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A colorimetric sensor for hydrogen sulfide detection using direct inhibition of active site in G-quadruplex DNAzyme

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ABSTRACT

Because of the toxicity of hydrogen sulfide and its important role in various physiological processes, the development of a selective and sensitive hydrogen sulfide sensing method is vital. In this study, a colorimetric method for hydrogen sulfide detection was developed by exploiting the direct inhibition of active site in G-quadruplex DNAzyme by hydrogen sulfide. Hydrogen sulfide inactivates the peroxidase-like activity of PS2.M-hemin G-quadruplex DNAzyme by directly blocking the active site of the DNAzyme, allowing the label-free colorimetric detection of hydrogen sulfide by the naked eye by using 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{2-}$) as a peroxidase substrate. From titration results, the detection limit of this colorimetric hydrogen sulfide sensor was estimated to be 410 nM. In addition, the sensor is highly selective for hydrogen sulfide over various anions and was used to determine hydrogen sulfide concentrations in tap water samples.

1. Introduction

Sulfide (S^{2-}) is a sulfur dianion formed as an industrial byproduct and a microbial reduction of sulfate or sulfur-containing amino acids, for example, cysteine or glutathione by anaerobic bacteria [1]. The detection of sulfide is crucial because of its high toxicity. Exposure to sulfide can lead to unconsciousness, irritation of mucous membranes, and, in cases of high-level exposure, respiratory paralysis [2,3]. In addition, several studies have demonstrated that H_2S (protonated S^{2-} in water) is involved in some physiological processes. For example, H_2S functions as both an endothelium-derived relaxing factor (EDRF) and an endothelium-derived hyperpolarizing factor (EDHF) [4]. In addition, sulfide acts as a relaxant of smooth muscle in a similar manner to carbon monoxide (CO) and nitric oxide (NO) [5]. Moreover, the levels of sulfide in human are affected by diseases such as Down's syndrome [6], Alzheimer's disease [7,8], and liver cirrhosis [9]; consequently, sulfide can be used as a diagnostic indicator of illness. Therefore, the development of sulfide detection methods is critical.

For this reason, many detection methods for hydrogen sulfide have been developed, including electrochemical methods [10–13], atomic fluorescence spectrometry [14], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [15], and ion chromatography [16]. However, these methods have drawbacks, often requiring expensive instrumentation and lengthy analysis. In contrast, colorimetric detection systems are very attractive because the target can be detected quickly by the naked eye without expensive instrumentation.

The G-quadruplex structure is a secondary DNA structure, stabilized by the G-tetrad, a square planar structure formed between four guanidine bases via Hoogsteen hydrogen bonds [17]. Interestingly, some G-quadruplex structure (such as PS2.M:GTGGGTAGGGCGGGTTGG) show peroxidase-like activities when bound to hemin, effectively catalyzing the H_2O_2-mediated oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salts ($ABTS^{2-}$) to a green colored radical cation ($ABTS^{\bullet+}$) [18,19]. This phenomenon facilitates the utilization of these G-quadruplex DNAzymes as label-free colorimetric probes for metal ions [20–22], DNA [23–25], or other biomolecules [26–28]. These sensor systems mostly utilize changes in G-quadruplex DNAzyme activity that arises on conformational changes to the DNAzyme induced by the analyte. For example, Dong et al. developed a colorimetric sensor for Pb^{2+} utilizing the Pb^{2+}-induced allosteric effect on G-quadruplex DNAzymes [29]. In addition, colorimetric Hg^{2+} sensors using changes in G-quadruplex DNAzyme activity induced by conformational changes on the formation of thymine-Hg^{2+}-thymine complex have been reported [30,31].

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Kong et al. reported a Ag^{+} and cysteine sensing platform based on G-quadruplex DNAzyme disruption [32].

Unlike biosensors that rely on conformational changes to alter the activity of the G-quadruplex DNAzyme, our simple colorimetric hydrogen sulfide sensor is based on the direct inhibition of the active site in G-quadruplex DNAzyme. As shown in Scheme 1, hydrogen sulfide binds directly to hemin, inactivating the peroxidase-like activity of G-quadruplex DNAzyme by blocking the active site. As a result, G-quadruplex DNAzyme cannot catalyze the H_2O_2-mediated oxidation of $ABTS^{2-}$, allowing label-free colorimetric detection of hydrogen sulfide.

