Peroxidase activity–structure relationship of the intermolecular four-stranded G-quadruplex–hemin complexes and their application in Hg$^{2+}$ ion detection

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The peroxidase activities of the complexes of hemin and intermolecular four-stranded G-quadruplexes formed by short-stranded $X_nG_mX_p$ sequences ($X = A, T$ or $C$), especially $T_nG_mT_p$ sequences, were compared. The results, combining with those of circular dichroism (CD) spectra and acid–base transition studies, indicate that the complexes of hemin and G-quadruplexes, provide some important information about DNAzymes based on G-quadruplex–hemin complexes, such as the formation of a G-quadruplex structure is an important factor for determining whether a DNA sequence can enhance the catalytic activity of hemin; both intramolecular parallel G-quadruplexes and intermolecular four-stranded parallel G-quadruplexes can enhance the catalytic activity of hemin; the addition of T nucleotides to the 5′-end of a G-tract confers corresponding G-quadruplex greatly enhanced catalytic activity, whereas the addition of T nucleotides to the 3′-end of the G-tract has little effect; the high catalytic activity of hemin in the presence of some short-stranded G-rich sequences may be a result of the reduction of the acidity of the bound hemin cofactor. These studies provide more information for the DNA–hemin peroxidase model system. May help to elucidate the structure–function relationship of peroxidase enzymes and to develop novel highly efficient peroxidase–liking DNAzymes. As a sequence of such an investigation, a new Hg$^{2+}$ detection method was developed.

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1. Introduction

DNAzymes are catalytically active DNA molecules that could show different enzymatic activities. With all else being equal, DNAzymes should be superior to many protein-based enzymes because DNAzymes are relatively less expensive to produce and more readily to mutate and self-replicate [1,2]. Therefore, the development of DNAzymes with interesting activities attracted increasing interest [3,4].

An important development in the field of DNAzymes has been the discovery of the peroxidase-like activity of some DNA–hemin complexes. It has been reported that the complexes formed by hemin and some G-quadruplexes can reveal enhanced peroxidase activity [5,6]. This class of DNAzymes has been used in the detection of telomerase activity [7,8], hemin [9] and cofactor units of DNAzymes (e.g. Pb$^{2+}$ ions, L-histidine and AMP) [10,11], the screening of G-quadruplex ligands [12] and the development of DNA sensors [13–15]. Compared with application studies, the basic studies (e.g. structure–function relationship study) on this class of DNAzymes are relatively backward. In DNA–hemin peroxidase model system studies, the most widely used catalytic DNA aptamer is PS2.M. PS2.M originally resulted from an in vitro selection for a reaction (porphyrin metallation) very different from peroxidation [16–19]. Travascio et al. reported that PS2.M–hemin can show peroxidase activity two orders of magnitude higher than that of hemin [6]. The ability of PS2.M to enhance the catalytic activity of hemin is attributed to the formation of a specific DNA structure, that is, a G-quadruplex. G-quadruplexes are unique higher-order structures formed by G-rich nucleic acid sequences based on stacked arrays of G-quartets connected by Hoogsteen-type base pairing. These structures are stabilized by monovalent cations (especially K$^+$) and can have different types dependent on the number of associated DNA strands, such as intermolecular four-stranded quadruplex formed by the association of four nucleic acid strands possessing a single pol(y)dG domain, intermolecular dual-stranded quadruplex formed by two nucleic acid strands possessing two pol(y)dG domains, and intramolecular single-stranded quadruplex formed by a nucleic acid strand possessing four or more than four pol(y)dG domains [20]. Even with the same number of associated molecules, G-quadruplexes can reveal many topological structures differing by mutual strand orientation (Scheme 1) [21].
It is generally accepted that PS2.M can fold to a G-quadruplex, but the details of the folded and catalytically active structure of PS2.M have not been adequately elucidated to date. In most cases, such G-quadruplexes are described as a unimolecular quadruplex with antiparallel structure [10,11,13,14], but no evidence for this structure was presented yet. Lee et al. constructed two closely related structural models for the folded PS2.M, which both contained two guanine quartets [22]. But it seems that these two models cannot be supported by results of circular dichroism (CD) spectroscopy. Majhi and Shafer concluded the catalytically active form of PS2.M may be the multistranded parallel quadruplex; (e) unimolecular parallel quadruplex; (f and g) unimolecular antiparallel quadruplex; (h) unimolecular mixed-parallel quadruplex. Arrows indicate 5′-3′ polarity.

