J Med Biosci Suppl 2025;7: S143 - S148 DOI: 10.58837/CHULA.JMBS.7.S1.21

Original article

The catalytic efficiency of peroxidase-mimicking G-quadruplex/hemin DNAzyme when using ABTS vs. TMB as a substrate

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Abstract

Background: Functional nucleic acids are a group of nucleic acids with functions beyond the natural genetic materials. G-quadruplex/hemin DNAzyme can mimic the catalytic activity of horseradish peroxidase (HRP) on various organic substrates.

Objective: To investigate the catalytic efficiency of G-quadruplex/hemin DNAzyme when using either ABTS or TMB as a substrate.

Methods: The catalytic activity of G-quadruplex/hemin DNAzyme was studied in different types of buffer solution by absorbance measurement. Topology of G-quadruplex structure formed in Tris-HCl buffer was analyzed by circular dichroism. The kinetics parameters of the DNAzyme were determined using KaleidaGraph software.

Results: The catalytic activities of G-quadruplex/hemin DNAzyme were different in different types of buffer solution. The CD analysis revealed that the parallel G-quadruplex could form in the presence of hemin even without monovalent ions. The kinetics studies, in the selected buffer, with different substrates (ABTS or TMB) showed comparable catalytic efficiency.

Conclusion: G-quadruplex/hemin DNAzyme could use either ABTS or TMB as a substrate with similar catalytic efficiency.

Keywords: ABTS, biocatalyst, G-quadruplex/hemin DNAzyme, HRP-mimicking activity, TMB

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Functional nucleic acids are a group of nucleic acids with functions beyond the natural genetic materials. Nucleic acids with catalytic function are called nucleic acid enzymes or DNAzymes.[1] Compared with protein enzymes, DNAzymes could offer higher thermal stability, lower production cost, and easier modification.[2] In 1998, Tavascio and coworkers reported the first G-quadruplex/hemin DNAzyme by using a systematic evolution of ligands by exponential enrichment (SELEX) method.[3] Single-stranded DNA (ssDNA) with guanine (G)-rich sequence could fold into a secondary G-quadruplex structure via Hoogsteen's hydrogen (H) bonds. After binding with hemin, the G-quadruplex/hemin DNAzyme can mimic the catalytic activity of horseradish peroxidase (HRP) enzyme. Various organic compounds, such as tyramine, luminol, (ABTS) 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt, and (TMB) 3,3',5,5'-tetramethylbenzidine sulfate could be used as a substrate for this DNAzyme.[4] It might be useful to have kinetics information of this artificial enzymes for substrate selection. In this study, we aimed to investigate the catalytic efficiency of G-quadruplex/hemin DNAzyme when using either ABTS or TMB as a substrate in the presence of hydrogen peroxide (H_2O_2). The experimental condition for activity assay was optimized and then the catalytic efficiency of G-quadruplex/hemin DNAzyme when using either ABTS or TMB as a substrate was examined.

Materials and Methods

Oligonucleotide and chemicals

Single-stranded oligonucleotide (5'GGGTTGGG CGGGATGGG 3') was purchased from Integrated DNA Technologies, IDT (Singapore) and used without further purification. All reagents; Tris, 2-morpholinoethanesulphonic acid (MES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium dihydrogen phosphate (NaH_2PO_4), triton X-100, dimethyl sulfoxide (DMSO), sodium chloride (NaCl), potassium chloride (KCl), hemin, and substrates; ABTS, TMB, H_2O_2, were purchased from Sigma-Aldrich (Germany).