2. Experimental section

2.1. Materials and methods

The G-quadruplex sequence, PS2.M (5'-GTGGGTAGGGCGGGTTGG-3'), was purchased from Bioneer (South Korea). Bovine hemin, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{2-}$), Triton X-100, H_2O_2 (35 wt %), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), potassium chloride, and sodium salts of the anions (S^{2-}, F^{-}, Cl^{-}, Br^{-}, I^{-}, CN^{-}, HSO_4^{-}, NO_3^{-}, OAc^{-}, CO_3^{2-}, and SO_4^{2-}) were purchased from Sigma-Aldrich. All chemicals were used without further purification. UV-Vis spectrometry studies were carried out on a JASCO V-630 spectrophotometer. CD spectra were obtained with JASCO J-815 spectropolarimeter.

2.2. Preparation of G-quadruplex DNAzymes

G-quadruplex DNAzymes were prepared following a literature procedure [29]. To prepare the K^{+}-stabilized G-quadruplex DNAzyme, the oligonucleotide (10 μM) was heated at 88 °C for 10 min and then cooled to room temperature in Tris buffer (20 mM, pH 7.5). After cooling, KCl (10 mM) was added to the solution, which was incubated for 40 min. Then, the mixture was incubated with Triton X-100 (0.05% (w/v)) and hemin (10 μM) over 1 h.

2.3. Colorimetric assay for hydrogen sulfide detection

Mixtures of KCl (10 mM), Triton X-100 (0.05% (w/v)), $ABTS^{2-}$ (2 mM), Na_2S (0, 0.5, 0.8, 1, 2, 5, 8, 10, 15, 20, 30, and 50 μM), and DNAzyme (0.2 μM) were prepared in HEPES buffer (20 mM, pH 8.0). After transferring the mixtures to a cell, H_2O_2 (1 mM) was added to start the peroxidation reaction. The mixtures were incubated for 10 min, and then analyzed using a UV-Vis spectrophotometer at a wavelength of 418 nm to determine the changes to $ABTS^{2-}$.

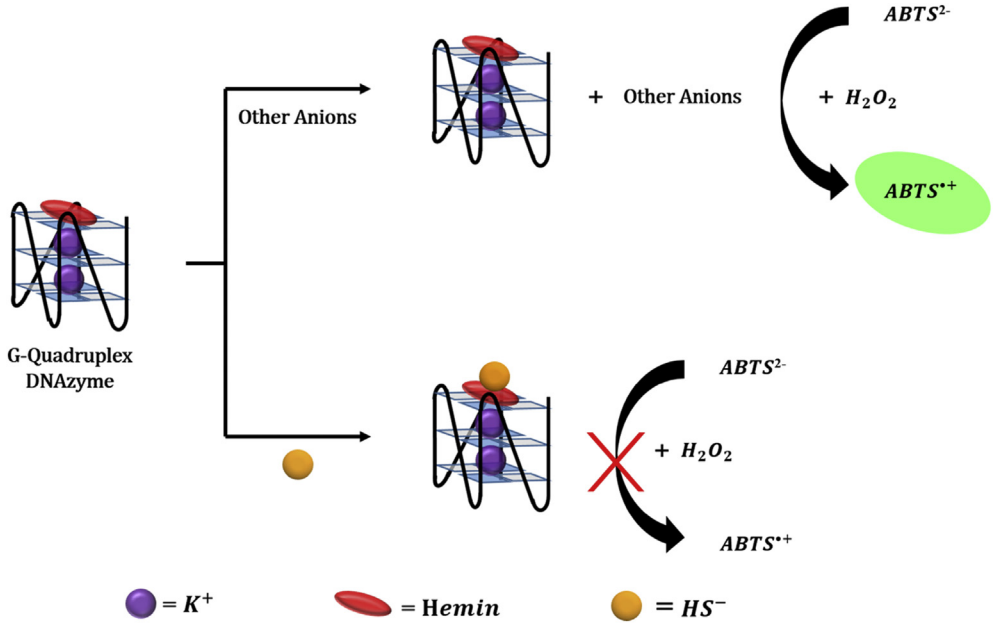
2.4. Mechanistic studies of hydrogen sulfide detection using DNAzyme

To obtain absorbance spectra of the DNAzyme, Na_2S (0, 10, 20, 50, 80, 100, 200, and 250 μM) was added to HEPES buffer (20 mM, pH 8.0) containing DNAzyme (1 μM), KCl (10 mM), and Triton X-100 (0.05% (w/v)) and the mixtures were incubated for 10 min. Then changes to the DNAzyme were analyzed by UV-Vis spectrophotometer.

