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G-Quadruplexes Form Ultrastable Parallel Structures in Deep Eutectic Solvent

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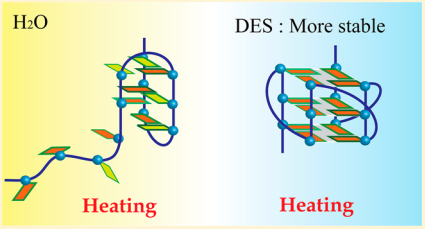
Supporting Information

ABSTRACT: G-quadruplex DNA is highly polymorphic. Its conformation transition is involved in a series of important life events. These controllable diverse structures also make G-quadruplex DNA a promising candidate as catalyst, biosensor, and DNA-based architecture. So far, G-quadruplex DNA-based applications are restricted done in aqueous media. Since many chemical reactions and devices are required to be performed under strictly anhydrous conditions, even at high temperature, it is challenging and meaningful to conduct G-quadruplex DNA in water-free medium. In this report, we systemically studied 10 representative G-quadruplexes in anhydrous room-temperature deep eutectic solvents (DESs). The results indicate that intramolecular, intermolecular, and even higher-order G-quadruplex structures can be formed in DES. Intriguingly, in DES, parallel structure becomes the G-quadruplex DNA preferred conformation. More importantly, compared to aqueous media, G-quadruplex has ultrastability in DES and, surprisingly, some G-quadruplex DNA can survive even beyond 110 °C. Our work would shed light on the applications of G-quadruplex DNA to chemical reactions and DNA-based devices performed in an anhydrous environment, even at high temperature.



1. INTRODUCTION

Guanine-rich DNA sequences can fold into unique four-stranded helices known as G-quadruplexes formed by the stacking of planar quartets composed of four guanines that interact by Hoogsteen hydrogen bonding.[1−3] Bioinformatic analysis has identified more than 350 000 candidate sequences, in the human genome, with propensity to form G-quadruplexes.[4] In vivo, G-quadruplexes have been shown to potentially form in regions of biological significance, such as human telomeres and oncogene promoter regions. Many studies have revealed that G-quadruplex structures appeared to be involved in several significant biological processes.[5] One important example is that the telomere G-quadruplex formed at the chromosomal extremities can inhibit telomerase activity and is viewed as emerging therapeutic targets in oncology.[6−8] Recently reports showed that G-quadruplexes DNA also can be the inhibitor of HIV-1 integrase.[9−11]

G-quadruplex structures are highly polymorphic. Human telomeric sequences, for instance, can adopt at least five different intramolecular G-quadruplexes: a parallel-stranded conformation in the crystalline state, a basket-type antiparallel-stranded structure in Na^+ solution, while at least three other forms were seen in K^+ solution (Figure 1).[12−17] Owing to the crowded environment in living cells, molecular crowding studies have suggested that parallel quadruplex form may be more biologically relevant, while parallel structure hardly formed in dilute solution.[18]

Besides the biological importance, the controllable diverse structures and robust physicochemical nature of G-quadruplex DNA also make it a promising candidate as a catalyst, biosensor, nanomachine, and in the construction of functional DNA nanostructure.[19−24] So far, these G-quadruplex DNA-based applications have been realized in aqueous media. However, many important chemical reactions or devices are normally conducted under strictly anhydrous conditions.[25] In a recent report, Ito et al. covalently attached a poly(ethylene glycol) unit (PEG) to the G-quadruplex DNA to realize its catalytic functions in organic solvents.[26] Exploring a water-free medium, in which G-quadruplex is favored, will open a new promising avenue for applications. Room-temperature ionic liquids (RTILs) provide favorable environments for a series of reactions and are considered to be green solvents.[27] Currently, RTILs are being used for a variety of bioapplications including catalytic reactions, biosensors, protein stabilization, and nucleic acids storage.[28−30] It was reported that DNA exhibited exceptional long-term stability in hydrated ILs, and more interestingly, for duplex DNA, A-T base pairs were more stable than G-C base pairs in hydrated ILs.[31,32] Deep eutectic solvents (DESs) are a new family of ionic fluids that exhibit similar physicochemical properties to traditional ionic liquids.[33] Using DESs as anhydrous and high-viscosity solvents, Hud and co-workers investigated the properties of several DNA and RNA secondary structures.[34] Very recently, they reported that the folding of G-quadruplex in the anhydrous DES and mixed DES-water solvents revealed diffusion-limited kinetics consistent with Kramers rate theory.[35] Thus, if it is possible to introduce