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Herein, the peroxidase activities of the complexes formed by hemin and different four-stranded G-quadruplexes were compared to provide more information for the DNA–hemin peroxidase model system. The four-stranded G-quadruplexes are formed by different short-stranded DNAs that have a single G-tract with different numbers of T residues attached at the 5′ end and 3′ ends. The results demonstrated that some four-stranded G-quadruplexes can increase the peroxidase activity of hemin to some extent, and the position of T residues in the sequence has a great influence on the level of activity.

2. Experimental

2.1. Materials

All Oligonucleotides that were used in this study were purchased from Jinsite Co., Ltd. (China). The concentrations of these oligonucleotides were represented as single-stranded concentrations. Single-stranded concentrations were determined by measuring the absorbance at 260 nm. Molar extinction coefficients were determined using a nearest neighbor approximation (http://www.idtdna.com/analyzer/Applications/OligoAnalyzer).

H2O2, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Triton X-100 and hemin were obtained from Sigma. All chemical reagents were of reagent grade and used without further purification.

2.2. Peroxidase activity measurements

DNA solutions (0.86, 1.72 and 3.44 μM for one-stranded, two-stranded and four-stranded G-quadruplexes, respectively) were prepared in buffer A (10 mM Tris–HCl, pH 8.0, 16 mM KCl, 0.8 mM MgCl2, 0.0017% (v/v) Triton X-100). In order to ensure the formation of G-quadruplex structures, the mixtures were heated to 90 °C for 5 min, cooled slowly to room temperature, and then incubated at room temperature for 30 min. 1.72 μM of hemin was added to the mixtures. The reaction mixtures were held for another 1 h at room temperature, then 1.28 mM of H2O2 were added. The color of the reaction mixtures was recorded by a digital camera, and the absorption intensity at λ = 414 nm was followed using a TU-1901 UV–vis spectrophotometer.

2.3. Circular dichroism (CD) study

CD spectra were measured on a Jasco J-820 spectropolarimeter at room temperature. DNA solutions (0.86, 1.72 and 3.44 μM for one-stranded, two-stranded and four-stranded G-quadruplexes, respectively) were prepared in buffer A. To ensure the formation of quadruplex structures, the solutions were heated to 90 °C for 5 min, cooled slowly to room temperature, and then incubated at room temperature overnight. Spectra were recorded between 200 and 320 nm in 1 mm pathlength cuvettes. Spectra were averaged from three scans, which were recorded at 50 nm/min with a response time of 1 s and a bandwidth of 0.2 nm.

2.4. Acid–base transition study for DNA–hemin complexes

The mixtures of 1.72 μM hemin and individual DNAs (0.86, 1.72 and 3.44 μM for one-stranded, two-stranded and four-stranded G-quadruplexes, respectively) were prepared in different buffers: glycine–HNO3 (pH 2.0–4.0), MES–Tris (pH 4.6–7.0), HPO42−–H2PO4− (pH 5.8–6.8), Tris–HNO3 (pH 7.0–8.9), and glycine–OH− (pH 8.5–10.5). The electronic absorption spectra of various DNA–hemin complexes were recorded between 300 and 600 nm. The ratio of A360/A404 was plotted as a function of pH value, and corresponding pKa value was determined from the position of the maximum of the first derivative of the plot.

2.5. Detection of Hg2+ ion

Aliquots of 3.44 μM T4G3 (5′-TTTTGGG) solutions containing 10 mM Tris–HCl (pH 8.0), 16 mM KCl, 0.8 mM MgCl2, 0.0017% (v/v) Triton X-100 were heated to 90 °C for 5 min, cooled slowly to room temperature. Then, different concentration of Hg2+ ion was added and the mixtures were incubated at room temperature for 30 min. 1.72 μM of hemin was added to the mixtures and the reaction mixtures were held for another 1 h at room temperature. Then 1.28 mM of ABTS and 1.28 mM of H2O2 were added. 10 min later, the absorption spectra of the reaction product ABTS+ were recorded. The absorbance at 414 nm (the maximal absorption of ABTS+) was used for quantitative analysis.
Table 1
Potential G-quadruplex-forming sequences used in this work.