Buffer solutions

Tris-HCl buffer: 25 mM Tris-HCl buffer pH 8.0 with and without 20 mM NaCl, 10 mM KCl, 1% DMSO, and 0.05% triton X-100

MES buffer: 25 mM MES buffer pH 5.1 with and without 20 mM NaCl, 10 mM KCl, 1% DMSO, and 0.05% triton X-100, MES buffer at 50 mM, 75 mM, 100 mM, 125 mM, and 150 mM, pH 5.1 with 20 mM NaCl, 10 mM KCl, 1% DMSO, and 0.05% triton X-100

PBS buffer: 25 mM PBS buffer pH 7.2 with and without 20 mM NaCl, 10 mM KCl, 1% DMSO, and 0.05% triton X-100

HEPES buffer: 25 mM HEPES buffer pH 7.2 with and without 20 mM NaCl, 10 mM KCl, 1% DMSO, and 0.05% triton X-100

Assembly of G-quadruplex/hemin DNAzyme

G-quadruplex/hemin DNAzyme was assembled by mixing the G-quadruplex structure in selected buffers with hemin (using 1:10 molar ratio of oligonucleotide:hemin) and then left at room temperature for 30 minutes before using in further experiments.

Catalytic activity assay

The catalytic activity of G-quadruplex/hemin DNAzyme (25 nM) was determined in selected buffers using peroxidase substrates, either ABTS or TMB (2 mM) and H_2O_2 (2.5 mM). After incubation with substrates for 20 minutes, the absorbance at 420 nm (for ABTS) or 650 nm (for TMB) was recorded using a microplate reader. The results were averaged from three replicates.

Circular dichroism (CD) analysis

The topology of G-quadruplex structure was investigated using a JASCO J-815 CD spectrometer. G-quadruplex structures were prepared in selected buffers at 2 μM. CD spectra were recorded from 225 nm to 325 nm at 0.2 nm intervals. The data was averaged over three scans.

Kinetics study

To initiate the catalytic reaction, G-quadruplex/hemin DNAzyme (0.625 nM) was mixed with selected substrates. The concentrations of ABTS was varied from 5-300 μM, the concentration of TMB was varied from 20-300 μM, and the concentration of H_2O_2 was fixed at 0.15 M. The absorbance intensity at 420 nm (for ABTS) and 650 nm (for TMB) was measured as a function of time (3 minutes). The kinetic parameters were calculated using KaleidaGraph software (version 4.0), employing the Marquardt-Levenberg algorithm.

Results

The optimization of catalytic activity assay

According to previous study with a minor modification, G-quadruplex/hemin DNAzyme was annealed in Tris-HCl buffer pH 8.0 and the activity assay was done in MES buffer pH 5.1 containing 20 mM NaCl, 10 mM KCl, 1% DMSO, and 0.05% triton X-100,[5] as shown in **Figure 1**. To optimize the activity assay condition, the preparation of G-quadruplex/hemin DNAzyme and the activity assay were tested in different types of buffer solutions, includings Tris-HCl, MES, PBS, and HEPES buffers. G-quadruplex/hemin DNAzyme was annealed and assayed in selected buffers either with or without 20 mM NaCl, 10 mM KCl, 1% DMSO, and 0.05% triton X-100. Then, the catalytic activity assay was initiated by the addition of substrates, ABTS and H_2O_2. After 20-minute incubation at room temperature, the absorbance at 420 nm was measured. The DNAzyme annealed in Tris-HCl buffer pH 8.0 and the activity assay was done in MES buffer pH 5.1 containing 20 mM NaCl, 10 mM KCl, 1% DMSO, and 0.05% triton X-100 was used as control.

