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ARTICLE TYPE

G-quadruplex DNAzymes-induced highly selective and sensitive colorimetric sensing of free heme in rat brain

Ruimin Li,[†a] Qin Jiang,[†,b] Hanjun Cheng,[†,b] Guoqiang Zhang[†,a] Mingming Zhen,[†,a] Daiqin Chen,[†,a] Jiechao Ge,[‡] * Lanqun Mao,[†,b] Chunru Wang[†,a] and Chunying Shu[†,a]*

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Direct selective determination of free heme in the cerebral system is of great significance due to the crucial roles of free heme in physiological and pathological processes. In this work, a G-quadruplex DNAzymes-induced highly sensitive and selective colorimetric sensing of free heme in rat brain is established. Initially, the conformation of an 18-base G-rich DNA sequence, PS2.M (5'-GTGGGTAGGGCGGGTTGG-3'), in the presence of K^+, changes from a random coil to a "parallel" G-quadruplex structure, which can bind free heme in cerebral system with high affinity through π-π stacking. The resulted heme/G-quadruplex complex exhibits high peroxidase-like activity, which can be used to catalyze the oxidation of colorless $ABTS^{2-}$ to green $ABTS^{\bullet-}$ by H_2O_2. The concentration of heme can be evaluated by the naked eye and determined by UV-vis spectroscopy. The signal output showed a linear relationship for heme within the concentration range from 1 nM to 120 nM with a detection limit of 0.637 nM. The assay demonstrated here was highly selective and free from the interference of physiologically important species such as dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), ascorbate acid (AA), cysteine, uric acid (UA), glucose and lactate in the cerebral system. The basal dialysate level of free heme in the microdialysate from the striatum of adult male Sprague-Dawley rats was determined to be 32.8±19.5 nM (n=3). The analytic protocol possesses much more advantages, including theoretical simplicity, low-cost technical and instrumental demands, and the responsible detection of heme in rat brain microdialysate.

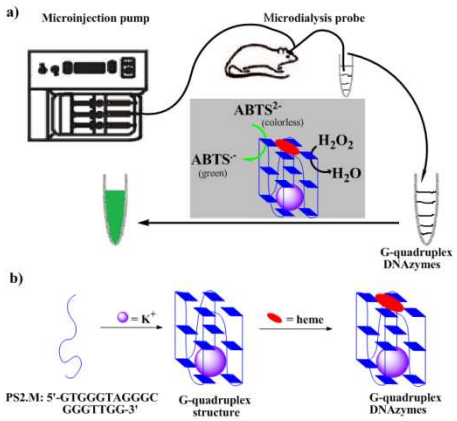
Introduction

Cerebrovascular diseases and their consequences, such as strokes, have been the second most common cause of death, after ischemic heart diseases.[1] Intracerebral hemorrhage (ICH) is a frequent cause of disability in adults, with a significant amount of the permanent brain damage arising in weeks after a stroke.[2] A growing number of experimental evidences suggest that free heme released therein is related with the brain damage, even the pathophysiology of ICH is complicated.[3-6] Heme (Fe-protoporphyrin IX) is an iron-containing prosthetic group among a diverse group of proteins.[7] Free heme, a protein-unbound form of heme, which can be released from hemoglobin following hemolysis, is pro-inflammatory and contributes to iron-derived reactive oxygen species, potentially causing oxidative damage to cells and tissues.[8,9] Free heme level can be up-regulated under pathological conditions and lead to various inflammatory lesions including vascular disorders, renal failure, and immune-mediated disorders.[4,10-12] In addition, recent studies have revealed that free heme is also responsible for the progression of cerebral malaria,[13] colon cancer[14] and Alzheimer's disease.[15,16]

Highly selective and sensitive detection of physiological free heme involved in brain function is of great importance for understanding its nature involved in the physiological and pathological events and therefore providing a platform for the diagnosis and therapy of related diseases.[17,18] Up to date, a variety of techniques have been developed to satisfy this purpose,

including mass spectrometry,[19] absorption spectrophotometry,[20,21] chemiluminescence,[22] and fluorescent spectrometry.[23,24] However, these methods are either time-consuming, lacking specificity and selectivity, or depending on expensive instruments. In recent years, colorimetric biosensing has attracted much attention due to its low cost, simplicity, practicality and its potential application in field analysis and point-of-care diagnosis.[25-27] Therefore, colorimetric biosensing is very competitive.