<table>
<thead>
<tr>
<th>DNA aptamer</th>
<th>Sequences</th>
<th>G-quadruplex structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS2.M</td>
<td>5'-GTTGCGTATGGCCTGG-3'</td>
<td>e</td>
</tr>
<tr>
<td>Apt</td>
<td>5'-GTGCGTAATGGGTGG-3'</td>
<td>g</td>
</tr>
<tr>
<td>Hum21</td>
<td>5'-G(TTAG)-3'</td>
<td>h</td>
</tr>
<tr>
<td>Hum12</td>
<td>5'-(T(AG)-3'</td>
<td>b</td>
</tr>
<tr>
<td>Oxy12</td>
<td>5'-G4T4G4</td>
<td>d</td>
</tr>
<tr>
<td>TG4T</td>
<td>5'-TG4T</td>
<td>a</td>
</tr>
<tr>
<td>T4G4</td>
<td>5'-T4G4</td>
<td>a</td>
</tr>
</tbody>
</table>

* Refers to Scheme 1.

3. Results and discussion

3.1. Catalytic activity and structural studies of various DNAs

Although the structure of the DNA aptamers that can provide hemin with enhanced peroxidase activity is unclear, it is generally acknowledged that they may form a G-quadruplex structure. The peroxidase activity of hemin in the presence of seven different G-quadruplexes (Table 1) was compared to investigate the abilities of these G-quadruplexes to enhance the peroxidase activity of hemin. The results are shown in Fig. 1, where it can be seen that PS2.M and T4G4 have markedly enhanced peroxidase activity compared with hemin alone. They catalyze the oxidation of 2,2'-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid (ABTS) by H2O2, and the absorption intensity of reaction mixtures at λ = 414 nm are greatly increased. T4G4T enhances the peroxidase activity of hemin much less than PS2.M and T4G4. The other four G-quadruplexes induce very little enhancement of peroxidase activity.

The CD spectra of the seven G-quadruplexes were recorded in order to investigate whether there is any relationship between the peroxidase activity of a G-quadruplex and its conformation. Earlier studies showed that the CD spectra of quadruplexes can be used to indicate whether they fold in a parallel or an antiparallel conformation. The CD spectrum of a parallel G-quadruplex has a positive peak near 265 nm and a negative peak near 240 nm, whereas an antiparallel G-quadruplex has a positive peak near 295 nm and a negative peak near 285 nm [25]. The results shown in Fig. 1B suggest that Apt and Oxy12 can form antiparallel quadruplexes, whereas PS2.M, T4G4, TG4T and Hum12 can form parallel quadruplexes. The CD spectrum of Hum21 has a positive peak at around 295 nm and a negative peak at around 240 nm, suggesting the co-existence of parallel and antiparallel conformations in solution, or the presence of a hybrid structure containing both syn and anti bonds as in Scheme 1h [26–29]. It is interesting that the three G-quadruplexes (PS2.M, T4G4 and TG4T) that can enhance the peroxidase activity of hemin adopt the parallel G-quadruplex conformation and have a strong positive peak at around 265 nm in CD spectra. This result suggests that the conformation of G-quadruplexes may be an important factor in determining their effect on peroxidase activity.

3.2. Catalytic activity and structural studies of TnGmTp sequences

The results described above indicate that both unimolecular parallel G-quadruplexes and intermolecular four-stranded parallel G-quadruplexes can enhance the catalytic activity of hemin. A detailed study of the catalytic activity of four-stranded quadruplexes formed by TnGmTp sequence is reported here for the first time.