The results showed that G-quadruplex/hemin DNAzyme has very low activity in all conditions of Tris-HCl buffer pH 8.0 and PBS buffer pH 7.2, (1) – annealed and assayed in buffer without KCl, NaCl, DMSO, and triton X-100, (2) – annealed and assayed in buffer with KCl, NaCl, DMSO, and triton X-100, and (3) – annealed in buffer without KCl, NaCl, DMSO, and triton X-100 and assayed in buffer with KCl, NaCl, DMSO, and triton X-100 (**Figure 2A and 2C**). While the DNAzyme has higher activity in HEPES buffer pH 7.2 (**Figure 2D**) than those in Tris-HCl buffer pH 8.0 and PBS buffer pH 7.2, its activity is still lower than that in MES buffer pH 5.1 (**Figure 2B**). It seems like the one annealed in MES buffer pH 5.1 without KCl, NaCl, DMSO, and triton X-100 and assayed in buffer with KCl, NaCl, DMSO, and triton X-100 showed similar catalytic activity as the control. Then, the initial velocity (V_o) of G-quadruplex/hemin DNAzyme in each condition was determined. Only the conditions with positive V_o were showed in **Figure 2E**. However, DNAzyme annealed and assayed in MES buffer containing with KCl, NaCl, DMSO, and triton X-100 has similar V_o as the control one, the control condition was selected for further experiments. Also, the catalytic activity was tested in different MES concentrations. The results indicated that the higher the MES concentrations, the lower the catalytic activity of G-quadruplex/hemin DNAzyme (**Figure 2F**). Therefore, G-quadruplex/hemin DNAzyme will be annealed in 25 mM Tris-HCl buffer pH 8.0 and activity assayed in 25 mM MES buffer containing 20 mM NaCl, 10 mM KCl, 1% DMSO, and 0.05% triton X-100 for further activity analysis.

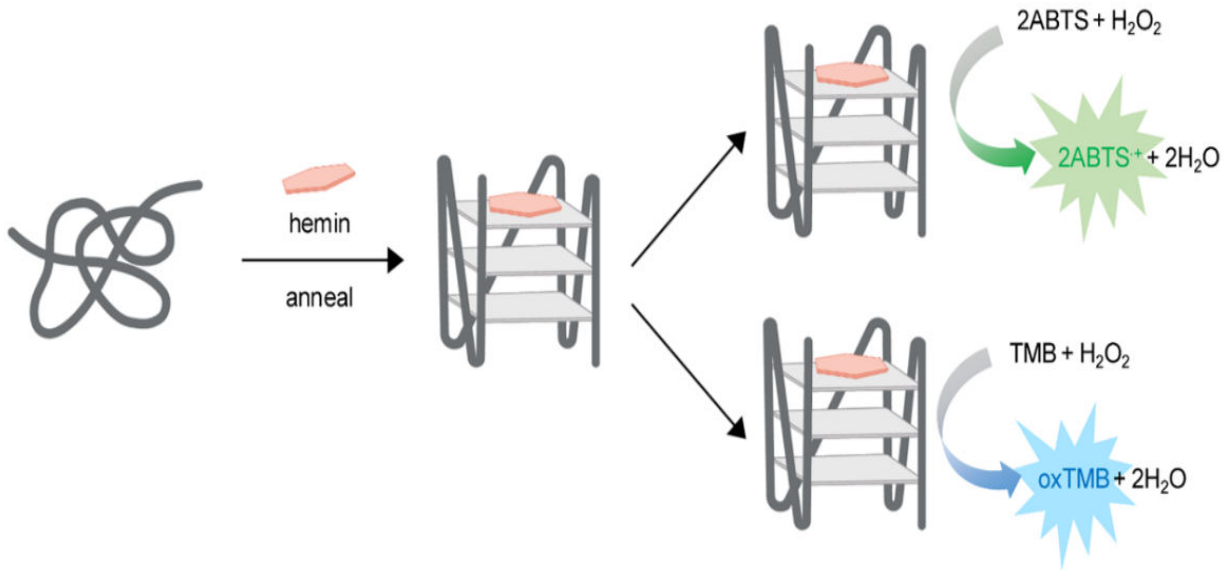

Figure 1. The preparation of G-quadruplex/hemin DNAzyme and the HRP-like catalytic activity when using ABTS or TMB in the presence of H_2O_2.