To obtain circular dichroism spectra of the DNAzyme, Na_2S (0, 100, 500 μM and 1, and 2.5 mM) was added to the HEPES buffer (20 mM, pH 8.0) containing DNAzyme (10 μM), KCl (10 mM), and Triton X-100 (0.05% (w/v)) and the mixtures were incubated for 10 min. Changes to the DNAzyme were analyzed by CD spectropolarimeter.

2.5. Selectivity tests for hydrogen sulfide detection

Mixtures of KCl (10 mM), Triton X-100 (0.05% (w/v)), $ABTS^{2-}$ (2 mM), various anions (S^{2-}, F^{-}, Cl^{-}, Br^{-}, I^{-}, CN^{-}, HSO_4^{-}, NO_3^{-}, OAc^{-}, CO_3^{2-}, and SO_4^{2-}, 50 μM), and DNAzyme (0.2 μM) were prepared in HEPES buffer (20 mM, pH 8.0). After transferring the mixtures to a cell, H_2O_2 (1 mM) was added to start the peroxidation reaction. The mixtures were incubated for 10 min, and then analyzed using a UV-Vis spectrophotometer at a wavelength of 418 nm to determine the changes to $ABTS^{2-}$.



Scheme 1. A schematic illustration of the colorimetric hydrogen sulfide sensor using G-quadruplex DNAzyme.

2.6. Interference effects of other anions

Mixtures of KCl (10 mM), Triton X-100 (0.05% (w/v)), $ABTS^{2-}$ (2 mM), Na_2S (50 μM), and DNAzyme (0.2 μM) were prepared in the presence of various anions (500 μM) in HEPES buffer (20 mM, pH 8.0). After transferring the mixtures to a cell, H_2O_2 (1 mM) was added to start the peroxidation reaction. The mixtures were incubated for 10 min, and then analyzed using a UV-Vis spectrophotometer at a wavelength of 418 nm to determine the changes to $ABTS^{2-}$.

2.7. Determination of concentrations of hydrogen sulfide in tap water samples

To investigate the applications to real samples, Na_2S (0.2 M) solutions were prepared in tap water. Mixtures of KCl (10 mM), Triton X-100 (0.05% (w/v)), $ABTS^{2-}$ (2 mM), Na_2S (5, 15, and 20 μM), and DNAzyme (0.2 μM) were prepared in HEPES buffer (20 mM, pH 8.0). After transferring the mixtures to a cell, H_2O_2 (1 mM) was added to start the peroxidation reaction. The mixtures were incubated for 10 min, and then analyzed using a UV-Vis spectrophotometer at a wavelength of 418 nm to determine the changes to $ABTS^{2-}$. The absorbance was compared with titration results of S^{2-} in deionized water.

3. Results and discussion

3.1. Colorimetric assay for hydrogen sulfide detection

Of the various G-quadruplex structures, we selected PS2.M to develop our colorimetric hydrogen sulfide sensor because its peroxidase activity has been studied well under different conditions [18]. As shown in Fig. 1(a), PS2.M-hemin complexes show peroxidase-like activity and efficiently oxidize $ABTS^{2-}$ to $ABTS^{\bullet+}$. Furthermore, the addition of sodium sulfide induced inactivation of G-quadruplex DNAzyme activity, as demonstrated by a decrease in the absorbance at 418 nm; as a result, the solution changed from green to colorless. As shown in Fig. 1(b), the observed absorbance at 418 nm decreased proportionally with the sodium sulfide concentration. From the titration results, the detection limit of our colorimetric sensor system for hydrogen sulfide was estimated to be 410 nM (Fig. S1), an acceptable value for the determination of