3.2.1. G4Tn and TnG4

G4 has little effect, and G4Tn and TnG4 (n = 0–5) enhance the catalytic activity of hemin to different extents (Fig. 2). The increase is much greater in the presence of TnG4 sequences than that in the presence of G4Tn sequences; i.e. the addition of T residues to G4 increases the catalytic activity of hemin, and the greatest effect is obtained when the T residues are added to the 5'-end. However, the number of T nucleotides added to the same side of G4 has little effect on the catalytic activity.

The CD spectra of the oligonucleotides with sequences of G4Tn and TnG4 are shown in Fig. 2. The CD spectra of both TnG4 and G4Tn sequences have a major positive peak around 265 nm and a minimum around 240 nm, which is typical of a parallel arrangement of the strands. These results suggest that the ability of the intermolecular G-quadruplexes to enhance the catalytic activity of hemin is correspond not only to G-quadruplex structure, but also to the position of added T nucleotides.

3.2.2. T4G4Tn and TnG4Tn

The results described above show that the position of T nucleotides in the sequence has a marked influence on the catalytic activity. Two experiments were done to further investigate the effect of the location of the T nucleotides. In the first experiment, different numbers of T nucleotides were added to the 3'-end of T4G4, and the catalytic activity of the resulting TnG4Tn sequences (n = 0–5) was determined (Fig. 3). The addition of T nucleotides at the 3'-end dramatically decreases the catalytic activity of T4G4, but there was little difference in the effect when different numbers of T nucleotides were added. In the second experiment, the catalytic activity of the TnG4Tn sequences (n = 0–4) was investigated. As shown in Fig. 3, the TnG4Tn sequences have a level of catalytic activity comparable to that of the T4G4Tn sequences. That is to say,
although the T₄G₃T₇ sequences have slightly higher catalytic activity than G₄, it is much weaker than those of the T₄G₄ sequences (n = 1–4).

The CD spectra of both T₄G₄T₆ and T₇G₄T₇ sequences have a major positive peak around 265 nm with a minimum around 240 nm, indicating that all of the G-quadruplexes formed by these sequences adopt the parallel quadruplex conformation.

On the basis of the results described above, the catalytic activity of the T₄G₄Tₘ sequences is ranked in the order: T₄G₄ > G₄Tₘ ≈ T₄G₇Tₘ (m > 0) > G₄.

3.2.3. T₄Gₘ

The number of quarters in a G-quadruplex is an important factor in determining the stability of the G-quadruplex. From the results described above, it can be seen that the addition of T nucleotides to the 5′-end of a G tract can provide corresponding G-quadruplexes with strong catalytic activity. So, in this experiment, the position of the T nucleotides was fixed at the 5′-end of the G tract, the number of T nucleotides was fixed at 4, and the number of G nucleotides in the G tract was changed from 3 to 9, and the catalytic activity and CD spectra of the resulting T₄Gₘ sequences (n = 3–9) were compared. As shown in Fig. 4, T₄G₃ has no catalytic activity, whereas each of the other T₄Gₘ sequences has very strong catalytic activity, which increases as the number of G nucleotides increases from 4 to 6; after that, the catalytic activity reaches a plateau and there is no change as the number of G nucleotides increases.

T₄G₇ did not show any CD signal in the range of wavelengths studied here, implying that it cannot form a G-quadruplex structure. Under the same conditions, as the CD spectra display a positive peak around 265 nm with a minimum around 240 nm, the T₄G₉–T₄G₉ sequences can form a typical parallel G-quadruplex structure.
From these results, it can be concluded that: (1) the formation of a stable G-quadruplex structure is an important factor for determining whether T4Gn sequences can enhance the catalytic activity of hemin; (2) stable intermolecular four-stranded G-quadruplexes cannot be formed when the number of G nucleotides in the G tract is less than 4; (3) in the range of n = 4–6, the catalytic activity of the T4Gn sequences gradually increased as n increased. Any further increase of n has very little effect on the catalytic activity.

3.3. Catalytic activity and structural studies of X4G4 (X = T, A or C) sequences

The catalytic activity of the X4G4 sequences (X = A, T or G) were compared to investigate the effect of the identity of the nucleotide that was added to the 5′-end of the G tract. As shown in Fig. 5, both T4G4 and A4G4 have strong catalytic activity, and the activity of A4G4 is a little higher than that of T4G4. The catalytic activity of C4G4 is much lower than that of either T4G4 or A4G4.

