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High-Activity DNAzymes

The Effect of Adenine Repeats on G-quadruplex/hemin Peroxidase Mimicking DNAzyme Activity

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Abstract: The catalytic activity of G-quadruplex/hemin is much lower than that of proteinous enzymes, so it is very important to increase its activity. Very recently, flanking sequences, which can be regarded as an external part of G-quadruplexes, were found to enhance the activity of G-quadruplex/hemin DNAzyme. However, little is known about the effect of internal parts, such as loop sequences and linkers, on the activity. In the present study, adenine repeats were incorporated into several designed G-quadruplex structures either in the loops, bulges, or linkers, and the constructed G-quadruplex/hemin DNAzyme exhibit about five-fold improvement in peroxidase-mimicking activity in some cases. The enhancement effect may result from the formation of compound I, protoporphyrin·Fe^{IV}═O$^{\bullet+}$, accelerated by dA repeats, which was demonstrated by H_2O_2 decay kinetics and pH dependency analysis. The novel enhancement methods described here may help in the development of high-activity DNAzymes, illustrated by a dimer G-quadruplex with flanking adenine at one end, a relatively long adenine run in one loop, and another adenine run in the linker.

Introduction

Since the discovery in the late 1990s that G-quadruplex/hemin complexes have peroxidase-mimicking DNAzyme activity,[1,2] their potential for practical applications, such as catalysts and signal amplification for biosensing, have been extensively exploited. G-quadruplexes are tertiary structures formed by guanine-rich DNA sequences, which are composed of two or more stacked G-quartet cores bridged by loop sequences (Figure 1). Hemin is an anionic porphyrin, Fe^{III} protoporphyrin IX. G-quadruplex/hemin complexes can catalyze the oxidation of substrates (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 3,3′,5,5′-tetramethylbenzidine (TMB), or luminol) in the presence of H_2O_2, and the subsequent color change (ABTS, $\lambda_{max}=415$ nm when oxidized) has been used in colorimetric and/or chemiluminescent detection of various targets. Compared with proteinous enzymes, G-quadruplex/hemin DNAzymes have many advantages. For example, it has traits of highly selective, easy synthesis and excellent biocompatibility. Furthermore, it is a simple and sensitive biosensor and molecular machine that is suitable for the detection of multiple targets such as metal ions,[3] small molecules,[4] and biomolecules (DNA and proteins),[5,6] which were described in recent reviews.[7–12]

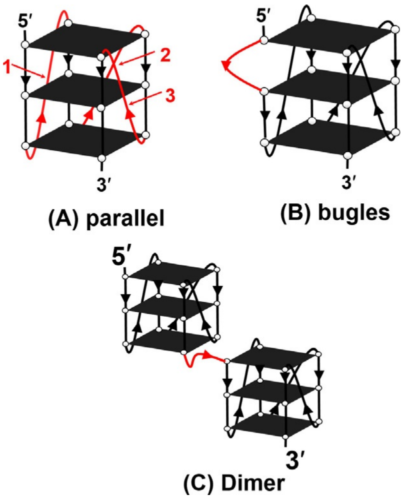

Figure 1. Schematic representation of G-quadruplex structures investigated in the study. A) parallel G-quadruplex (Arabic numerals indicate the loop position); B) parallel G-quadruplex with bulges, and C) dimer parallel G-quadruplex. The arrows indicate the DNA 5′- to 3′-polarity. The black quadrilaterals represent G-quartets and red lines indicate adenine repeats that merged into the G-quadruplex structures.

To enhance the performance and activity of peroxidase-mimicking DNAzymes, different strategies have been proposed. Small molecule additives (ATP or spermine),[13–15] may be added to the G-quadruplex/hemin system; alternatively, covalent conjugation of a cationic peptide[16] or hemin to G-quadruplex,[17] or chemical modification of G-quadruplexes[18] have been used. Recently, flanking sequences such as d(CCC)[19] and dA,[20] in intramolecular parallel G-quadruplexes, were found to enhance the activity of G-quadruplex/hemin DNAzymes. However, the

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role of the loops on DNAzyme activity remains unclear. Furthermore, it is well established that multimeric quadruplexes, called “beads-on-the-string”[21] or “pearl-necklace” structures,[22] can be formed by successive G-quadruplex units connected by the nucleotide(s) linkers, and the effect of linkers on DNAzyme has not been investigated.

Investigations on the relationship between the G-quadruplex structures and the peroxidase-like activity reveal that loops on the sides of the G-quadruplex structures allow hemin end-stacking to G-quartets in parallel form, but the loops over the top and bottom G-quartets inhibit end-stacking in the nonparallel (antiparallel and/or hybrid) forms, with the former exhibiting high and the latter low peroxidase activity.[23–26] Here, by using ABTS as the model substrate, we demonstrate that adenine repeats in the loops of intramolecular parallel G-quadruplex and the linkers between dimer parallel G-quadruplex enhance the activity of G-quadruplex/hemin DNAzyme and we show that the degree of enhancement depends on the length of the adenine repeats. In contrast, when incorporated in the bulges[27] of intramolecular parallel G-quadruplex, adenines did not display the activity enhancement.