G-quadruplex DNAzymes are usually formed by the G-rich nucleic acid sequences in the presence of alkali metal ions, which can easily combine with hemin,[28-31] especially when it exists as an oxidized state in vitro.[19] Considering the purpose of our study, heme herein is used as the general term for the oxidized form. Most importantly, the resulting heme-G-quadruplex complexes possess peroxidase-like activity and exhibit catalytic activity towards the H_2O_2-mediated oxidation of 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS).[32,33] In view of this unique property, the heme-G-quadruplex DNAzymes have shown great potential in colorimetric detection of various targets, including metal ions,[34,35] small molecules,[36,37] DNAs[38,39] and proteins.[40,41] Herein, we report a novel colorimetric assay for highly selective and sensitive sensing of cerebral free heme. The fundamental for this sensing is essentially on the strength of in vivo microdialysis, and the peroxidase-like activity of G-quadruplex DNAzymes induced by free heme in cerebral system, which can catalyze the oxidation of colorless $ABTS^{2-}$ to green $ABTS^{\bullet-}$ by H_2O_2 (Scheme 1). To the best of our knowledge, this is the first example for the direct selective sensing of cerebral free heme based on G-quadruplex DNAzymes, which may provide a facile strategy for monitoring brain chemistry in a primitive style.



Scheme 1. G-quadruplex DNAzymes-based colorimetric sensing of cerebral free heme coupled with in vivo microdialysis.

Materials and methods

Materials

The purified G-rich oligonucleotide 5'-GTGGGTAGGGCGGGTTGG-3' (PS2.M), which could form G-quadruplex structure in the presence of K^+, is designed for the following study. All oligonucleotides were obtained from Sangon Biotech. Co. Ltd. (Beijing, China). 2, 2'-azinobis (3-ethylbenzothiozoline)-6-sulfonic acid (ABTS) was purchased from Tokyo Chemical Industry Co. Ltd. Hydrogen peroxide (H_2O_2, 30%) was obtained from Beijing Chemical Works. Glucose was purchased from Sinopharm Chemical Reagent Co., Ltd. Hemin (recognized as the oxidized state of heme in vitro), cysteine, dopamine (DA), ascorbate acid (AA), uric acid (UA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were purchased from Sigma-Aldrich. L(+)-lactate acid was obtained from Acros Organics. All reagents were used as received without further purification.

The stock solution of oligonucleotide (100 μM) was prepared in 10 mM Tris-Ac buffer (pH 7.0) and accurately quantified using UV-vis absorption spectrum with the following extinction coefficients (260 nm, $M^{-1}cm^{-1}$): A) 15400, G) 11500, C) 7400, T) 8700.[36] The oligonucleotide solutions were diluted to required concentrations with the buffer prior to use. The stock solution of 1 mM heme was prepared in dimethyl sulfoxide (DMSO), and stored in darkness at -20 °C. Concentration of H_2O_2 was accurately quantified using UV-vis absorption spectrum at 240 nm by the characteristic molar extinction coefficient (ε = 43.6 $dm^3 M^{-1}cm^{-1}$). Artificial cerebrospinal fluid (aCSF) was prepared by mixing NaCl (126 mM), KCl (2.4 mM), KH_2PO_4 (0.5 mM), $MgCl_2$ (0.85 mM), $NaHCO_3$ (27.5 mM), Na_2SO_4 (0.5 mM), and $CaCl_2$ (1.1 mM) into Milli-Q water. Aqueous solutions of DA, AA, UA, DOPAC, lactate, cysteine, and glucose were freshly prepared with aCSF prior to experiment. All aqueous solutions were prepared with Milli-Q water (18.2 MΩ cm^{-1}).

