Biophysics

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


*E. Andronikashvili Institute of Physics, Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia

(Preseented by Academy Member Tengiz Beridze)

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: $\Delta H^{\text{VH}} = 2.15$ kcal/mol, $T_m = 34.0^\circ$ C and $\Delta T_m = 34.5^\circ$ C. In the range of K$^+$ ion concentrations from 12 $\mu$M to 3 mM inclusive, an intensive increase in quadruplex melting temperature $T_m$, moderate decrease in melting width $\Delta T_m$ and intensive increase in melting enthalpy $\Delta H^{\text{VH}}$ to 6.6 kcal/mol are observed. On the basis of CD melting curves we have calculated $\Delta H^{\text{VH}}$; dependence curve $\Delta H^{\text{VH}} = f(T_m)$ was built; and heat capacity increment $\Delta C_m = (1.56 \pm 0.17)$ 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 that 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.
Influence of K⁺ Ions on Thermodynamic Stability of DNA G-Quadruplex

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 µM 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 ∆T. On the basis of the presented data, the

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, 15, 20, 25, 30, 30, 35, 60; G3T concentration is 3 µM

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

dependence $T_m = \text{Log}[K^+]$ is presented in Fig.4. This dependence show 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 $\Delta H^{\text{VH}}$ 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-canonic 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, presence 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 mged. 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 3µM oligomer i.e not more than 30 molecules of $K^+$ ions are bound with G3T quadruplex.

**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°/min

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

**Fig. 5.** Dependence of $\Delta H^{\text{VH}}$ of G3T quadruplex on melting temperature
Influence of K⁺ Ions on Thermodynamic Stability of DNA G-Quadruplex

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 Δ_H^V = (1.56 ± 0.17) kcal/K.mol or 130 kcal/K.mol re-calculated 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.

K⁺ იონების გამოვლინება რით-აი G-კვადრუპლექსის თერმოდინამიკური სტაბილურობის ჩადება

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 Δ_H^V = (1.56 ± 0.17) kcal/K.mol or 130 kcal/K.mol re-calculated 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.
REFERENCES


Received February, 2017