Results and Discussion

To investigate the role played by adenine repeats in peroxidase-mimicking DNAzyme, several G-quadruplex structures were designed (Figure 1); their sequences are listed in Table 1. As shown in Figure 1, adenine or adenine repeats (shown in red) were incorporated into parallel G-quadruplex (**I**), parallel G-quadruplex with bulges (**II**), and dimer parallel G-quadruplex (**III**) as loops, bulges, and linkers, respectively.

First, the effect of A, T, or C repeats in the loops of a parallel G-quadruplex formed by GGGTGGGX_nGGGTGGG (X = A, T or C, and n represents the number of nucleotides, sequences shown in Series I, Table 1) on G-quadruplex/hemin DNAzyme activity was explored. The parallel G-quadruplex was chosen to avoid steric hindrance by the loops, which prevent hemin binding to the G-quartet. To confirm the structures of these sequences formed, circular dichroism (CD) spectra were collected. As shown in Figure S1 in the Supporting Information, the CD spectra of all sequences exhibit main positive and negative peaks around 260 and 240 nm, respectively, which is indicative of the formation of parallel G-quadruplexes, as designed.[28,29] Exceptions are the **C9–L2** and **C12–L2** sequences with nine and twelve cytosines, respectively, which exhibit a shoulder peak around 280 nm, suggesting a certain amount of duplex formed by guanines and cytosines (Figure S1 B). Duplex formation between G-C rich regions is not unexpected given the stability of the G-C base-pair, as demonstrated previously,[30,31] even under high concentration of K$^+$ (100 mM used here). We do not describe in detail the structures formed by these two sequences, which is beyond the scope of the present study. In short, the CD results support previous reports concluding that two short loops (one or two nucleotides, one nt used herein) favor parallel G-quadruplex structures,[32,33] even if the third loop is much longer.[34,35]

Table 1. The oligonucleotides examined in this work.

Name	Sequence (5′–3′)
Parent sequence[a]	
G3T	GGGTGGGTGGGTGGG
(I) parallel G-quadruplex with different loops (n = 1, 2, 3, 4, 5, 6, 9, 12)	
The second loop (L2)	
An-L2	GGGTGGGA$_n$GGGTGGG
Tn-L2	GGGTGGGT$_n$GGGTGGG
Cn-L2	GGGTGGGC$_n$GGGTGGG
The first loop (L1)	
An-L1	GGGA$_n$GGGTGGGTGGG
Tn-L1	GGGT$_n$GGGTGGGTGGG
Cn-L1	GGGC$_n$GGGTGGGTGGG
The third loop (L3)	
An-L3	GGGTGGGTGGGA$_n$GGG
Tn-L3	GGGTGGGTGGGT$_n$GGG
Cn-L3	GGGTGGGTGGGC$_n$GGG
(II) parallel G-quadruplex with bulges (n = 2, 5)	
An-B1	GA$_n$GGTGGGTGGGTGGG
An-B3	GGGTGA$_n$GGTGGGTGGG
An-B5	GGGTGGGTGA$_n$GGTGGG
An-B7	GGGTGGGTGGGTGA$_n$GG
(III) dimer G-quadruplex (n = 1, 3, 5, 7, 9, 11, 13, for T and C, only 1, 3, 5)	
D-G3T-An	GGGTGGGTGGGTGGGA$_n$GGGTGGGTGGGTGGG
D-G3T-Cn	GGGTGGGTGGGTGGGC$_n$GGGTGGGTGGGTGGG
D-G3T-Tn	GGGTGGGTGGGTGGGT$_n$GGGTGGGTGGGTGGG
(IV) high activity DNAzyme	
D-G3T-A7	GGGTGGGTGGGTGGGA7GGGTGGGTGGGTGGG
A-D-G3T-A7	AGGGTGGGTGGGTGGGA7GGGTGGGTGGGTGGGA
A-D-G3T-A7-L4	AGGGTGGGA4GGGTGGGA7GGGTGGGA4GGGTGGGA
A-D-G3T-A7-L7	AGGGTGGGA7GGGTGGGA7GGGTGGGA7GGGTGGGA

[a] It should be noted that the sequence **G3T** can also be named **T1–L1**, **T1–L2**, **T1–L3** in other sets. To keep it consistent in the paper, we use **G3T** throughout the text.