Preparation of the G-quadruplex DNA solution modulated by K^+

The PS2.M solution was heated at 88 °C for 10 min to dissociate any intermolecular interaction, and gradually cooled to room temperature. Then KAc with an appropriate concentration was added into the DNA solution, allowing the DNA sequences folding properly for 40 min to form the quadruplex structures in the presence of K^+.

Colorimetric sensing of heme in aCSF

All the samples were prepared by dissolving the 1 mM heme stock solution directly into the aCSF. The aCSF was used to prepare heme solutions with different concentrations prior to each measurement. For the colorimetric detection of heme in aCSF, a reaction mixture was first prepared by mixing 50.0 μL of different concentration of heme in an aCSF, which was added into 430 μL of G-quadruplex DNA solution containing K^+ (10 mM) in a vial. After 1.0 hour, 10 μL of H_2O_2 (5 mM) and 10 μL of ABTS (50 mM) were added into the above mixture. The resulting mixtures were then incubated for 2.0 hours (see ESI) at 30 °C and subsequently for UV-vis spectrometric measurements. The final concentrations of heme in 500 μL of the resulting mixtures were 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150, 180 and 200 nM.

In vivo microdialysis

Animal surgery and in vivo microdialysis were carried out according to the procedures reported.[18] Briefly, adult male Sprague-Dawley mice (250-300 g) obtained from Health Science Center, Peking University, were fed casually with food and water in a 12:12 h light-dark schedule. Based on standard stereotaxic procedures, the microdialysis guide cannula (BAS/MD-2250, BAS) was implanted into the rat's striatum. The body temperature of the animal was kept at 37 °C with a heating pad during the whole procedure of the surgery. A microdialysis probe was implanted into the rat's striatum and then perfused with aCSF at 1.0 μL/min after the rat recovered for at least 24 h. Subsequently perfusing ceaselessly for at least 90 min for the sake of equilibration, the microdialysate was collected for the colorimetric sensing.

Colorimetric sensing of cerebral free heme

For colorimetric detection of free heme in the brain microdialysate, 50 μL of the microdialysate sample from striatum was added into 430 μL of the G-quadruplex DNA solution containing K^+ (10 mM), and then incubated for about 1.0 hour. Afterwards, 10 μL of H_2O_2 (5 mM) and 10 μL of ABTS (50 mM) were added into the above mixture. For the quantitative assay of free heme in the striatum microdialysate, the resulting mixture was incubated for 2.0 hours at 30 °C for UV-vis spectrometric measurements.

CD measurements

Circular dichroism (CD) spectroscopy of PS2.M (1.0 μM) was measured on a J-815 spectropolarimeter under different conditions at room temperature. PS2.M solutions were prepared in 10 mM of Tris-Ac buffer (pH 7.0). Three scans (100 nm/min) from 225 nm to 325 nm at 0.2 nm intervals were accumulated and averaged. The background of the buffer solution was subtracted from the CD data.

Measurement of UV-vis spectra

All UV-vis absorption spectra were measured on a UV-2550 UV-vis spectrophotometer (Shimadzu, Japan) equipped with a quartz cell (1×0.33 cm cross-section) at room temperature. The maximum absorption wavelength for the radical anion ABTS•$^-$ (the oxidation product of $ABTS^{2-}$) is about 420 nm.

