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Relations between the loop transposition of DNA G-quadruplex and the catalytic function of DNAzyme

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ABSTRACT

The structures of DNA G-quadruplexes are essential for their functions *in vivo* and *in vitro*. Our present study revealed that sequential order of the three G-quadruplex loops, that is, loop transposition, could be a critical factor to determinate the G-quadruplex conformation and consequently improved the catalytic function of G-quadruplex based DNAzyme. In the presence of 100 mM K^+, loop transposition induced one of the G-quadruplex isomers which shared identical loops but differed in the sequential order of loops into a hybrid topology while the others into predominately parallel topologies. ^{1}D NMR spectroscopy and mutation analysis suggested that the hydrogen bonding from loops residues with nucleotides in flanking sequences may be responsible for the stabilization of the different conformations. A well-known DNAzyme consisting of G-quadruplex and hemin (Ferriprotoporphyrin IX chloride) was chosen to test the catalytic function. We found that the loop transposition could enhance the reaction rate obviously by increasing the hemin binding affinity to G-quadruplex. These findings disclose the relations between the loop transposition, G-quadruplex conformation and catalytic function of DNAzyme.

1. Introduction

DNA G-quadruplex structures are formed by repetitive guanine-rich sequences and stabilized by monovalent cations (such as, K^+). G-rich sequences that have the potential to form G-quadruplexes are widely distributed in genomes [1] and appear to be involved in gene regulation [2,3]. Due to the structural polymorphism and high thermal stability, G-quadruplexes are extensively used as scaffolds in DNAzymes [4–6], aptamers [7], biosensors [8,9] and therapeutic agents [10,11]. Unravelling the nature of the G-quadruplex folding is of fundamental and widespread importance.

There are two main components of G-quadruplex structure: the G-quartet cores and the loops. The G-quartet cores are composed of two or more stacked G-quartets with strands in parallel, antiparallel, or hybrid orientations [12,13]. Loops are the sequences between two adjacent G-tracts. G-quadruplex structures are influenced by the environmental factors such as cation type and concentration [14,15], dehydrating or crowding agent concentration [16,17], targeting ligand [18], and DNA concentration [19,20]. Besides the above external factors, G-quadruplex folding is determined by its primary sequence. G-quadruplex topology can be tuned by the length of G-tracts [21,22]. The flanking sequence can contribute to or limit conformational heterogeneity [23–28]. What's more, the influence of loop length on structural polymorphism and thermal stability has been extensively studied [29–35]. Moreover, Plavec and colleagues even designed novel G-quadruplex structure by optimizing the length of each loop individually [36,37].

Loop transposition denotes that two or more G-quadruplexes share identical loops, G-tracts and flanking sequences but only differ in the sequential orders of their three loops. As far as we known, there is no evidence to show that the loop transposition can induce two G-quadruplexes into different conformations. For example, Fox et al. reported that d[$(TG_3)_2T_4(G_3T)_2$] and d[$TG_3T_4(G_3T)_3$], which differed in the sequential orders of T and TTTT loops, adopted parallel topologies equally [38]. The two T loops constrained the G-quadruplexes into parallel topologies [29,30]. In addition, Mergny et al. reported that

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three terminal truncated sequences d[$(G_3T_2)_2G_3T_3G_3$], d[$G_3T_3(G_3T_2)_2G_3$] and d($G_3T_2G_3T_3G_3T_2G_3$), sharing two TT and one TTT loops, also folded into predominantly parallel topologies without structural difference [30]. However, we demonstrated for the first time that the G-quadruplex conformations could be switched by the loop transposition alone.

DNAzyme was chosen to test whether the loop transposition could alter the functions through structural control. G-quadruplex-based DNAzymes (or DNA enzymes) are drawing more and more attentions from both chemists and biologists [39,40]. The catalytic repertoire included porphyrin metallation [41], one-electron and two-electron (oxygen transfer) oxidation [42–46], photoreversion of thymine dimers [39,47] and Aldol reactions [48]. Even more, the chiral catalysis, for instance, carbon-carbon bond formation [49,50] and oxidation reactions [40], could be achieved by G-quadruplex based metalloenzymes.

The G-quadruplex/hemin catalyst is one of the most famous DNAzymes because of its versatility and accessibility in biological and chemical analyses [4]. It was accepted that parallel G-quadruplex promoted the peroxidase activity of hemin more efficient than that of non-parallel (hybrid or antiparallel) G-quadruplex [8,51–54]. Considering that loop transposition could alter the conformation of G-quadruplex, the relations between the loop transposition and catalytic activity of G-quadruplex/hemin DNAzyme were also investigated. Interestingly, loop transposition promoted the catalytic efficiency (k_{cat}/K_m) by increasing the hemin binding affinity to G-quadruplex.

2. Materials and methods

2.1. DNA samples

DNA oligonucleotides were purchased from Sangon Biotech, Co., Ltd. and purified by PAGE or HPLC. The concentration of each DNA sample was determined from the absorbance at 260 nm, and concentrations were determined using the extinction coefficients obtained from IDT website (http://www.idtdna.com/calc/analyzer). Samples were prepared in distilled and deionized H_2O (18.2 MΩ, Milli-Q A10). Sequences are given in the 5′ to 3′ direction in Table 1 in the main text. The samples were heated in the relevant solution for 3 min at 95 °C and annealed slowly over the course of 2 h to room temperature before spectroscopy measurements.