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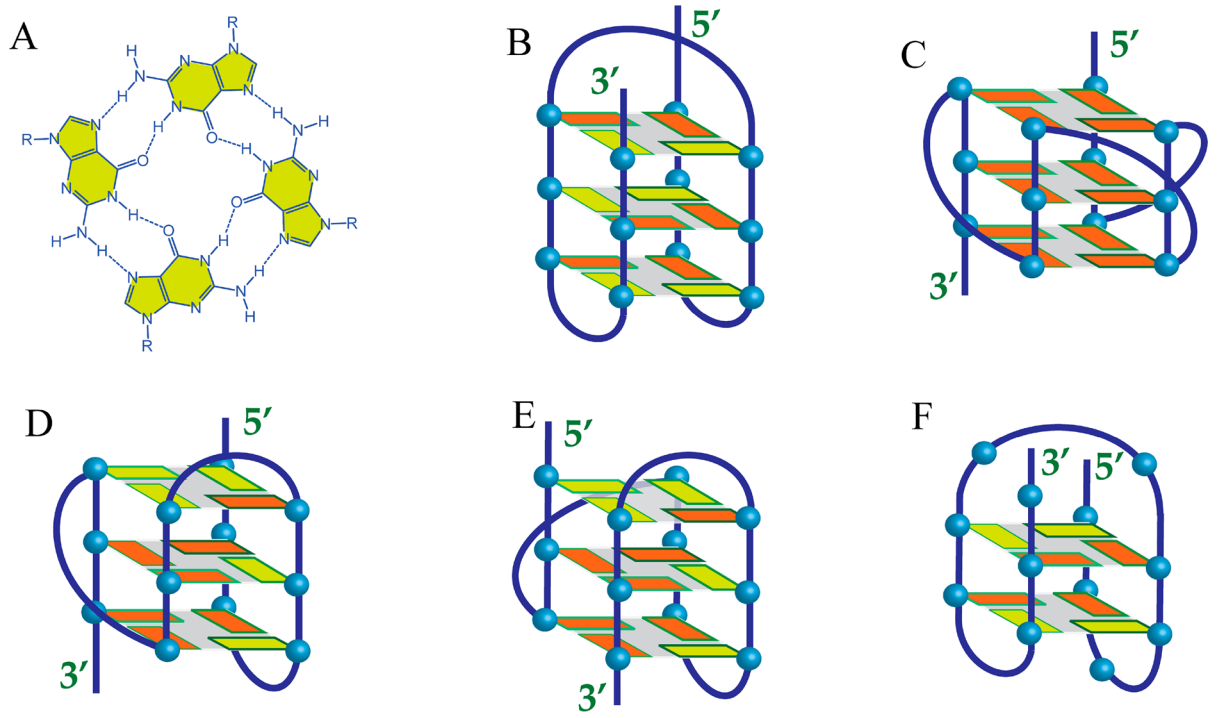

Figure 1. (A) G-tetrad structure. Various human telomeric G-quadruplexes in different conditions: (B) basket-type antiparallel G-quadruplex observed in Na^+-containing solution; (C) propeller-type parallel G-quadruplex observed in a K^+-containing crystal; (D) Hybrid-1 G-quadruplex observed in K^+-containing dilute solution; (E) Hybrid-2 G-quadruplex observed in K^+-containing dilute solution; (F) basket-type G-quadruplex with two G-tetrad layers observed in K^+-containing dilute solution.

various G-quadruplex DNA into water-free ILs or DESs, their properties may be improved or even changed, and thereby this may expand the applications of G-quadruplexes in anhydrous environment.

Herein, we systematically studied 10 representative G-quadruplexes in anhydrous DES. Our results revealed that intramolecular, intermolecular, and higher-order G-quadruplex structures can be formed in DES. In DES, parallel structure was G-quadruplex preferred conformation. More importantly, compared to water solution, G-quadruplex exhibited ultrastability in DES.

2. EXPERIMENTAL SECTION

Materials. DNA was synthesized by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China). The concentrations of DNA were determined by ultraviolet absorbance measurements. For the fluorescent conjugates, ε_{260} = 13000 M^{-1} cm^{-1} for FAM and ε_{260} = 34000 M^{-1} cm^{-1} for TMR were added into the calculations. Chemicals were purchased from Sigma-Aldrich and used without further purification. All DNA samples were heated at 95 °C for 5 min, and then slowly cooled to room temperature unless otherwise indicated. All water used to prepare buffer solutions was obtained by using a Milli-Q water system. The choline chloride/urea DES was prepared by heating a 1:2 molar mixture of choline chloride and urea at 100 °C until a liquid was formed. After heating for 2 h, the DES was naturally cooled to room temperature. Either 0.585 g (10 mM) of NaCl or 0.745 g of KCl (10 mM) was added to 100 mL DES and was heated at 100 °C for 2 h to prepare 100 mM/L NaCl or KCl-containing DES. Solutions of DNA in the DES were prepared by mixing aqueous stock nucleic acid solutions with the DES and then removing the great mass of water by vacuum evaporation.

UV Melting. UV melting experiments were carried out on a Cary 300 UV/vis spectrophotometer equipped with a Peltier temperature control accessory. Experiments were measured in 1.0-cm path-length cell with the same percent of DES solution as the reference. Absorbance changes at 295 nm versus temperature were collected at a heating rate of 1 °C min^{-1}.

Thermodynamic Parameters. The enthalpy change, ΔH°, was determined from the temperature dependence of equilibrium association constant, where ΔH° was the slope of the ln K_a versus $1/T$ plot according to the equation ln $K_a = -(\Delta H^\circ/RT) + \Delta S^\circ/R$, where ΔS° was the entropy change that was calculated according to the y axis intercept. The free energy change (ΔG°_{25}) at 25 °C was calculated from the standard Gibbs's equation, $\Delta G^\circ_{25} = \Delta H^\circ - T\Delta S^\circ$.

Circular Dichroism (CD) Measurements. CD spectra and CD melting experiments were carried out on a JASCO J-810 spectropolarimeter equipped with a temperature controlled water bath. The optical chamber of CD spectrometer was deoxygenated with dry purified nitrogen (99.99%) for 45 min before use,and kept the nitrogen atmosphere during experiments.[31] Three scans were accumulated and automatically averaged. In CD melting experiments, signals at 292 and 260 nm versus temperature were collected at a heating rate of 1 °C min^{-1}.