Results and discussion

Principle of colorimetric sensing heme by G-quadruplex DNAzyme

PS2.M (5'-GTGGGTAGGGCGGGTTGG-3'), an 18-base G-rich DNA sequence, can form DNAzyme with high peroxidase-like activity.[42] Since K^+ has individual effects on the peroxidase like activity of G-quadruplex DNAzymes,[43] K^+-stabilized PS2.M was utilized to sense free heme in the cerebral system (see Scheme 1). In the presence of K^+, the conformation of PS2.M changes from a random coil to a "parallel" G-quadruplex structure, which can bind free heme in cerebral system with high affinity.[29] Importantly, the resulting heme/G-quadruplex complex exhibits much higher peroxidase-like activity as compared to pristine heme, which can catalyze the oxidation of colorless $ABTS^{2-}$ to green ABTS•$^-$ in the presence of H_2O_2.[30,42] The presence of free heme in cerebral system is therefore detected indirectly by the absorption enhancement of green ABTS•$^-$. The analytic protocol of heme based on the G-quadruplex structure was assessed by UV-vis absorption spectrum as described in the Experimental Section.

Figure 1 shows the color and the absorption spectra of the H_2O_2-ABTS reaction at different cases. In the presence of PS2.M itself (curve a), the mixture of H_2O_2 and ABTS has no apparent absorption at the range of the wavelength 390-470 nm as well as that in the coexistence of PS2.M and K^+ (curve b), which suggest that neither the random coil of PS2.M nor the "parallel" G-quadruplex structure of PS2.M induced by K^+ have any peroxidase-like catalytic activity. In the presence of PS2.M and heme, the mixture of H_2O_2 and ABTS has weak absorption at about 420 nm (curve c), due to the weak peroxidase-like catalytic property of heme.[36] However, the mixture of H_2O_2 and ABTS shows strong absorption at about 420 nm (curve d) when PS2.M, K^+ and heme were added, suggesting that heme/G-quadruplex

complex possess enhanced peroxidase-like catalytic activity. These results have good accordance with that of corresponding color changes.

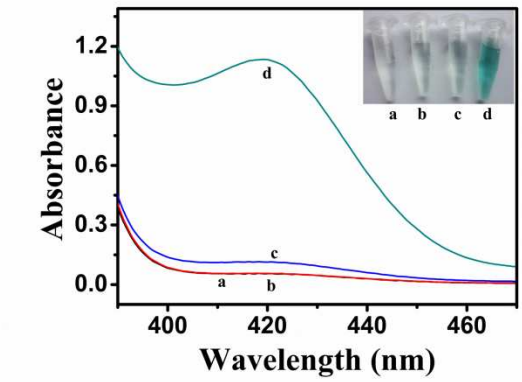


Figure 1. Color and absorption spectra of the H_2O_2-ABTS reaction in different cases. (a. 0.1 mM H_2O_2 + 1 mM $ABTS^{2-}$ + 0.25 μM PS2.M; b. 0.1 mM H_2O_2 + 1 mM $ABTS^{2-}$ + 0.25 μM PS2.M + 10 mM K^+; c. 0.1 mM H_2O_2 + 1 mM $ABTS^{2-}$ + 0.25 μM PS2.M + 100 nM heme; d. 0.1 mM H_2O_2 + 1 mM $ABTS^{2-}$ + 0.25 μM PS2.M + 10 mM K^+ + 100 nM heme.)

To explore the principle of enhanced peroxidase-like catalytic activity of heme/G-quadruplex complex toward H_2O_2-ABTS reaction, CD spectra of PS2.M under different conditions were measured. As shown in Figure 2, the CD spectrum of PS2.M upon addition of K^+ demonstrates a typical characteristic of parallel G-quadruplex structure, a positive peak near 265 nm and a negative peak near 240 nm.[36] This phenomenon illustrates the structural transition of the 18-base G-rich DNA sequence PS2.M in the presence of K^+ in comparison with that in the absence of K^+. When heme, as a contrast, was added into the solution of PS2.M, the CD spectrum of the mixture doesn't show apparent change compared with that of PS2.M alone, which indicates that the heme itself can't promote the formation of G-quadruplex structure. Notably, CD spectrum of PS2.M in the coexistence of K^+ and heme gives rise to a significant change. The intensity near 290 nm has greatly reduced than that in the presence of K^+ alone, which suggests the addition of heme is able to externally stack on the terminal G-tetrad owning to strong π-π interaction.[44,45] These results indicate that heme and G-quadruplex structure formed by G-rich DNA sequence PS2.M in the presence of K^+ are very important to the enhanced color reaction of H_2O_2-ABTS.