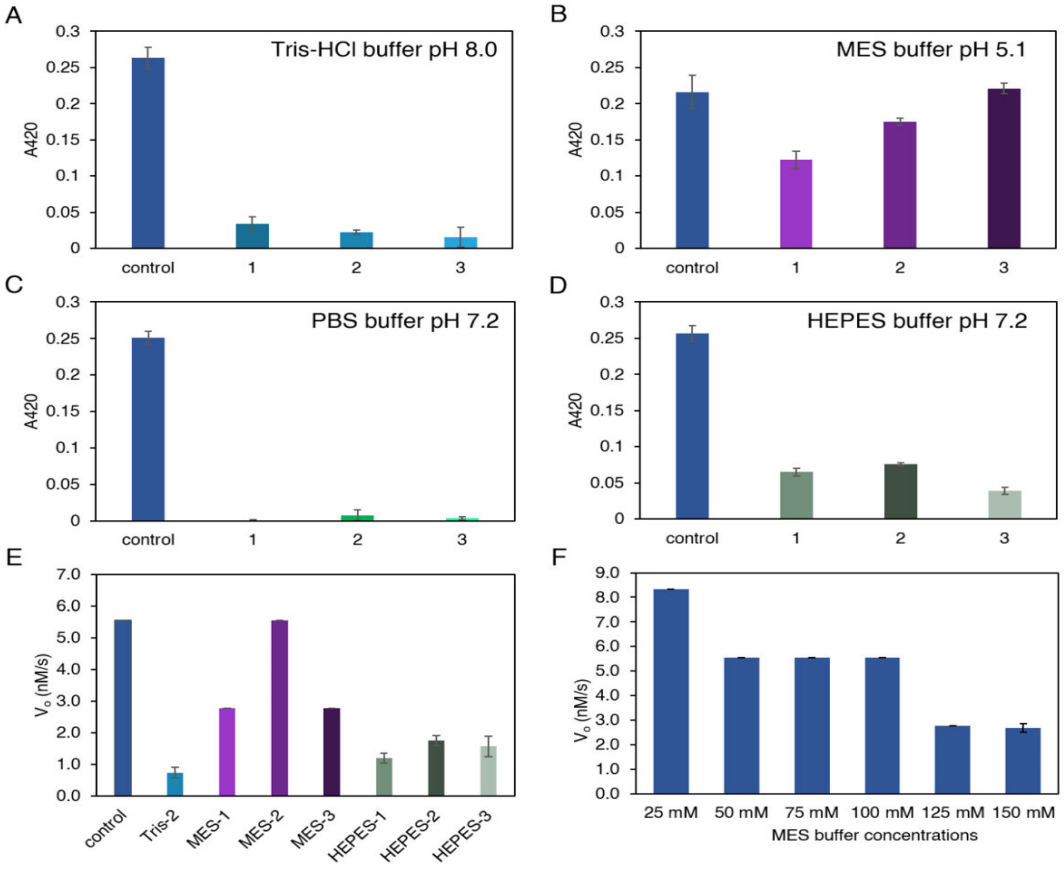

Figure 2. The catalytic activity of G-quadruplex/hemin DNAzyme in different buffers; Tris-HCl buffer pH 8.0 (**A**), MES buffer pH 5.1 (**B**), PBS buffer pH 7.2 (**C**), and HEPES buffer pH 7.2 (**D**); 1 – annealed and assayed in buffer, 2 – annealed and assayed in buffer with KCl, NaCl, DMSO, and triton X-100, and 3 – annealed in buffer and assayed in buffer with KCl, NaCl, DMSO, and triton X-100. The initial velocity (v_o) of G-quadruplex/hemin DNAzyme in different buffers (**E**) and in different MES concentrations (**F**).