The CD spectrum of T4G4 has a positive peak around 265 nm with a minimum around 240 nm, indicating that T4G4 adopts a parallel G-quadruplex structure. The CD spectrum of C4G4 shows a positive peak at 263 nm, and a negative peak is at 235 nm. The secondary structure of C4G4 cannot be determined directly from the CD spectrum. It is well known that the presence of divalent cations (e.g., Mg2+) will aid the formation of duplex DNAs, and the presence of monovalent cations (especially K+) will aid the formation of G-quadruplex structures. Under the buffer conditions used here (0.8 mM Mg2+; 16 mM K+), both duplex and G-quadruplex structures may be formed, but only the G-quadruplex DNA can enhance the catalytic activity of hemin. Therefore, the catalytic effect of the C4G4 sequence is much lower than that of either T4G4 or A4G4.

The CD spectrum of T4G4 has a positive peak around 265 nm with a minimum around 240 nm, indicating that T4G4 adopts a parallel G-quadruplex structure. The CD spectrum of C4G4 shows a positive peak at 263 nm, and a negative peak is at 235 nm. The secondary structure of C4G4 cannot be determined directly from the CD spectrum. It is well known that the presence of divalent cations (e.g., Mg2+) will aid the formation of duplex DNAs, and the presence of monovalent cations (especially K+) will aid the formation of G-quadruplex structures. Under the buffer conditions used here (0.8 mM Mg2+; 16 mM K+), both duplex and G-quadruplex structures may be formed, but only the G-quadruplex DNA can enhance the catalytic activity of hemin. Therefore, the catalytic effect of the C4G4 sequence is much lower than that of either T4G4 or A4G4. The CD spectrum of the T4G4 sequence has a positive peak at 283 nm, but fluctuates sharply at short wavelengths, so it is difficult to identify the presence of the G-quadruplex structure. Considering that no base-pair can be formed between A and G, it is very possible that the A4G4 sequence will adopt the intermolecular parallel G-quadruplex structure. Considering that two hydrogen bonds may be formed between T and G, and the stability of the T:G mismatch is the highest of all base mismatches, the possibility of the formation of the G-quadruplex structure for A4G4 is even higher than that for T4G4. That is to say, the possibility of the formation of G-quadruplex structures for the three sequences follows the order: A4G4 > T4G4 ≫ C4G4, which is quite consistent with the results of catalytic activity studies.

3.4. The “acid–base transition” of the DNA–hemin complexes

Recently, Travascio et al. demonstrated that the high catalytic activity of PS2.M may be a result of the reduction of the acidity of the bound hemin cofactor (e.g., a water molecule coordinated to the iron atom of the hemin that is bound to the DNAzyme scaffolds) [16]. Here, the acid–base titrations for hemin and DNA–hemin complexes were also followed by monitoring the absorption curve at various pH values, and corresponding pKa values could be derived from A360/A404–pH plots. As shown in Fig. 6 (and see supporting information Fig. S6), regardless of hemin or the DNA–hemin complexes, the absorbance ratio of A360/A404 increases greatly with increasing pH, and pKa can be determined from the position of the maximum of the first derivative of the transition curves. According to the relationship between catalytic activities and pKa values (Table 2), hemin and the DNA–hemin complexes that were studied can be clearly divided into two groups (Fig. 7). Hemin has a pKa value of 4.36 ± 0.15, which is very similar to those of the DNA–hemin complexes whose catalytic activities are comparable to hemin itself, but is much lower than those of the DNA–hemin complexes (pKa > 7.20) that have much higher catalytic activities. Our results are gen-

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**Fig. 4.** Oxidation of ABTS by H2O2 in the presence of hemin and the T4Gn (A), and the CD spectra of T4Gn (B). Corresponding color figures can be found in supporting information.