2.2. Other reagents

TBAP buffer (10 mM) was prepared by titration of monobasic tetrabutylammonium phosphate (98%, Alfa) to pH 7.0 with tetrabutylammonium hydroxide (40% wt% in water, Macklin). KCl (99%) and ethylenediamine tetraacetic acid (EDTA, 99.4%) were obtained from Alfa.

2.3. Circular dichroism (CD) spectroscopy

CD spectra were recorded on a dual beam DSM 1000 CD spectrophotometer (Olis, Inc.) equipped with Peltier temperature controller, using quartz cells of 10-mm path length. Each measurement was the average of ten scans recorded from 230 to 320 nm at 20 °C. The scanning rate was automatically selected by the Olis software as function of the signal intensity to optimize data collection. All DNA samples were prepared at 5 μM concentration in 10 mM TBAP buffer (pH 7.0) and 1 mM EDTA supplemented with various concentrations of KCl.

2.4. ^1H NMR spectroscopy

Samples were prepared by dissolving 450 μM DNA in 10 mM TBAP buffer (pH 7.0) in 90%/10% H_2O/D_2O supplemented with either 10 or 100 mM KCl. ^1H NMR experiments were performed on a 700 MHz Bruker Avance III HD spectrometer equipped with a cryogenic QCI probe at 20 °C. The WATERGATE pulse program was used in recording ^1H spectra.

Table 1
Sequences of DNA oligonucleotides used in this work and their conformations in the presence of 10 and 100 mM K^+ respectively.

Abbreviation[a]	Sequence (5′ → 3′)	Conformation (K^+/mM)[c]	
		10	100
H1 series			
H1-223	TT G_3 TT G_3 TT G_3 TTA G_3 A	P	NP
H1-232	TT G_3 TT G_3 TTA G_3 TT G_3 A	P	P
H1-322	TT G_3 TTA G_3 TT G_3 TT G_3 A	P	P
H2 series			
H2-223	TA G_3 TT G_3 TT G_3 TTA G_3 TT	P	P
H2-232	TA G_3 TT G_3 TTA G_3 TT G_3 TT	P	P
H2-322	TA G_3 TTA G_3 TT G_3 TT G_3 TT	P	NP
T to A mutation[b]			
H1-223T	TT G_3 TT G_3 TT G_3 TT<u>T</u> G_3 A	P	P
H1-232T	TT G_3 TT G_3 TT<u>T</u> G_3 TT G_3 A	P	P
H1-322T	TT G_3 TT<u>T</u> G_3 TT G_3 TT G_3 A	P	P
H0 series			
H0-223	G_3 TT G_3 TT G_3 TTA G_3	P	P
H0-232	G_3 TT G_3 TTA G_3 TT G_3	P	P
H0-322	G_3 TTA G_3 TT G_3 TT G_3	P	P

[a] Three consecutive numbers in the abbreviations refer to the lengths of the three loops respectively.
[b] The A base in the loop is replaced by a T.
[c] "NP" is short for non-parallel (including hybrid and antiparallel) conformation and "P" is short for parallel conformation. Parallel and non-parallel here refer to two distinct predominant conformations, which is differentiated by $\gamma = 0.5$ ($\gamma = CD_{265}/(CD_{265} + CD_{290})$; $\gamma > 0.5$, parallel G-quadruplex as predominant conformation; $\gamma < 0.5$, non-parallel G-quadruplex as predominant conformation), and the precise topology can't be characterized by CD spectrum.

2.5. UV melting experiment

UV melting experiments were carried out on a Shimadzu 2450 spectrophotometer equipped with a Peltier temperature control accessory in sealed quartz cells of 0.5-mL volume and 10-mm path length. DNAs were generally tested at 5 μM strand concentration in 10 mM TBAP (pH 7.0) supplemented with either 10 or 100 mM KCl. The melting curves were obtained at 295 nm with a temperature gradient of 0.5 °C/min [55].

2.6. Kinetics of DNAzyme catalyzing oxidative reactions

The experiments were carried out using a Shimadzu 2450 spectrophotometer equipped with a Peltier temperature controller at 20 °C using 3-mL quartz cells with 10-mm path length. Stock solutions containing hemin (10 mM in DMSO), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS, 90 mM in water), and H_2O_2 (1.0 or 3.0 M in H_2O) were stored at 4 °C. Hemin and Triton X-100 were added to DNA samples, and the solutions were incubated for at least 9 h at 20 °C. The reaction system was composed of 10 mM TBAP buffer (pH 7.0), 100 mM KCl, 250 nM DNA, 500 nM hemin, 0.05% (v/v) Triton X-100, and 0.01% (v/v) DMSO, and final volume was 2 mL. Initial reaction rates (v_{int}) were calculated from the slope of the initial linear portion of the curve (45 s) obtained by monitoring the absorbance at 414 nm as a function of time. Molar extinction coefficients (ε) of oxidation product $ABTS^{\cdot+}$ is 36,000 M^{-1} cm^{-1} at 414 nm [45]. All experiments were performed in triplicate.

2.7. UV–visible spectroscopy and titration

Stock solution of hemin (1.0 mM in DMSO) was stored at 4 °C. The