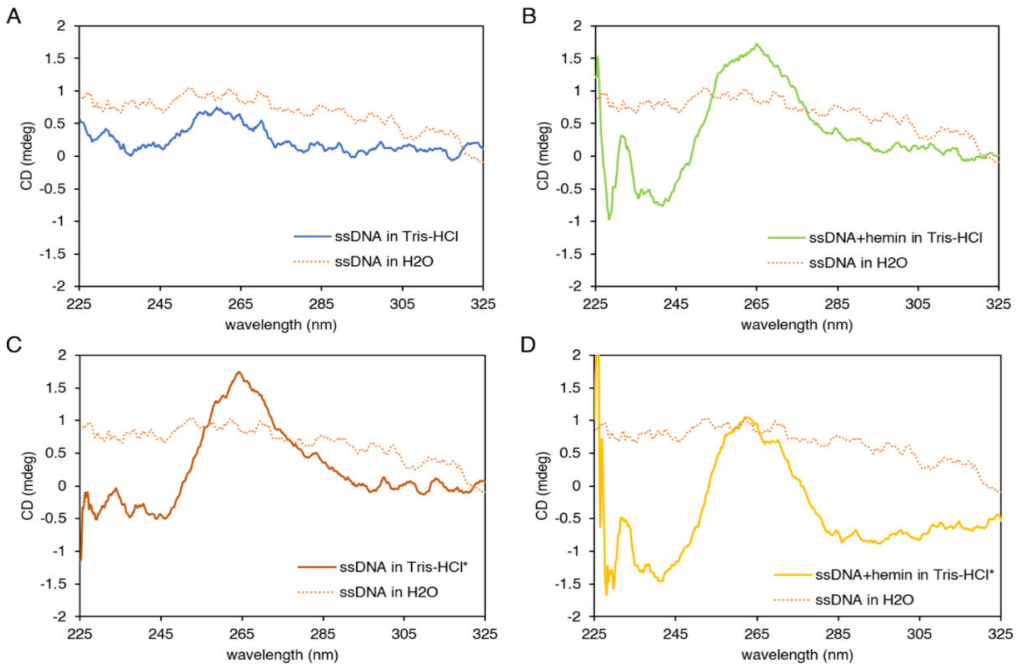

Figure 3. CD analysis of G-quadruplex structures; ssDNA in Tris-HCl buffer (**A**), ssDNA+hemin in Tris-HCl buffer (**B**), ssDNA in Tris-HCl buffer containing NaCl and KCl (**C**), and ssDNA+hemin in Tris-HCl buffer containing NaCl and KCl (**D**).

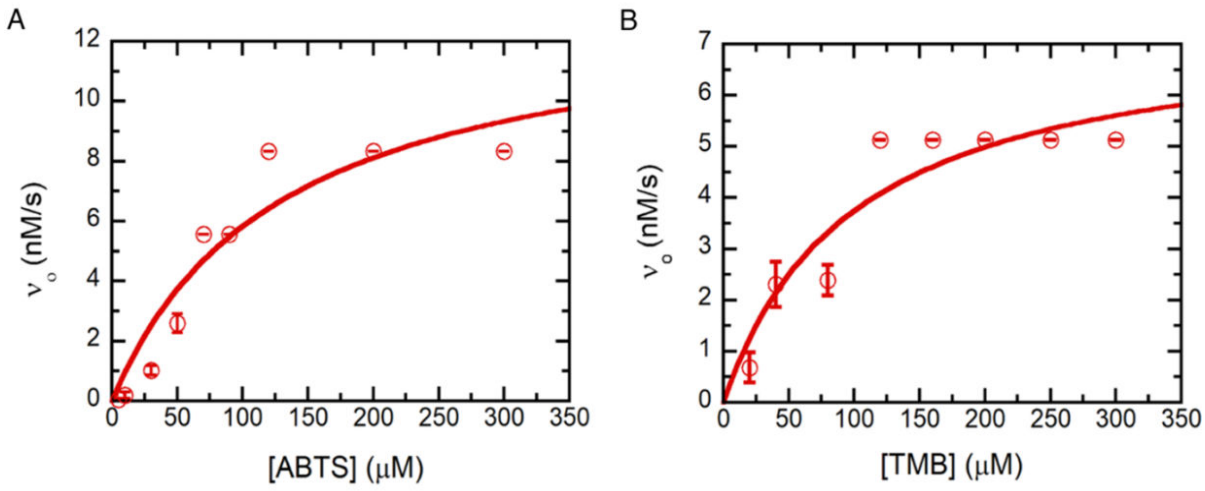

Figure 4. Michaelis-Menten plot of G-quadruplex/hemin DNAzyme with varied ABTS concentrations (**A**) and with varied TMB concentrations (**B**) in the presence of 0.15 M H_2O_2.