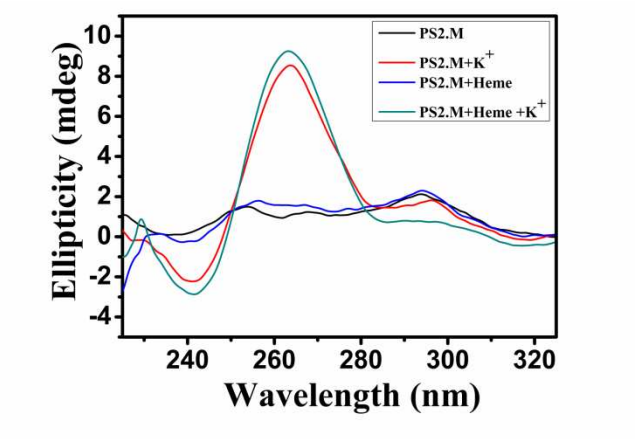


Figure 2. CD spectra of PS2.M under different conditions. (1 μM PS2.M，0.4 μM heme, 40 mM K^+)

Optimization of experimental conditions

The colorimetric biosensor for heme was established based upon G-quadruplex DNAzymes catalyzed the oxidation-reduction reaction of $ABTS^{2-}$ and H_2O_2. Therefore, any factors involved in this reaction may affect the sensitivity of the sensor, such as concentrations of H_2O_2, $ABTS^{2-}$, PS2.M and K^+, temperature and pH of Tris-Ac buffer.

H_2O_2 and ABTS are key components in this color reaction. Herein, the optimization of their concentrations is important for the assay of heme. The effect of the concentrations of H_2O_2 and ABTS were studied over the range of 0-400 μM and 0-2 mM, respectively. As shown in Figure 3a, the absorption of reaction mixture (100 nM heme, 1 mM $ABTS^{2-}$, 0.25 μM PS2.M, 10 mM K^+, 30 °C, pH 7.0) at 420 nm increased with the increase of H_2O_2 during the range of 0-80 μM, and kept a plateau when H_2O_2 varied from 80 μM to 120 μM, and then decreased with the increase of H_2O_2 concentration. The absorption of reaction mixture (100 nM heme, 100 μM H_2O_2, 0.25 μM PS2.M, 10 mM K^+, 30 °C, pH 7.0) at 420 nm increased with the increase of ABTS during the range of 0-0.8 mM, and then kept a plateau at the range of 0.8-2.0 mM (Figure 3b). According to the above results, the optimal concentration of H_2O_2 and ABTS for the detection of heme is 100 μM and 1 mM, respectively.

PS2.M and K^+ are necessary to form G-quadruplex-based DNAzyme. The peroxidase-like catalytic activity of this DNAzyme is closely related with the concentrations of PS2.M and K^+. The concentrations of PS2.M and K^+ were investigated over the range of 0-0.4 μM and 0-25 mM, respectively, as demonstrated in Figure 3c and Figure 3d. The absorption of reaction mixture (100 nM heme, 100 μM H_2O_2, 1 mM $ABTS^{2-}$, 10 mM K^+, 30 °C, pH 7.0) at 420 nm increased rapidly with the

increase of PS2.M at the range of 0-0.2 μM, reached a plateau at concentration of 0.2 μM, and kept constant even when the concentration of PS2.M increased to 0.4 μM. With the increase of K^+, the absorption of reaction mixture (100 nM heme, 100 μM H_2O_2, 1 mM $ABTS^{2-}$, 0.25 μM PS2.M, 30 °C, pH 7.0) at 420 nm increased sharply in the range of 0-7.5 mM and then kept a plateau at the concentration of K^+ even as 25 mM. Based on above mentioned, 0.25 μM PS2.M and 10 mM K^+ were used as optimal conditions for the detection of heme.