We then measured the G-quadruplex/hemin DNAzyme activity of all sequences with dA/adenine repeats, dC/cytosine repeats, and dT/thymine repeats in the second loop, expressed in initial velocity (V_0, nM s^{-1}).[36] Structures are schematically represented in Figure 1 A and the activity results are depicted in Figure 2. As shown in Figure 2, the peroxidase-mimicking activity was enhanced by adenine repeats and depended on the

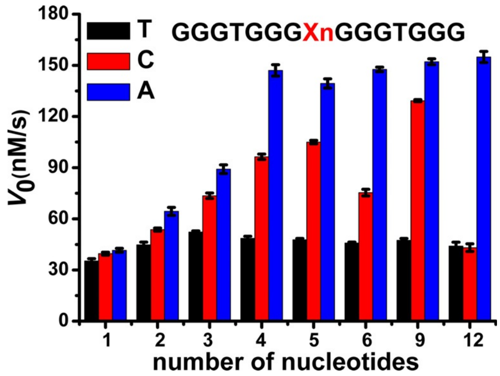

Figure 2. DNAzyme activity of intramolecular parallel G-quadruplexes with different loop compositions (black: dT/dT repeats, red: dC/dC repeats, and blue: dA/dA repeats) in the second loop. Experiments were carried out in 10 mM Tris-HCl buffer (pH 7.0) at 25 °C with the following concentrations: DNA (0.4 μM), H_2O_2 (0.6 mM), ABTS (0.6 mM), and hemin (0.8 μM).

number of dA nucleotides. For example, the initial velocity was improved gradually from 41.6 to 147.0 nM s^{-1} when the number of adenine bases was increased from one to four. Moreover, compared with the **G3T** parent sequence (one dT in the loop; initial velocity of 35.3 nM s^{-1}), a 4.2× activation was observed with a four-adenine loop; addition of more dA residues did not result in further increases. To our knowledge, this is the first report to note that the loop composition of the G-quadruplex can enhance G-quadruplex/hemin DNAzyme activity.

Similar to adenine repeats, cytosines also exhibit activation effect, but to a lesser extent. Notably, cytosines can interact with guanines to form a duplex by Watson–Crick base pair, based on previous CD results (Figure S1 B). It is well known that hemin bound to G-quadruplexes has peroxidase-like activity, whereas single-stranded and double-stranded DNA sequences have not. The potential reason is that π–π interaction between G-quartets and hemin overcomes the electrostatic repulsion between the carboxyl groups of hemin and the phosphate groups, which result in the specific binding between G-quartets and hemin.[37] Therefore, the activity decrease observed in **C12–L2** may result from duplex formation between cytosines and guanines. However, unlike dA and dC, dT repeats in the second loop of the parallel G-quadruplex did not show prominent activity enhancement, even though the number of thymines varied from one to twelve. These results demonstrate that, when forming a complex with hemin, parallel G-quadruplex with dA (and to a lesser extent dC) repeats in the second loop can catalyze peroxidatic reactions to a significant level. Therefore, we focused our attention on adenine repeats.

The loop position dependency of dA, dC, and dT runs on G-quadruplex/hemin peroxidase-mimicking DNAzyme was then investigated. As shown in Figure S2 (see the Supporting Information), the guanine-rich sequences with dA, dC, and dT repeats loop in either the first or the third positions (schematically presented in Figure 1 A), formed parallel G-quadruplex structures, as confirmed by CD spectra. Interestingly, they showed similar enhancement effect in the second position as described above (Figure S3), indicating that the dA-dependent DNAzyme activity enhancement is independent of loop position.

G-quadruplex can be formed with bulges, as recently demonstrated by Phan et al.[27] Parallel G-quadruplex and parallel G-quadruplex structures with bulges have similar topology (parallel), but the latter has an additional bulge (Figure 1 A and B). The bulges can be regarded as "extra loops" and the analogues of propeller-type loops in parallel G-quadruplexes. Inspired by this new type of topology, several G-quadruplex structures containing bulges with two or five adenines were designed (Figure 1 B). The CD spectra of these sequences (sequence information is shown in Series II, Table 1), demonstrated that they still formed parallel G-quadruplex structures, although one G-tract was interrupted by dA repeats (Figure S4). These results are in agreement with data obtained previously, which used dT/dT repeats as bulges in the G-quadruplexes.[27] As described above, whereas adenine runs in the loop of intramolecular parallel G-quadruplex exhibited notable DNAzyme activity enhancement, they did not exhibit an enhancement when incorporated in the bulges (Figure S5). When these bulges were incorporated at various positions in the G-quadruplexes, no enhancement in activity was observed (Figure S5). Moreover, similar experiments replacing dA runs by dT or dC sequences as the bulges in parallel G-quadruplex did not exhibit DNAzyme activity enhancement (results not shown).