Fluorescence Assays. Human telomeric DNA (Tel_{22}) labeled with fluorescein (FAM) at the 5′ end as the donor and tetramethylrhodamine (TAM) at the 3′ end as the acceptor (5′-FAM-d(AGGG[TTAGGG]$_3$)-TAM-3′), was termed as F-Tel_{22}. With a 5′ end FAM donor and a 3′ end TAM receptor, the formation of an intramolecular G-quadruplex brings the two fluorophores into close proximity and allows fluorescence

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Table 1. G-Quadruplex Structures in Water Solution and in Water-Free DES

DNA name	sequence	structure[a]				
		Na^+/water	K^+/water	DES	Na^+/DES	K^+/DES
human telomeric (Tel_{22})	$AG_3(T_2AG_3)_3$	antiparallel	hybrid			parallel
long human telomeric	$AG_3(T_2AG_3)_3TTAG_3(T_2AG_3)_3$	antiparallel	hybrid			parallel
oxytricha telomeric	$(T_4G_4)_4$	antiparallel	antiparallel			parallel
G_3T_4	$(G_3T_4)_3G_3$	antiparallel	antiparallel			parallel
tetrahymena telomeric	$(G_4T_2)_3G_4$	hybrid	hybrid	parallel	parallel	parallel
c-myc	$(AG_3TG_3)_2A_2T_2$	parallel	parallel	parallel	parallel	parallel
c-kit	$(AG_3)_2CGCTG_3AG_2AG_3$	parallel	parallel			parallel
KRAS	$AG_3CG_2TGTG_3(A_2GAG_3)_2G_2AG_2$	parallel	parallel			parallel
TBA	$G_2T_2G_2TGTG_2T_2G_2$	antiparallel	antiparallel			antiparallel
A_4G_6	A_4G_6	parallel	parallel	parallel	parallel	parallel

[a]The results were summarized from Figure 2.

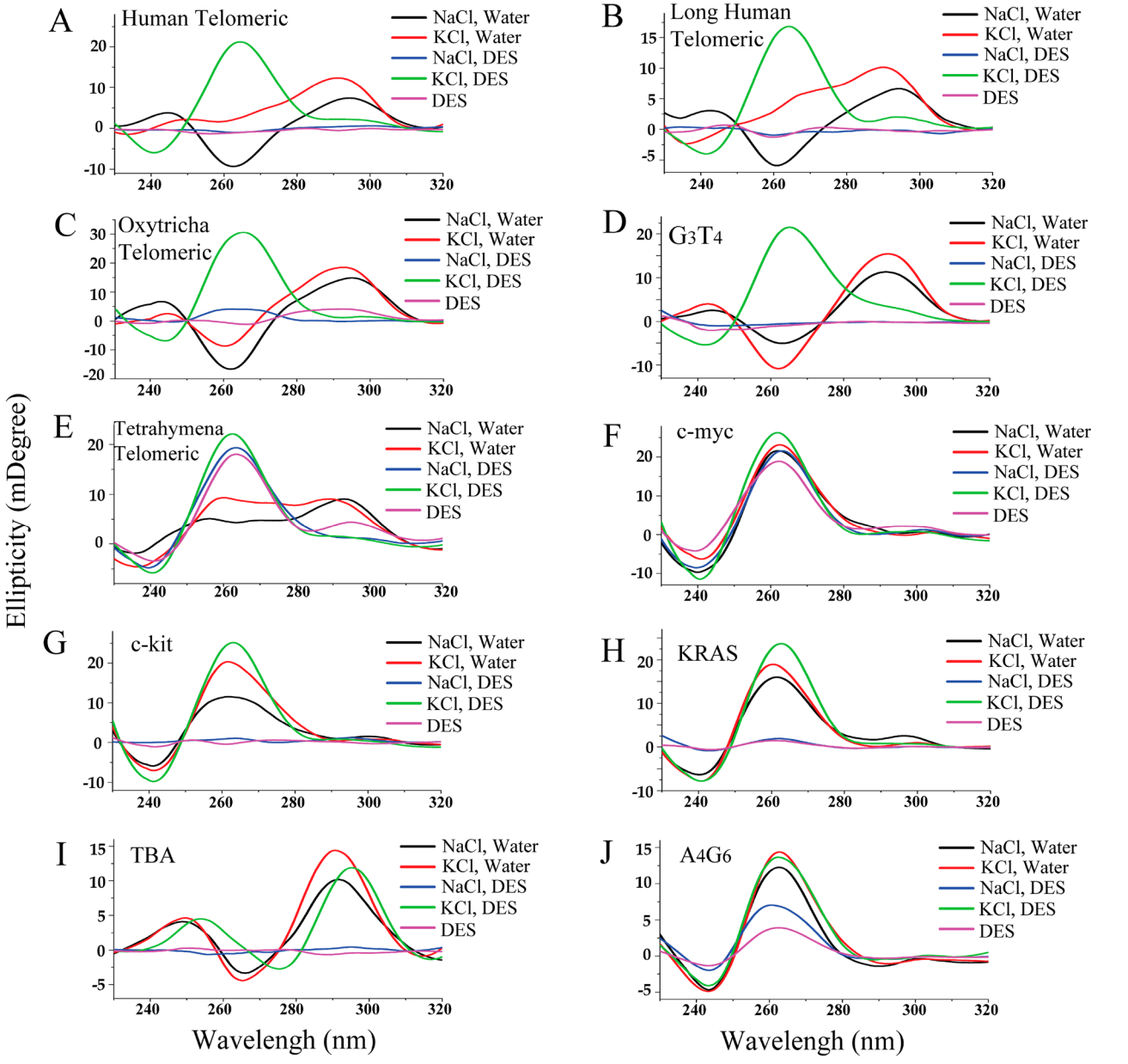

Figure 2. CD spectra of various G-quadruplex DNAs in different conditions as indicated in the figure. Aqueous solutions contain 10 mM Tris, 100 mM NaCl or 100 mM KCl (pH = 7.2). Anhydrous assays were carried out in DES, or DES containing 100 mM NaCl or 100 mM KCl. CD spectra were acquired at 25 °C.

