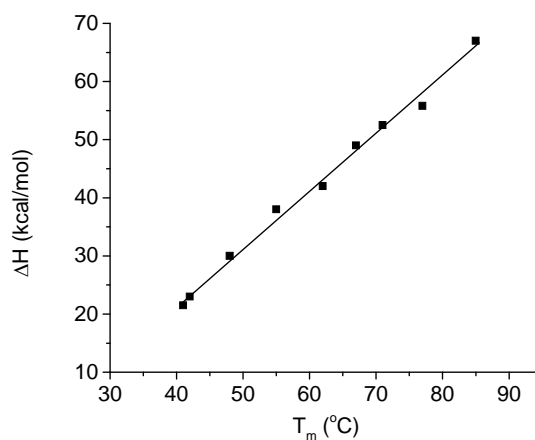


Fig. 5. Dependence of ΔH^{VH} of G3T quadruplex on melting temperature

Our study showed that GGG(TGGG)₃ sequence forms G3T quadruplex even at absence of K^+ ions or when K^+ concentration is so small that it equals the atmospheric content of K^+ (see Figs.1,2). See also [10].

CD melting curve of G3T at different K^+ concentrations gave a possibility to calculate ΔH^{VH} enthalpy, to build the dependence $\Delta H^{VH} = f(T_m)$, and to calculate the heat capacity increment

$\Delta C_m = (1.56 \pm 0.17)$ kcal/K.mol or 130 kcal/K.mol recalculated per GG base pair. These values are more than 4 times higher than the values observed for stable globular proteins, and they are 2.2 times higher than those for 12-bp GC rich DNA duplex [11]. The high value increments demonstrated that the G3T quadruplex is extremely flexible, and this flexibility is caused by quadruplex fluctuations and partially with its ends.

ბიოფიზიკა

K^+ იონების გავლენა დნმ-ის G-კვადრუპლექსის თერმოდინამიკურ სტაბილურობაზე

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ითვლება, რომ ერთფაგენტიანი კატიონები საჭიროა G-კვადრუპლექსების ინ ვიტრო ფორმირებისთვის, ხოლო ამ მხრივ ყველაზე ეფექტურია K^+ იონი. ჩვენ ვაჩვენეთ, რომ ისეთ 15-წევრიან მიმდევრობას, როგორცაა GGG(TGGG)₃ (შემოკლებით - G3T) შეუძლია ბიდესტილირებულ წყალშიც კი წარმოქმნას სტაბილური კვადრუპლექსები, რომელთა სითბური გადასვლის პარამეტრებია: $\Delta H^{VH} = 2,15$ კკალ/მოლი, $T_m = 34,0^\circ C$ და $\Delta T_m = 34,5^\circ C$. როდესაც K^+ იონის კონცენტრაცია არის 12 მიკრომოლიდან 3 მილიმოლამდე, ინტენსიურად იზრდება კვადრუპლექსის დნობის ტემპერატურა თმ, ზომიერად მცირდება დნობის სიგანე ?თმ და ინტენსიურად იზრდება დნობის ენტალპია ΔH^{VH} 6,6 კკალ/მოლამდე. დნობის წრიული დიქროიზმის მრუდების ანალიზზე დაყრდნობით, ჩვენ გამოთვალეთ ΔH^{VH} ; ავაგეთ $\Delta H^{VH} = f(T_m)$ დამოკიდებულების მრუდი და განვსაზღვრეთ დნობით გამოწვეული სითბოტევადობის ნახტომი $\Delta C_m = (1,56 \pm 0,17)$ კკალ/გრადუსი.მოლი. უკიდურესად მაღალი სითბოტევადობის ნახტომი კარგად თანხვედება თეორიულ მოსაზრებებს, რომელთა მიხედვითაც დნმ-ის G-კვადრუპლექსები ხასიათდება მაღალი მოქნილობით.

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Received February, 2017