Remarkably, multiple G-quadruplex structures can be formed by contiguous G-quadruplex units with linkers.[21,22,38] Interestingly, DNAzymes that are complexed by multiple G-quadruplexes and hemin exhibit higher peroxidase-mimicking catalytic activity than single G-quadruplex and hemin systems.[39] A detailed investigation by Monchaud et al. revealed that the junction region between two quadruplex units constitutes the high activity center because it is a hydrophobic hemin-binding pocket, leading to better DNAzyme results.[39] However, the role of linkers between/among multiple G-quadruplex on DNAzyme activity was not investigated before. Enlightened by our results on dA repeats activation in the loops of parallel G-quadruplex, we studied dA, dT, or dC runs as the *linkers* of dimeric G-quadruplexes, formed by <u>GGGTGGGTGGGTGGG</u>X_n<u>GGGTGGGTGGGTGGG</u> (X = A, T or C, and n represents the number of nucleotides, sequences shown in Series III, Table 1), which consist of two intramolecular parallel G-quadruplexes (underlined parts in the sequences) (Figure 1 C). The dimeric G-quadruplex formation of these sequences in the presence of 100 mM K$^+$ were confirmed by CD (Figure S6).

To compare the activity of double and single G-quadruplexes, we chose to keep G-quadruplex units at the same concentration. For example, for the single **G3T** G-quadruplex formed by GGGTGGGTGGGTGGG, its concentration is twice as high (shown by 2× in Figure 3) as for a dimeric G-quadruplex. As shown in Figure 3, when the linker was composed of dT/dT repeats, an actual inhibitory effect was observed, whereas dC/dC repeats exhibited slight inhibitory or activating effects, and dA/dA runs showed enhancement effect. For example, in contrast

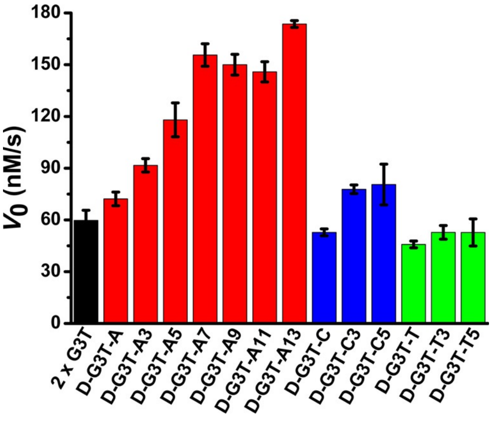

Figure 3. DNAzyme activity of parallel double G-quadruplexes with different linkers (black: control; red: dA/dA repeats; blue: dC/dC repeats; and green: dT/dT repeats). The experiments were carried out in 10 mM Tris-HCl buffer (pH 7.0) at 25 °C with a DNA concentration of 0.4 μM, H_2O_2 (0.6 mM), ABTS (0.6 mM), and hemin (0.8 μM). It should be noted that the G-quadruplex units are at the same concentrations, so single G-quadruplex was two times (0.8 μM) higher, see text for details.

to the enhancement of DNAzyme activity by a linker composed of five adenines (118.1 nM s^{-1}), thymines and cytosines exhibit slightly detrimental (52.8 nM s^{-1}) or indistinct activation (80.6 nM s^{-1}) effects, respectively, compared to 2×**G3T** (62.5 nM s^{-1}). We further investigated the length effect of adenine linkers on DNAzymes. Interestingly, the DNAzyme activity was further enhanced when the number of adenines was increased from five to thirteen (118.1 to 173.6 nM s^{-1}), with initial velocities around 150 nM s^{-1} for A7, A9, A11, and A13 sequences.

It should be noted that each individual G-quadruplex unit adopts a parallel topology in dimeric forms. Very recently, it has been shown that adenines in the 3′-terminal end of parallel G-quadruplex structures enhance the G-quadruplex/hemin DNAzyme activity.[20] We have checked this in our designed parallel G-quadruplex formed by **G3T**, and observed similar adenine enhancement of DNAzyme activity (Figure S7, Table S1, details seen in the Supporting Information). However, Li et al. found that the activation effect was not affected by the length of the flanking adenines, whereas we found a length-dependent effect (Figure 3).

Compared with hemoproteins, the catalytic activity of G-quadruplex/hemin complexes is low.[10] Therefore, it is desirable to design high-activity DNAzyme. Based on the sequences described here, we constructed high-performance G-quadruplex/hemin DNAzymes with flanking adenines at one end,[20] a relatively long adenine run in one loop, and another adenine run in the linker, when studying double G-quadruplexes. The formation of these sequences in the G-quadruplexes were confirmed by CD (Figure S8). As shown in Figure 4, the activity of the designed DNAzyme with flanking dA and seven adenines linker (A-D-G3T-A7, 294.5 nM s^{-1}), was nearly five times higher than single G-quadruplex **G3T** (62.5 nM s^{-1}, the concentration of single G-quadruplex is twice that of the dimeric G-quadruplex). It should be noted here that the number of linker adenines was chosen as seven because a plateau was observed in the double system (Figure 3). Interestingly, when further adenine repeats were incorporated into the loop of the parallel G-quadruplex unit (A-D-G3T-A7-L4 and A-D-G3T-A7-L7), the activity was further enhanced.