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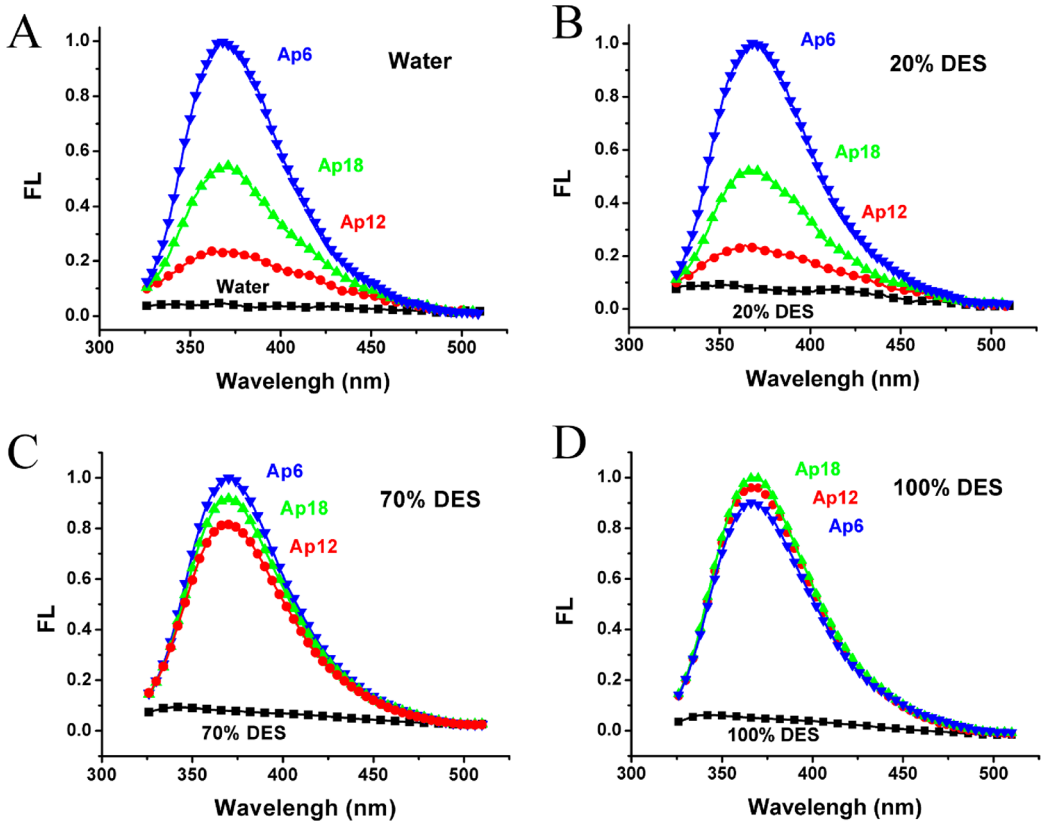

Figure 3. Fluorescent emissions of Ap substitutes at different positions in the $G_3(T_2AG_3)_3$ G-quadruplex (DNA sequences are listed in the Supporting Information). Sample treatment before emission measurement: Heated at 95 °C for 5 min in the presence of 0% (A), 20% (B), 70% (C), 100% (D), then cooled to 25 °C. All samples contained 100 mM KCl.

resonance energy transfer (FRET) to occur between them, which results in a decrease in the emission intensity of the FAM and an increase in the emission intensity of the TAM. The assays were carried out on a JASCO FP-6500 spectrofluorometer. Fluorescence spectra were measured by using an excitation wavelength of 480 nm and recorded from 490 to 650 nm. The concentration of F-Tel_{22} was fixed at 0.7 μM in strand. Emission spectra of oligonucleotides containing Ap were collected from 325 to 510 nm with excitation at 305 nm.

3. RESULTS AND DISCUSSION

Structures of G-Quadruplexes in DES. CD spectroscopy was employed to characterize the structure of G-quadruplex DNAs that exhibit characteristic CD signals depending on their strand composition.[36] Ten widely investigated G-quadruplex DNAs (short and long human telomeric DNA, oxytricha telomeric DNA, G_3T_4, tetrahymena telomeric DNA, c-myc DNA, c-kit DNA, KRAS, TBA, and artificial DNA A_4G_6) were studied (Table 1). Their CD spectra in water solution and in water-free DES are compared in Figure 2. First, 22-mer short human telomeric DNA (Tel_{22}) was analyzed.[37,38] Figure S1 (Supporting Information) indicated that DNA was well dissolved in DES. It was reported that Tel_{22} adopted hybrid G-quadruplex structure in K^+ aqueous solution[3,13] and antiparallel G-quadruplex structure in Na^+ aqueous solution.[12] However, it was different in cation-containing DES. Clearly, Tel_{22} adopted parallel G-quadruplex structure in K^+ DES, while it could not form G-quadruplex in Na^+ DES (Figure 2A). The formation of G-quadruplex in K^+ DES was further evidenced by FRET assay.[38] Dye-labeled F-Tel_{22} in K^+ DES displayed efficient FRET, indicating the formation of G-quadruplex (Figure S2). In addition, the G-quadruplex in K^+ DES remained stable at room temperature even for three months (Figure S3).