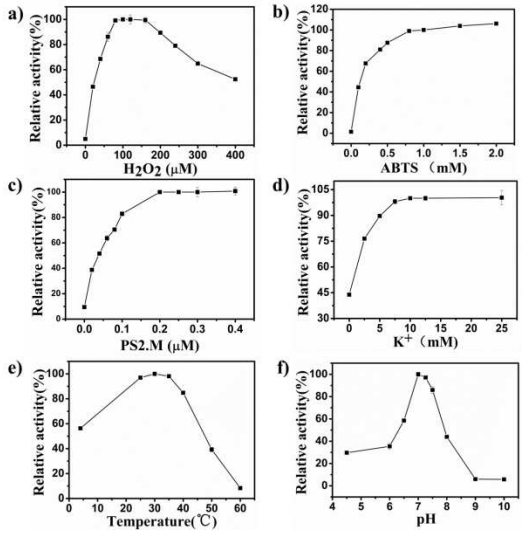


Figure 3. Effect of different conditions on the absorption of the solution, including concentrations of H_2O_2 (a), $ABTS^{2-}$ (b), PS2.M (c) and K^+ (d), temperature (e) and pH value of Tris-Ac buffer (f). The error bars represent the standard deviation of the three measurements. The optimal point in each curve was set as 100 %. (a. 100 nM heme + 1 mM $ABTS^{2-}$ + 0.25 μM PS2.M + 10 mM K^+, 30 °C, pH 7.0; b. 100 nM heme + 100 μM H_2O_2 + 0.25 μM PS2.M + 10 mM K^+, 30 °C, pH 7.0; c. 100 nM heme + 100 μM H_2O_2 + 1 mM $ABTS^{2-}$ + 10 mM K^+, 30 °C, pH 7.0; d. 100 nM heme + 100 μM H_2O_2 + 1 mM $ABTS^{2-}$ + 0.25 μM PS2.M, 30 °C, pH 7.0; e. 100 nM heme + 100 μM H_2O_2 + 1 mM $ABTS^{2-}$ + 0.25 μM PS2.M + 10 mM K^+, pH 7.0; f. 100 nM heme + 100 μM H_2O_2 + 1 mM $ABTS^{2-}$ + 0.25 μM PS2.M + 10 mM K^+, 30 °C.)

Temperature and pH value of Tris-Ac buffer are crucial to the catalytic activity of G-quadruplex-based DNAzymes. In view of the sensitivity of the method, temperature and pH value of Tris-Ac buffer were optimized as well. Herein, the influence of temperature in the range of 4-60 °C and pH value of Tris-Ac buffer in the range of 4.5-10 on the reaction system were demonstrated in Figure 3e and Figure 3f, respectively. The absorption of reaction mixture (100 nM heme, 100 μM H_2O_2,1 mM $ABTS^{2-}$, 0.25 μM PS2.M, 10 mM K^+, pH 7.0) at 420 nm increased with the temperature over the range of 4-25 °C, kept a plateau when the temperature varied between 25-35 °C, and declined rapidly at temperature above 40 °C. The absorption of the reaction mixture (100 nM heme, 100 μM H_2O_2, 1 mM $ABTS^{2-}$, 0.25 μM PS2.M, 10 mM K^+, 30 °C) at 420 nm increased with the pH value of Tris-Ac buffer over the range of 4.5-7.0, and decreased with the increase of pH above 7.0. Therefore, 30 °C and pH 7.0 of Tris-Ac buffer were selected as the optimal reaction environment.