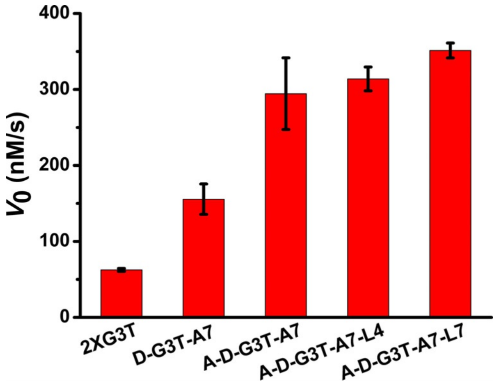

Figure 4. Constructed high-activity DNAzyme with flanking dA, dA repeat linker or/and loop (sequence information shown in Table 1). The experiments were carried out with DNA (0.4 μM; total G-quadruplex concentration 0.8 μM), H_2O_2 (0.6 mM), ABTS (0.6 mM), and hemin (0.8 μM) in 10 mM Tris-HCl buffer (pH 7.0) at 25 °C.

The reason why dA runs activate DNAzymes remains to be determined. Here, conventional analyses such as structural alternation, thermal stability, and hemin-binding affinity failed to provide information. For example, according to CD spectra, dA runs insertion into the second loops in parallel G-quadruplex did not alter the conformation of the parent sequence **G3T** (Figure S1A). Moreover, we also measured the number of hemin binding to the G-quadruplex, and found no difference among these sequences (all ca. 1:1 ratio; Figure S9 and Table S2 in the Supporting Information). To explore the mechanism of activation by adenine runs, we monitored the decay kinetics of the G-quadruplex/hemin DNAzyme by recording absorbance at 404 nm in the presence of H_2O_2 because previous reports indicated that the hemin-activation step to form compound I is rate-determining (Scheme S1 in the Supporting Information).[14,20,40] As shown in Figure 5 A, all DNAzymes displayed hypochromicity effect at 404 nm upon addition of H_2O_2, suggesting the fast degradation of G-quadruplex/hemin complexes by H_2O_2. We noted that the decay rate of **G3T** is very similar to that of **A1–L2** (one adenine in the second loop), whereas it is remarkably slower than that of **A2–L2**, **A3–L2**, and **A4–L2** to **A12–L2** (more than two adenines in the second loop). Interestingly, the decay rates were constant when the number of adenines reached four. These results are in agreement with data on peroxidase activities of these DNAzymes (Figure 2), suggesting that higher degradation efficiency is correlated with higher peroxidase activities for these DNAzymes. Moreover, the degradation rates of dimer series were tested and similar results were obtained (Figure 5 B), implying that the activity enhancement found with adenine runs may result from the accelerated formation of compound I proph·$Fe^{IV}=O^{\bullet +}$ (Scheme S1). Furthermore, we also evaluated the influence of pH on the catalytic efficiency of G-quadruplex/hemin DNAzymes in Tris-HCl buffer,[41] and notable differences were observed (Figure 5 C and D). For instance, sequences **G3T** and **A1–L2** exhibited a similar behavior in that their activity increased when the pH was over 6.5, whereas **A4–L2** showed two parts, one sharply increased region between pH 3.5 and pH 6, and the second gradually over pH 6. The same results were obtained in phosphate solution (Figure S10), demonstrating that adenine runs play an important role in activity enhancement because the pK_a of the adenine is 4.2.[42] These results are in agreement with a recent work on flanking adenine(s) in intramolecular parallel G-quadruplex;[20] however, an adenine in the loop did not show any difference with a thymine in activity, but when the number of adenine units increased to four, clear activity enhancement was observed (Figure 5 C). Similar results were found for dimer G-quadruplexes (Figure 5 D).