To further verify the parallel conformation of human telomeric DNA in K^+ DES, another fluorescence experiment was carried out using $G_3(T_2AG_3)_3$ with 2-aminopurine (Ap) substitutions at adenine residue positions 6, 12, and 18. A pevious study showed that Ap6, Ap12, and Ap18 had different fluorescence when the corresponding G-quadruplex was in a hybrid conformation, whereas all of them displayed similar fluorescence when the G-quadruplex was in the parallel conformation.[39] In our study, the fluorescence intensity of Ap in the water and 20% DES, followed the order Ap6 > Ap18 > Ap12 (Figure 3A,B) as previously reported for the hybrid structure. However, in 70% DES, their intensities were a little different (Figure 3C). In 100% DES, their intensities became very similar (Figure 3D). This result confirms that the conformation of Tel_{22} in K^+ DES is the parallel conformation. It should be noted that dye-labeled DNA exhibited higher fluorescence in DES than that in water solution (Figure S4). In addition, it has been reported that Tel_{22} adopted a parallel structure in 40% PEG 200.[18] The CD spectrum of Tel_{22} in K^+ DES was similar with that in 40% PEG 200 (Figure S5A), which provided further evidence that Tel_{22} adopted a parallel structure in K^+ DES.

Previous reports showed that G-quadruplex formation was strongly depended on the solvation free energy of the cation.[40] Thus, Na^+ DES did not support the G-quadruplex formation of Tel_{22} might result from the strong solvation free energies of Na^+ in the DES. Several groups have reported that long human

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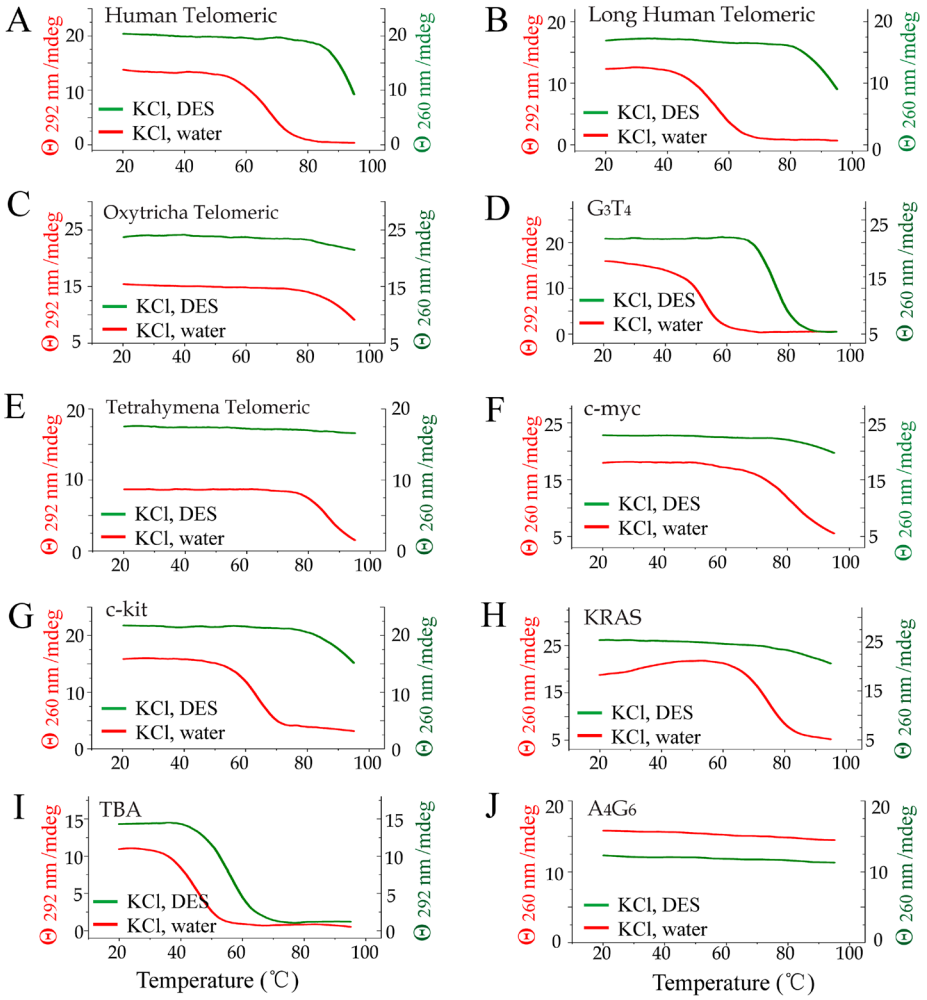

Figure 4. CD melting curves of various G-quadruplexes in DES containing 100 mM KCl (green) and in water containing 100 mM KCl (10 mM Tris, pH = 7.2) (red). Oligonucleotide name and the T_m values are listed in the figure.

telomeric DNA formed a higher-order G-quadruplex structure by connecting individual G-quadruplex motifs.[41−44] We then studied the structure of 46-mer long human telomeric DNA in DES (Figure 2B). Similar to Tel_{22}, long telomeric DNA could form higher-order G-quadruplex structure composed two individual parallel G-quadruplexes in K^+ DES, indicating that DES also provided a favorable environment for higher-order G-quadruplex structure.