Table 1. Kinetic parameters of G-quadruplex/hemin DNAzyme when varying either ABTS or TMB concentrations at fixed 0.15 M H_2O_2 concentration.

	ABTS as a substrate	TMB as a substrate
K_m (mM)	129.11 ± 3.87	99.45 ± 12.97
V_{max} (nM/s)	13.36 ± 0.13	7.47 ± 0.32
k_{cat} (s^{-1})	21.37 ± 0.21	11.95 ± 0.51
k_{cat}/K_m ($s^{-1}mM^{-1}$)	0.17 ± 0.06	0.12 ± 0.01

The circular dichroism (CD) analysis

The topology of G-quadruplex structure annealed in Tris-HCl buffer pH 8.0 with and without KCl, NaCl, DMSO, and triton X-100 was examined by using circular dichroism (CD) technique, using ssDNA in distilled water as a control. **Figure 3** showed that this G-quadruplex structure in all condition (except in water) was form a parallel G-quadruplex structure since the negative signal around 240 nm and the positive signal around 260 nm were observed.[6] In addition, it has been shown that monovalent cations such as K^+ and Na^+ are required for structure stabilization.[7] Our result showed that even without monovalent ions, the G-quadruplex structure could form in the presence of hemin.

The kinetics study of G-quadruplex/hemin DNAzyme

The kinetics of G-quadruplex/hemin DNAzyme when using different substrates, ABTS and TMB, were compared. To study the kinetics of G-quadruplex/hemin DNAzyme, the reaction rates were measured as v_o when the concentration of DNAzyme and H_2O_2 were held constant and the concentration of ABTS or TMB was varied. Then, the kinetics parameters were determined using KaleidaGraph software as shown in **Figure 4A** and **4B** for ABTS and TMB, respectively.

The results showed that the maximum velocity (v_{max}) of the reaction with ABTS substrate is almost two times higher than that of TMB substrate while both K_m values are not much different (**Table 1**). According to turnover number (k_{cat}), this DNAzyme can transform ABTS substrate into product faster than transform TMB substrate into product. However, the catalytic efficiency of G-quadruplex/hemin DNAzyme when using either ABTS or TMB as substrate is comparable.

Discussion

The reaction condition was optimized and DNAzyme annealed in Tris-HCl buffer and assayed in 25 mM MES buffer containing 20 mM NaCl, 10 mM KCl, 1% DMSO, and 0.05% triton X-100 showed the highest catalytic activity. The CD analysis indicated that parallel G-quadruplex structure was formed in the presence of hemin. The catalytic efficiency of G-quadruplex/hemin DNAzyme when using either ABTS or TMB as substrate is the same. These kinetics information might be useful for designing and developing a colorimetric G-quadruplex/hemin DNAzyme-based biosensor.

Acknowledgements

This research work was financially supported by the Office of the Permanent Secretary, Ministry of Higher Education, Science, Research and Innovation (OPS MHESI) and Thailand Research and Innovation (TSRI) (Grant No. RGNS63-141) and Burapha University. We also thank Mahidol University – Frontier Research Facility (MU-FRF) for technical assistance.

Conflict of interest statement

The authors declare no commercial or financial relationships that could be perceived as potential conflict of interest.

Data sharing statement

All data generated or analyzed during the present study are included in this published article. Further details are available for noncommercial purposes from the corresponding author on reasonable request.

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