In this study, we developed a high-performance peroxidase-mimicking DNAzyme for ABTS catalyze oxidation. To establish what role the dA repeats play in G-quadruplex/hemin DNAzyme, we have combined UV/Vis spectroscopy, CD, and reaction kinetics assays. Finally, we found that when dA repeats serve as loop sequences and linkers in parallel G-quadruplex

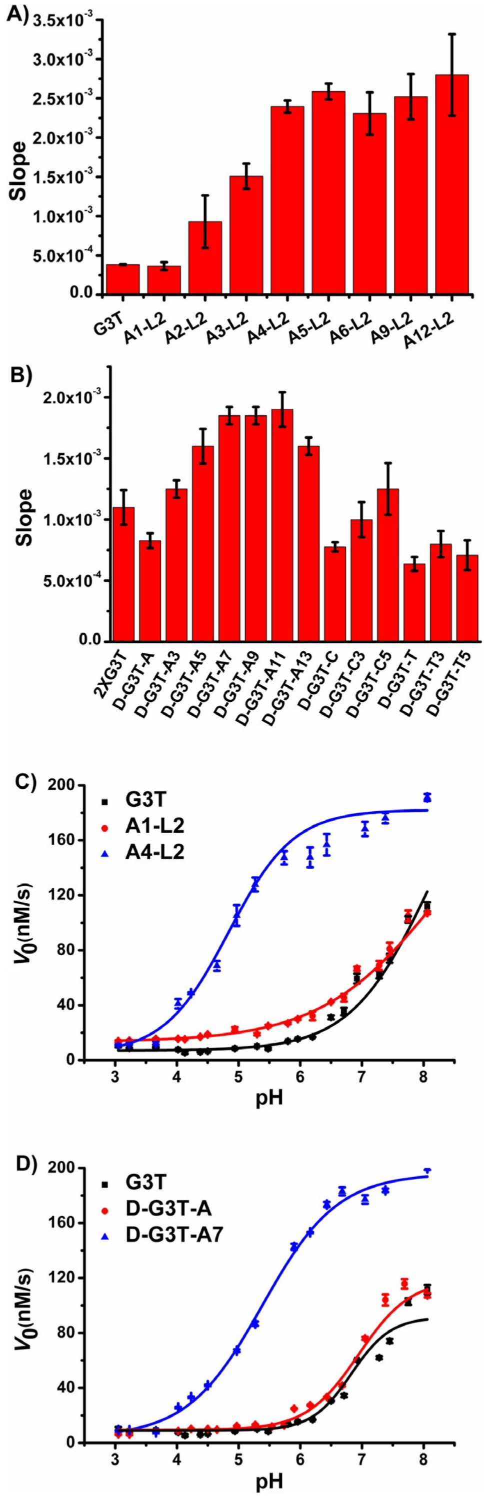

Figure 5. A, B) Decay kinetics of the absorbance of the DNAzymes at 404 nm in the presence of H_2O_2. A) G-quadruplex with dA repeats in the second loop; B) dimer G-quadruplex with adenine runs as linkers. C, D) Plots of the initial peroxidation rates of DNAzyme that have dA runs in the loop (C) or in the linker (D) as a function of pH values in Tris-HCl buffer. The experiments carried out with DNA (0.4 μM), H_2O_2 (0.6 mM), ABTS (0.6 mM), and hemin (0.8 μM) in Tris-HCl buffer (pH 7.0) at 25 °C.

structures, the dA repeats dramatically enhance the catalyzed oxidation activity by accelerating the formation of compound I. In this way, we can construct a novel DNAzyme without any requirement for exogenous species. Moreover, the G-quadruplex/hemin DNAzyme system constitutes a powerful toolkit for use in biosensing and biomolecular devices. Inspired by this, we can design some novel and rational sensors that combine the allosteric control of G-quadruplex and its property of chemiluminescence[43] and chemiluminescence resonance energy transfer.[44] Furthermore, there are a series of signal producers that have been developed with G-quadruplex/hemin DNAzymes, such as electrocatalyzed reduction of H_2O_2,[45] luminol,[46] NADH,[47] and dopamine.[48] The DNAzyme developed here facilitates the construction of systems that can generate intense signals for other chemical processes, and further studies of the aforementioned applications for G-quadruplex/hemin with dA repeats are being undertaken in our laboratory.

Conclusion

The results presented here show that dA repeats serve as loop sequences and that linkers in parallel G-quadruplex structures enhance the peroxidase-mimicking DNAzyme activity. Interestingly, the loops exhibit a position-independent but length-dependent effect. The enhancement effect may result from increased rate of formation of compound I. Furthermore, the novel enhancement method found here may help in the development of high-activity DNAzymes, which broaden the potential application of such systems in biosensing. The dA repeats present in the G-quadruplexes could be designed as addressable anchors for functional devices based on G-quadruplex/hemin peroxidase-mimicking DNAzyme activity.

Experimental Section

Materials and methods

PAGE purified oligonucleotides were purchased from Sangon (Shanghai, China) and used without further purification. Concentrations were determined by ultraviolet (UV) absorption at 260 nm by using the extinction coefficients obtained with an OligoAnalyzer 3.1 (http://sg.idtdna.com/calc/analyzer). All the chemicals used were purchased from Sigma unless otherwise stated.