Another six G-quadruplex DNAs were next tested. Oxytricha telomeric DNA and G_3T_4 formed antiparallel G-quadruplex in K^+ and Na^+ aqueous solution, while tetrahymena telomeric DNA formed hybrid G-quadruplex in K^+ and Na^+ water solution.[37] For c-myc, c-kit, and KRAS, all these DNAs formed parallel G-quadruplexes whether in K^+ solution or Na^+ water solution (Figure 2C−H).[45,46] However, similar to Tel_{22}, all these six DNAs formed parallel structure in K^+ DES (Table 1). From the results of the above six DNAs, we speculated that, for intramolecular G-quadruplexes, parallel structure was their preferred conformation in K^+ DES. However, TBA (one thrombin-binding aptamer) is an exception (Figure 2I). It adopted antiparallel structure in K^+ DES, similar to TBA in K^+ and Na^+ water solution (Table 1) . It was reported that, for TBA, parallel topology was not an appropriate topology, and there was no report to show that TBA adopted parallel quadruplex structure under any conditions.[47−50] This might be the reason for TBA adopting antiparallel structure in K^+ DES. In addition, we found that single-strand G-rich DNA (A_4G_6) could also form an intermolecular G-quadruplex in K^+ DES (Figure 2J), revealing that DES also supported the formation of intermolecular G-quadruplex. In combination with the above results, we can conclude that DES favors the formation of unimolecular G-quadruplex, intermolecular G-quadruplex, and even higher-order G-quadruplex, indicating that DES is an ideal interaction media for G-quadruplex. In addition, intriguingly, parallel structure became their preferred conformation in DES.

It should also be noted that, different from other DNAs, tetrahymena telomeric DNA, c-myc, A_4G_6 can form G-quadruplex structure in Na^+ DES and DES (Table 1). These G-quadruplex formations could be attributed to the dehydration of DNA in water-free DES, which coincided essentially with the report that the formation of the G-quadruplex was accompanied with water molecules releasing, and water depletion favored parallel-stranded G-quadruplex in the K^+-containing crowded solution.[51−54] In combination with the above results, we can conclude that DES favors the formation of unimolecular G-quadruplex, intermolecular G-quadruplex, and even higher-order G-quadruplex, indicating that DES is an ideal interaction medium for G-quadruplex. In addition, intriguingly, parallel structure became their preferred conformation in DES.

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Stability of G-quadruplexes in DES. Another attractive observation was that G-quadruplexes showed ultrastability in K^+ DES compared with that in water solution (Figure 4). For most of the G-quadruplexes, their T_m values were >90 °C in K^+ DES (Table 2). These results were consistent with the previous report that G-quadruplex was more stable in dehydration condition (Figure S5B).[18] To the best of our knowledge, there is no direct evidence to show the existence of any G-quadruplex beyond 100 °C because it is not suitable to do in aqueous solution. An intriguing feature of DES is that DES possesses no vapor pressure and high thermal and chemical stability, indicating that it would not be volatile and boiling when heated. These permit DES to be a more excellent medium for high-temperature reactions than water. In this view, we then tested the stability of tetrahymena telomeric G-quadruplex and A_4G_6 G-quadruplex at 110 °C. Obviously, these two G-quadruplex structures still held stable, even after being kept at 110 °C for 30 min (Figure 5A and Figure 5B). Undoubtedly, these ultrastable G-quadruplexes can be used for high-temperature biocatalytic reactions, biosensors, and construction of high-temperature-against DNA architectures.

Table 2. Melting Temperature of G-Quadruplex DNA in K^+ Water Solution and in Water-Free K^+ DES

DNA name	DNA T_m (°C)[a]	
	In K^+/ water	In K^+/ DES
human telomeric (Tel_{22})	67.5	>90
long human telomeric	56.3	>90
oxytricha telomeric	>90	>95
G_3T_4	53.3	76.3
tetrahymena telomeric	86.1	>95
c-myc	83.5	>95
c-kit	64.0	>95
KRAS	74.6	>95
TBA	45.7	55.7
A_4G_6	>95	>95

[a]The results were summarized from Figure 4.

Factors That Influence the Structure and Stability of G-Quadruplex in DES. Among G-quadruplex DNA, human telomeric DNA (Tel_{22}) was widely studied owing to its biological importance and its "tunable" conformation. Thus, we chose human telomeric DNA as a representative to study the factors that influenced the formation of G-quadruplex in DES. As depicted in Figure 5C, CD spectra of Tel_{22} in KCl solution containing different content of DES were monitored. With increasing percentage of DES, hybrid G-quadruplex slowly converted to parallel G-quadruplex. In about 80 wt % DES solution, parallel G-quadruplex completely formed. Next, we studied the effect of K^+ concentration on the formation of G-quadruplex. CD spectra of Tel_{22} in DES containing 30 mM K^+ was similar to that in DES containing 50 mM K^+ (Figure 5D), indicating that 30 mM K^+ was sufficient for the formation of G-quadruplex.

We then inquired about the effect of solvent components on the formation of G-quadruplex. It should be noted that the DES used here contained ChCl (3.7 M) and urea (7.4 M). In water solution containing ChCl (3.7 M) and KCl (100 mM) or NaCl (100 mM), Tel_{22} adopted a hybrid structure and antiparallel