**Fig. 5.** Oxidation of ABTS by H2O2 in the presence of hemin and the X4G4 (A), and the CD spectra of X4G4 (B). Corresponding color figures can be found in supporting information.
Fig. 7. The relationship between catalytic activities versus $pK_a$ values. The catalytic activities of hemin and the DNA–hemin complexes were represented as the absorption intensities for the reaction solutions of ABTS oxidation by $H_2O_2$.

very different $pK_a$ values for the corresponding DNA–hemin complexes.

3.5. Application in $Hg^{2+}$ ion detection

Mercury is an important toxic pollutant in the environment, and it is attractive to develop a reliable sensing system for mercury detection. To date, numerous studies concerning $Hg^{2+}$ ion assays have been reported [30]. Many of them are based on the fact that $Hg^{2+}$ ion can bind to two thymine (T) and stabilize T–T mismatches in a DNA duplex. Recently, Li et al. reported a new $Hg^{2+}$ ion detection method utilizing the $Hg^{2+}$–mediated T–T base pair to destroy the G-quadruplex structure and to inhibit corresponding catalytic activity [31]. Although this method gave a good detection result, from the sensing point of view, this is not a “turn-on” sensor. The results mentioned above indicate that the addition of T nucleotides to the 5′-end of the G-tract can confer enhanced catalytic activity, but the prerequisite is that a stable G-quadruplex can be formed. In the intermolecular G-quadruplex formed by $T_nG_m$ sequences, four $T_n$ tails locate at the 5′-terminals of the G-quadruplex. The addition of $Hg^{2+}$ ion can promote the formation of T–$Hg^{2+}$–T base pairs. The formation of T–$Hg^{2+}$–T base pairs may strengthen the intermolecular G-quadruplexes, resulting in the increase of catalytic

Scheme 2. Intermolecular G-quadruplex-based DNAzyme as sensing platform for colorimetric detection of $Hg^{2+}$ ion.
T₄G₃–hemin complex as a function of Hg²⁺ ion concentration was greatly enhanced catalytic activity can be observed. (4) The catalytic activity of hemin. When the number of G nucleotides exceeds 4, no stable G-quadruplex structure can be formed and the sequence is unable to enhance the catalytic activity of hemin. (2) When the number of G nucleotides in the G-tract is fixed at 4, the catalytic activity of T₄G₄ ion concentration up to 1 μM with a detection limit of 52 nM based on 3α/slope.

4. Conclusions

The catalytic activity and the secondary structure of short-stranded X₄GₜXₜ sequences (X = A, T or C), especially T₄G₄Tₜ sequences, were investigated. The results of these experiments suggest that: (1) both intramolecular parallel G-quadruplexes and intermolecular four-stranded parallel G-quadruplexes can enhance the catalytic activity of hemin. (2) When the number of G nucleotides in the G-tract is fixed at 4, the catalytic activity of the T₄G₄Tₜ sequence follows the order:

T₄G₄ > G₄Tₜ ≈ T₄G₄Tₜ(m > 0) > G₄

That is to say, the addition of T nucleotides to the 5′-end of the G-tract confers greatly enhanced catalytic activity, whereas the addition of T nucleotides to the 3′-end of the G-tract has little effect on the catalytic activity. (3) As for T₄G₄m, when the number of G nucleotides in the G-tract is 3, no stable G-quadruplex structure can be formed and the sequence is unable to enhance the catalytic activity of hemin. When the number of G nucleotides exceeds 4, stable intermolecular parallel G-quadruplexes can be formed, and greatly enhanced catalytic activity can be observed. (4) The catalytic activity of X₄G₄ follows the order A₄G₄ > T₄G₄ ≈ C₄G₄, which may be correlated with the competitive formation of G-quadruplex and duplex structures. (5) The high catalytic activity of hemin in the presence of some short-stranded G-rich sequences may be a result of the reduction of the acidity of the bound hemin cofactor. These studies may help to elucidate the structure–function relationship of peroxidase enzymes and the ways in which a DNAzyme might work to enhance the intrinsic activity of hemin. Also, these studies may be helpful for the development of novel, highly efficient DNAzymes with peroxidase activity. Based on these studies, a “turn-on” Hg²⁺ ion detection method with a detection limit of 52 nM was developed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2009.07.010.

References