DNA sample preparation

The DNA samples were heated to 95 °C for 5 min, cooled slowly to RT and then stored at 4 °C overnight or longer before use unless otherwise stated.

Instrumentation

UV-absorbance measurements were performed with a Cary 100 UV/Vis spectrophotometer (Agilent Technologies, Australia) or UV 2450 (Shimadzu, Japan) equipped with Peltier temperature control accessories. Circular dichroism (CD) spectra were recorded with a Chirascan equipped with a Peltier temperature control accessory (Applied Photophysics, U.K.). All experiments were carried out at 25 °C unless otherwise stated.

Peroxidase activity measurements

All of the experiments, except those addressing pH function, were performed in 10 mм Tris-HCl (pH 7.0) containing 0.05 % Triton X-100, 0.1 % DMSO, and 100 mм K^+. For valid comparison of the peroxidatic activities, the initial velocity (V_0, expressed in nм s^{-1}) was used. The definition of initial velocity is the concentration of $ABTS^{\bullet+}$ synthesized at the very beginning (30 s used in the present study) of the reaction as a function of time.[36] It should be noted that we keep the same buffer composition in pH dependency experiments to avoid its effect on DNAzyme activity.[2]

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Keywords: DNA structures · DNAzyme · peroxides · pi interactions · porphyrinoids