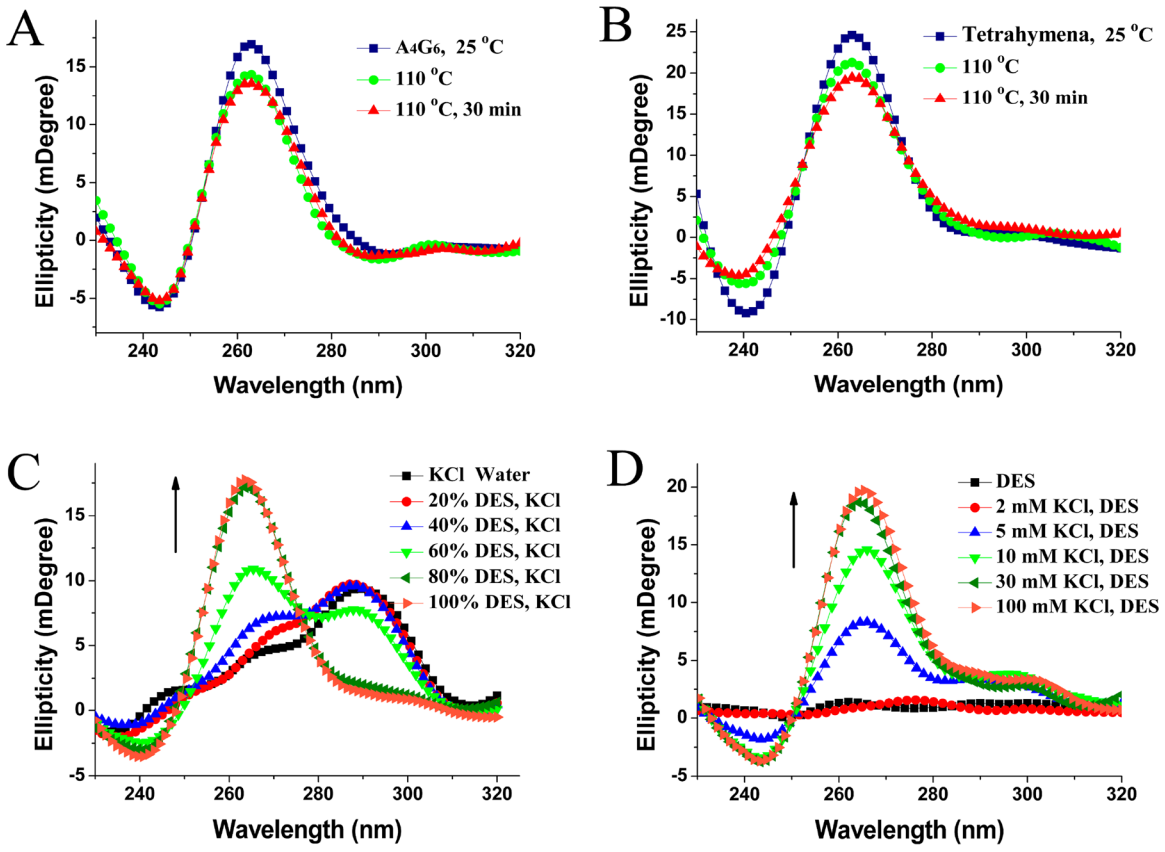

Figure 5. CD spectra of A_4G_6 G-quadruplex (A) and tetrahymena telomeric G-quadruplex (B) in K^+ DES at 25 and 110 °C. (C) CD spectra of human telomeric DNA (Tel_{22}) in KCl solution containing 0–100 wt % DES. (D) CD spectra of Tel_{22} in water-free DES containing 0–100 mM KCl.

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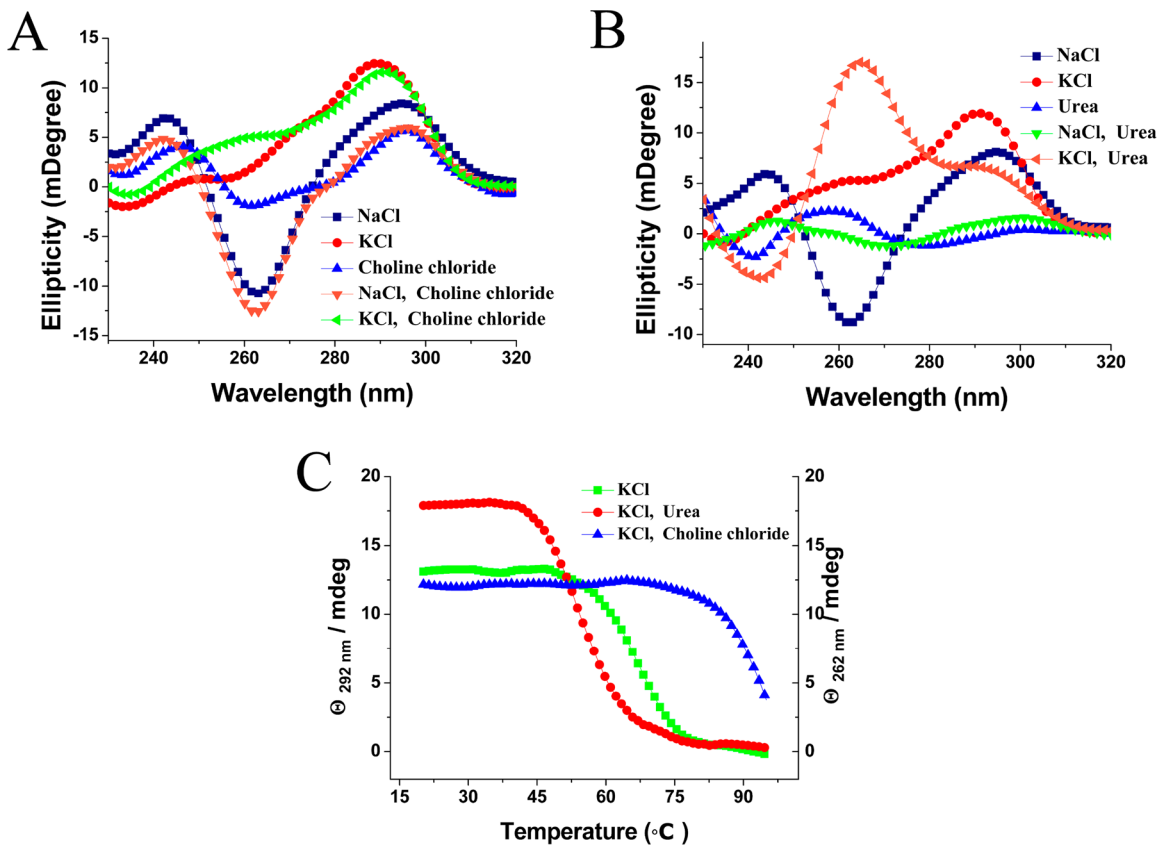
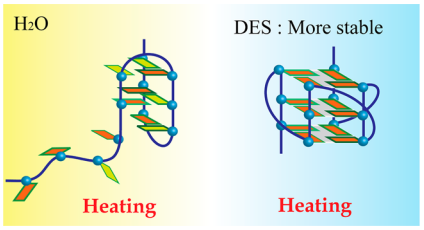