Selectivity and sensitivity

Using the G-quadruplex formation of PS2.M in the presence of K^+ as the probe, we studied the selectivity of the colorimetric method for heme sensing. Among the species that may potentially interfere with the selectivity of heme sensing, physiologically important species such as DA, DOPAC, AA, cysteine, UA, glucose and lactate in the cerebral system were taken into account (Figure 4). The existence of each of these species did not trigger the obvious changes of the H_2O_2-ABTS reaction with G-quadruplex DNAzymes except that heme demonstrated significant absorbance at 420 nm under the same conditions. These results substantially suggest that only the resulting heme/G-quadruplex complex can catalyze H_2O_2-ABTS reaction, which produces enhanced color reaction (green $ABTS^{\bullet-}$). That's to say, the present detection method based on the rational design of G-quadruplex formation of PS2.M in the presence of K^+ is very specific for cerebral heme sensing.

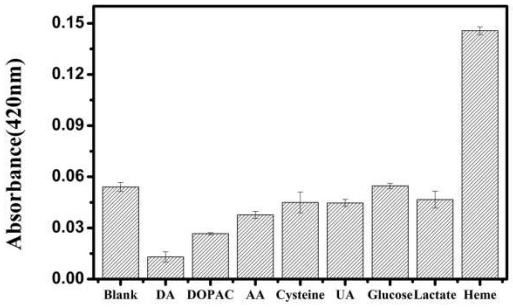


Figure 4. Selectivity of heme detection. All measurements were performed in 0.5 mL of 10 mM Tris-Ac buffer solutions (pH 7.0, 30 °C) containing 100 μM H_2O_2, 1 mM $ABTS^{2-}$, 0.25 μM PS2.M and 10 mM K^+. The error bars represent the standard deviation of three measurements. The concentration was 10 nM for heme, 50 nM for DA, DOPAC, AA, cysteine, UA, glucose and lactate.

To evaluate the sensitivity of the method, a series of heme in aCSF with different concentrations were added into the Tris-Ac buffer aqueous containing G-quadruplex DNAzymes to catalyze the H_2O_2-ABTS reaction. As shown in Figure 5, the absorbance

at 420 nm increased with the concentration of heme in aCSF and showed a linear response toward heme within a concentration range from 1 nM to 120 nM in Tris-Ac buffer solutions (pH 7.0, 30 °C) containing 100 μM H_2O_2, 1 mM $ABTS^{2-}$, 0.25 μM PS2.M and 10 mM K^+. The linear regression equation was A = 0.0111C + 0.0472 (C: nM, R^2=0.999), with a detection limit of 0.637 nM (3σ/slope). The enhanced color changes eventually form a straightforward basis for colorimetric sensing of heme.

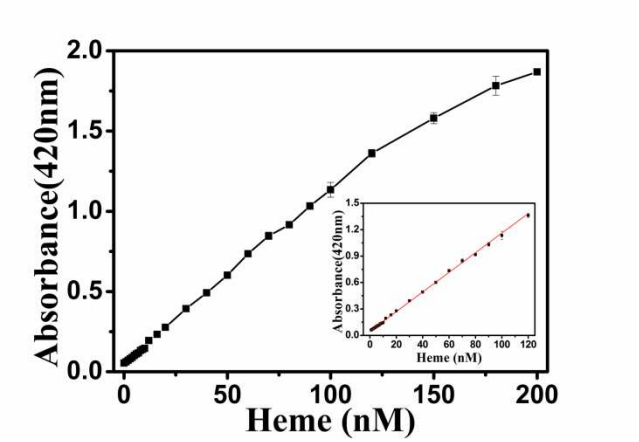


Figure 5. The heme concentration dependent UV-Vis absorption of ABTS-H_2O_2 system in the presence of G-quadruplex DNAzymes for colorimetric heme analysis at 420 nm. The inset shows a linear range of heme concentration from 1 nM to 120 nM. The error bars represent the standard deviation of the three measurements.