[1] P. Travascio, Y. F. Li, D. Sen, *Chem. Biol.* **1998**, *5*, 505 – 517.
[2] P. Travascio, A. J. Bennet, D. Y. Wang, D. Sen, *Chem. Biol.* **1999**, *6*, 779 – 787.
[3] Y. Tian, L. Zhang, J. Shen, L. Wu, H. He, D. L. Ma, C. H. Leung, W. Wu, Q. Fan, W. Huang, L. Wang, *Small* **2016**, *12*, 2913 – 2920.
[4] D. Li, B. Shlyahovsky, J. Elbaz, I. Willner, *J. Am. Chem. Soc.* **2007**, *129*, 5804 – 5805.
[5] M. Wang, Z. Mao, T. S. Kang, C. Y. Wong, J. L. Mergny, C. H. Leung, D. L. Ma, *Chem. Sci.* **2016**, *7*, 2516 – 2523.
[6] S. Lin, T. S. Kang, L. Lu, W. Wang, D. L. Ma, C. H. Leung, *Biosens. Bioelectron.* **2016**, *86*, 849 – 857.
[7] I. Willner, B. Shlyahovsky, M. Zayats, B. Willner, *Chem. Soc. Rev.* **2008**, *37*, 1153 – 1165.
[8] J. W. Liu, Z. H. Cao, Y. Lu, *Chem. Rev.* **2009**, *109*, 1948 – 1998.
[9] J. Kosman, B. Juskowiak, *Anal. Chim. Acta* **2011**, *707*, 7 – 17.
[10] D. Sen, L. C. H. Poon, *Crit. Rev. Biochem. Mol.* **2011**, *46*, 478 – 492.
[11] H. Xu, Q. Li, L. Wang, Y. He, J. Shi, B. Tang, C. Fan, *Chem. Soc. Rev.* **2014**, *43*, 2650 – 2661.
[12] S. Zhan, Y. Wu, L. Wang, X. Zhan, P. Zhou, *Biosens. Bioelectron.* **2016**, *86*, 353 – 368.
[13] D. M. Kong, J. Xu, H. X. Shen, *Anal. Chem.* **2010**, *82*, 6148 – 6153.
[14] L. Stefan, F. Denat, D. Monchaud, *Nucleic Acids Res.* **2012**, *40*, 8759 – 8772.
[15] C. Qi, N. Zhang, J. Yan, X. Liu, T. Bing, H. Mei, D. Shangguan, *RSC Adv.* **2014**, *4*, 1441 – 1448.
[16] L. Xiao, Z. Zhou, M. Feng, A. Tong, Y. Xiang, *Bioconjugate Chem.* **2016**, *27*, 621 – 627.
[17] Z. Wang, J. Zhao, J. Bao, Z. Dai, *ACS Appl. Mater. Interfaces* **2016**, *8*, 827 – 833.
[18] C. Li, L. Zhu, Z. Zhu, H. Fu, G. Jenkins, C. Wang, Y. Zou, X. Lu, C. J. Yang, *Chem. Commun.* **2012**, *48*, 8347 – 8349.
[19] T. Chang, H. Gong, P. Ding, X. Liu, W. Li, T. Bing, Z. Cao, D. Shangguan, *Chem. Eur. J.* **2016**, *22*, 4015 – 4021.
[20] W. Li, Y. Li, Z. L. Liu, B. Lin, H. B. Yi, F. Xu, Z. Nie, S. Z. Yao, *Nucleic Acids Res.* **2016**, *44*, 7373 – 7384.
[21] H. Q. Yu, D. Miyoshi, N. Sugimoto, *J. Am. Chem. Soc.* **2006**, *128*, 15461 – 15468.
[22] S. Amrane, M. Adrian, B. Heddi, A. Serero, A. Nicolas, J. L. Mergny, A. T. Phan, *J. Am. Chem. Soc.* **2012**, *134*, 5807 – 5816.
[23] D. M. Kong, L. L. Cai, J. H. Guo, J. Wu, H. X. Shen, *Biopolymers* **2009**, *91*, 331 – 339.
[24] X. H. Cheng, X. J. Liu, T. Bing, Z. H. Cao, D. H. Shangguan, *Biochemistry* **2009**, *48*, 7817 – 7823.
[25] D. M. Kong, W. Yang, J. Wu, C. X. Li, H. X. Shen, *Analyst* **2010**, *135*, 321 – 326.
[26] S. Nakayama, J. X. Wang, H. O. Sintim, *Chem. Eur. J.* **2011**, *17*, 5691 – 5698.
[27] V. T. Mukundan, A. T. Phan, *J. Am. Chem. Soc.* **2013**, *135*, 5017 – 5028.
[28] J. Kypr, I. Kejnovska, D. Renciuk, M. Vorlickova, *Nucleic Acids Res.* **2009**, *37*, 1713 – 1725.
[29] M. Vorlickova, I. Kejnovska, J. Sagi, D. Renciuk, K. Bednarova, J. Motlova, J. Kypr, *Methods* **2012**, *57*, 64 – 75.
[30] A. T. Phan, J. L. Mergny, *Nucleic Acids Res.* **2002**, *30*, 4618 – 4625.
[31] J. Zhou, S. Amrane, D. N. Korkut, A. Bourdoncle, H. Z. He, D. Ma, J. L. Mergny, *Angew. Chem. Int. Ed.* **2013**, *52*, 7742 – 7746; *Angew. Chem.* **2013**, *125*, 7896 – 7900.
[32] N. Smargiasso, F. Rosu, W. Hsia, P. Colson, E. S. Baker, M. T. Bowers, E. D. Pauw, V. Gabelica, *J. Am. Chem. Soc.* **2008**, *130*, 10208 – 10216.
[33] N. Q. Do, A. T. Phan, *Chem. Eur. J.* **2012**, *18*, 14752 – 14759.
[34] A. Guédin, J. Gros, P. Alberti, J. L. Mergny, *Nucleic Acids Res.* **2010**, *38*, 7858 – 7868.
[35] P. Agrawal, C. Lin, R. I. Mathad, M. Carver, D. Yang, *J. Am. Chem. Soc.* **2014**, *136*, 1750 – 1753.
[36] L. Stefan, H. J. Xu, C. P. Gros, F. Denat, D. Monchaud, *Chem. Eur. J.* **2011**, *17*, 10857 – 10862.
[37] R. T. Wheelhouse, D. K. Sun, H. Y. Han, F. X. G. Han, L. H. Hurley, *J. Am. Chem. Soc.* **1998**, *120*, 3261 – 3262.
[38] L. Petraccone, *Top. Curr. Chem.* **2012**, *330*, 23 – 46.
[39] L. Stefan, F. Denat, D. Monchaud, *J. Am. Chem. Soc.* **2011**, *133*, 20405 – 20415.
[40] T. Li, S. Dong, E. Wang, *Chem. Asian J.* **2009**, *4*, 918 – 922.
[41] P. Travascio, D. Sen, A. J. Bennet, *Can. J. Chem. Eng.* **2006**, *84*, 613 – 619.
[42] T. A. Evans, K. R. Seddon, *Chem. Commun.* **1997**, 2023 – 2024.
[43] V. Pavlov, Y. Xiao, R. Gill, A. Dishon, M. Kotler, I. Willner, *Anal. Chem.* **2004**, *76*, 2152 – 2156.
[44] R. Freeman, X. Liu, I. Willner, *J. Am. Chem. Soc.* **2011**, *133*, 11597 – 11604.
[45] G. Pelossof, R. T. Vered, J. Elbaz, I. Willner, *Anal. Chem.* **2010**, *82*, 4396 – 4402.
[46] Y. Xiao, V, Pavlov, R. Gill, T. Bourenko, I. Willner, *ChemBioChem* **2004**, *5*, 374 – 379.
[47] E. Golub, R. Freeman, I. Willner, *Angew. Chem. Int. Ed.* **2011**, *50*, 11710 – 11714; *Angew. Chem.* **2011**, *123*, 11914 – 11918.
[48] E. Golub, H. B. Albada, W. C. Liao, Y. Biniuri, I. Willner, *J. Am. Chem. Soc.* **2016**, *138*, 164 – 172.

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