Figure 6. (A) CD spectra of Tel_{22} in different water solutions as indicated in the figure; NaCl and KCl were 100 mM, choline chloride was 3.7 M. (B) CD spectra of Tel_{22} in different water solutions; NaCl and KCl were 100 mM, urea was 7.4 M. (C) CD melting curves of Tel_{22} in different water solution, KCl was 100 mM, chloride was 3.7 M, urea was 7.4 M.

structure, respectively (Figure 6A). These structures of Tel_{22} were similar to that in KCl solution and in NaCl solution not containing ChCl, respectively, indicating that ChCl had little influence on the conformation of Tel_{22}. However, in water solution containing urea (7.4 M), the results were different. As shown in Figure 6B, in water solution containing urea or solution containing urea and NaCl, Tel_{22} could not form G-quadruplex structure, while in water solution containing urea and KCl, parallel G-quadruplex structure was formed. In addition, fluorescence experiments of 2-Ap substituted $G_3(T_2AG_3)_3$ further identified the parallel structure formation of Tel_{22} in solution containing urea and KCl (Figure S6). These results were identical with that obtained in water-free DES. Thus, we presumed that, in DES, compared to ChCl, urea might be a main factor to change the conformation of Tel_{22}.

The effect of the two solvent components of ChCl and urea on the stability of G-quadruplex was evaluated by CD melting assays (Figure 6C). The T_m value of Tel_{22} measured in solution containing ChCl and KCl was significantly higher than the T_m value observed in KCl solution, indicating that ChCl had a positive effect on the stability of G-quadruplex. In striking contrast, the stability of Tel_{22} in solution containing both urea and KCl was decreased significantly as compared to that in KCl solution, revealing that urea had a negative effect on the stability of G-quadruplex. This is not surprising because urea has been commonly used as DNA denaturant. Thus, in combination with the effects of ChCl and urea on the structure and stability of Tel_{22}, we presume that, in DES, urea plays an important role for unfolding hybrid G-quadruplex to form parallel structure, and ChCl favorably contributes to the stability of Tel_{22}. Actually, DES is a water-free system. Thus, the stability and structure of G-quadruplex in the DES cannot be attributed entirely to either of the solvent components. Instead, it may be the result of distinct solvent properties of the DES and the dehydration during G-quadruplex formation (Scheme 1).

Scheme 1. Human Telomeric DNA, Tel_{22}, Adopting a More Stable Parallel G-Quadruplex Structure in Water-Free DES (Containing K^+)



Thermodynamic Studies on the Formation of Parallel G-Quadruplex. To further understand the structural transition, thermodynamic parameters for the formation of parallel G-quadruplex in K^+ DES were estimated from its thermal melting curves (Figure S7).[55,56] In view of Tel_{22} adopting different conformations in water solution and in DES, ultraviolet (UV) melting assays were carried out by recording the absorbance at 295 nm, which was widely used for monitoring the denaturation of G-quadruplex.[37] Figure S8 indicated that the denaturation of G-quadruplex was a two-state

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transition between G-quadruplex structure and the single strand. The thermodynamic parameters for the formation of G-quadruplex were summarized in Table 3. When the DES concentration increased from 0 to 100 wt %, the values of ΔH^{o}, ΔS^{o}, and ΔG^{o}_{25} of the G-quadruplex decreased from −56.7 to −146.0 kJ mol^{-1}, −168.2 to −414.9 J mol^{-1}, and −7.4 to −24.5 kJ mol^{-1}, respectively. These changes indicate that the formation of parallel G-quadruplex promoted by DES was enhanced by a favorable enthalpic contribution that exceeds an unfavorable entropic contribution.

Table 3. Thermodynamic Parameters for the Human Telomeric (Tel_{22}) G-Quadruplex Formation in Different Content of DES Solution

DES (%)[a]	ΔH^{o} (kJ mol^{-1})	ΔS^{o} (J mol^{-1} K^{-1})	ΔG^{o}_{25} (kJ mol^{-1})
0	−56.7 ± 1.9	−168.2 ± 3.6	−7.4 ± 0.2
20%	−58.7 ± 0.9	−172.1 ± 4.5	−8.1 ± 0.3
80%	−100.9 ± 4.6	−286.9 ± 7.3	−17.1 ± 0.5
100%	−146.0 ± 5.2	−414.9 ± 8.9	−24.5 ± 0.7

[a]Thermodynamic parameters were calculated from the UV melting curves shown in Figure S7.

4. CONCLUSIONS

In summary, we systemically studied 10 representative G-quadruplexes in anhydrous DES, and found that intramolecular, intermolecular, and higher-order G-quadruplex structures could form in DES. In DES, parallel structure was their favored conformation. More importantly, compared to water solution, G-quadruplex showed ultrastability in DES. Some G-quadruplexes could survive beyond 110 °C. Since many chemical reactions and devices are required to be performed under strictly anhydrous conditions, even at high temperature, our work would provide new insights into application of G-quadruplex DNA in anhydrous environments, even at high temperature.

■ ASSOCIATED CONTENT

Supporting Information

DNA sequences and Figures S1−S8. This information is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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