Colorimetric sensing of heme in rat brain

To explore the practicability of the as-established G-quadruplex-based assay for selectively sensing heme in the rat brain, the brain microdialysate (50 μL) was added into 430 μL of an Tris-Ac buffer aqueous containing formed G-quadruplex structure, which were incubated for 1.0 hour. Afterwards, 10 μL of H_2O_2 (5 mM) and 10 μL of ABTS (50 mM) were added into the above mixture. For the quantitative assay of free heme in the striatum microdialysate, the resulting mixture was incubated for 2.0 hours at 30 °C for UV-vis spectrometric measurements. In view of heme externally stacking on the terminal G-quadruplex structure through strong π-π interaction, the obtained heme/G-quadruplex DNAzyme exhibits high peroxidase-like activity, which can catalyze the oxidation of colorless $ABTS^{2-}$ to green $ABTS^{\bullet-}$ by H_2O_2. The presence of free heme in cerebral system is therefore detected indirectly by the absorption of green $ABTS^{\bullet-}$. It is important to note that the heme in cerebral system is called free heme since no concentrated proteins exist in the cerebral microdialysis as the result of the use of microdialysis for in vivo sampling. According to the calibration curve described above A = 0.0111C + 0.0472 (C: nM, R^2=0.999) (Figure 5), the initial value of the basal level of heme in rat brain microdialysates was determined to be 32.8 ± 19.5 nM (n=3) , which was almost consistent with the reported values.[46] These results substantially demonstrated that the colorimetric assay developed in this study would offer an effective way to directly selective sensing of heme in the cerebral system. In order to demonstrate the universal applicability of this method, we also detect heme in rat serum samples. (see ESI)

A comparison of various methods for heme estimation shows that the heme assay based on the high peroxidase-like activity of G-quadruplex/heme complexes is more sensitive than other methods and, the low detection limit and high selectivity have made our approach a potential method amplifier for several biomarker events based on heme. In addition, this assay does not need expensive instruments, sophisticated material synthysis and toxic reagents. The labeled free G-rich nucleic acid sequences for aptamer-based heme analysis made the process simple and easy.

Table 1 Comparison of different methods for the determination of heme

Technique	Linear range (nM)	Detection limit (nM)	Reference
Mass spectrometry	400-8000	400	19
Fluorescent spectrometry	10-500	5	23
	310-2500	50	24
Absorption spectrophotometry	4-130	---	21
	1-120	0.637	this work

Conclusions

In the present work, a highly selective and sensitive colorimetric method for directly sensing of free heme in cerebral system was established based on the rational design of G-quadruplex DNAzymes. In the presence of K^+, the conformation of PS.2M changes from a random coil to a "parallel" G-quadruplex structure, which can bind free heme in cerebral system with high affinity. Importantly, the resulting heme/G-quadruplex complex exhibits high peroxidase-like activity, which can catalyze the oxidation of colorless $ABTS^{2-}$ to green $ABTS^{\bullet-}$ by H_2O_2. As a result, the content of free heme in cerebral system is detected indirectly and easily by the absorption enhancement of green $ABTS^{\bullet-}$. As far as we know, this is the first example of the direct sensing of heme in rat brain. In a word, the simple quantification of heme in the cerebral system herein not only gives us a simple model to monitor brain chemistry, but also facilitates us to understand the chemical essence involved in some physiological and pathological events. This study

unambiguously provides a new analytical platform to comprehend brain chemistry by G-quadruplex structure.

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Notes and references

[†] *Beijing National Laboratory for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China.* [a]*Key Laboratory of Molecular Nanostructure and Nanotechnology,* [b]*Key Laboratory of Analytical Chemistry for Living Biosystems.*

[‡]*Key Laboratory of Photochemical Conversion and Optoelectronic Materials, Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing 100190, P.R. China.*

***** *Corresponding author. Tel: 86-10-62561085; Fax:86-10-62561085. E-mail:shucy@iccas.ac.cn*

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