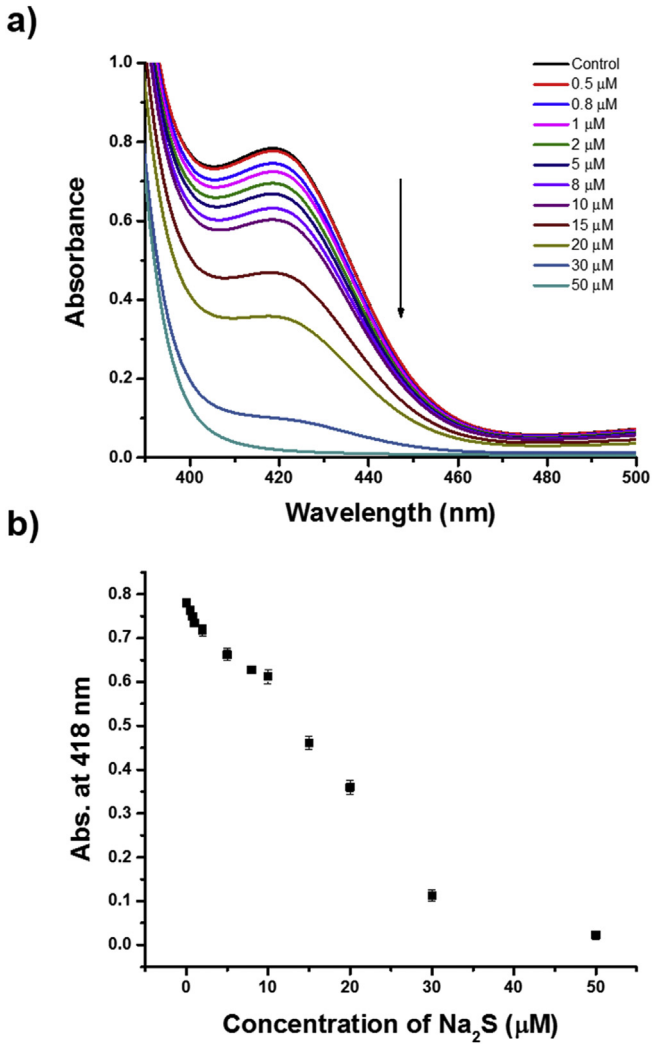


Fig. 1. a) UV-Vis spectra obtained 10 min after the addition of the H_2O_2 (1 mM) to the HEPES buffer (20 mM, pH 8.0) containing KCl (10 mM), Triton X-100 (0.05% (w/v)), $ABTS^{2-}$ (2 mM), DNAzyme (0.2 μM), and various concentrations of Na_2S; b) Plot of the absorbance at 418 nm against concentrations of Na_2S.

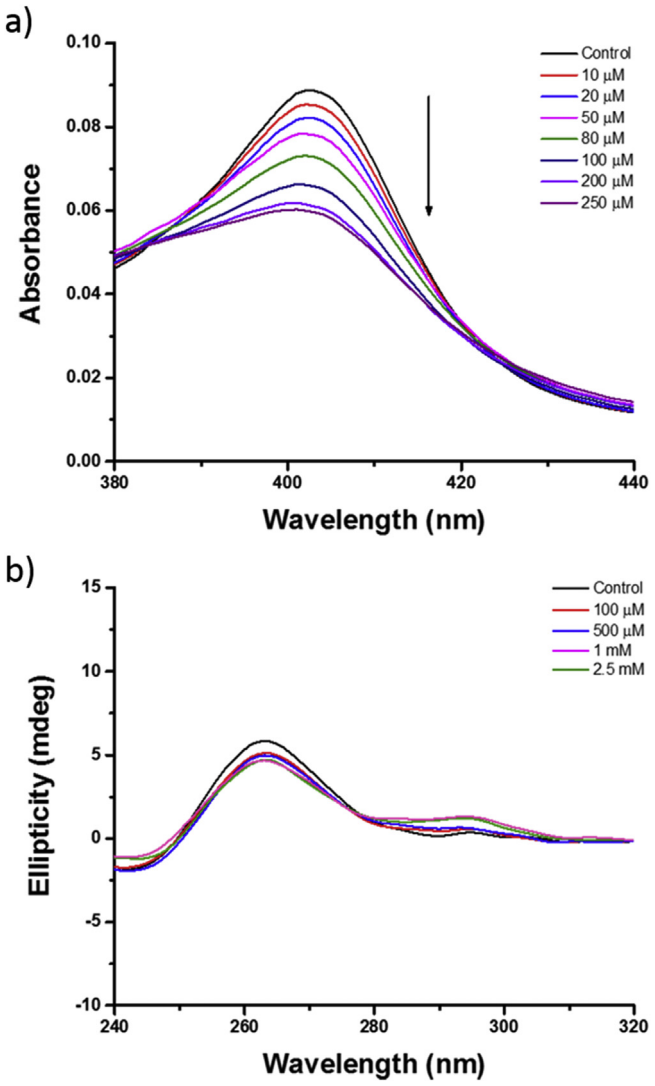


Fig. 2. a) UV-Vis spectra of the DNAzyme (1 μM) 10 min after the additions of Na_2S in HEPES buffer (20 mM, pH 8.0) containing KCl (10 mM) and Triton X-100 (0.05% (w/v)); b) The CD spectra of DNAzyme (10 μM) obtained 10 min after the addition of Na_2S to the HEPES buffer (20 mM, pH 8.0) containing KCl (10 mM) and Triton X-100 (0.05% (w/v)).

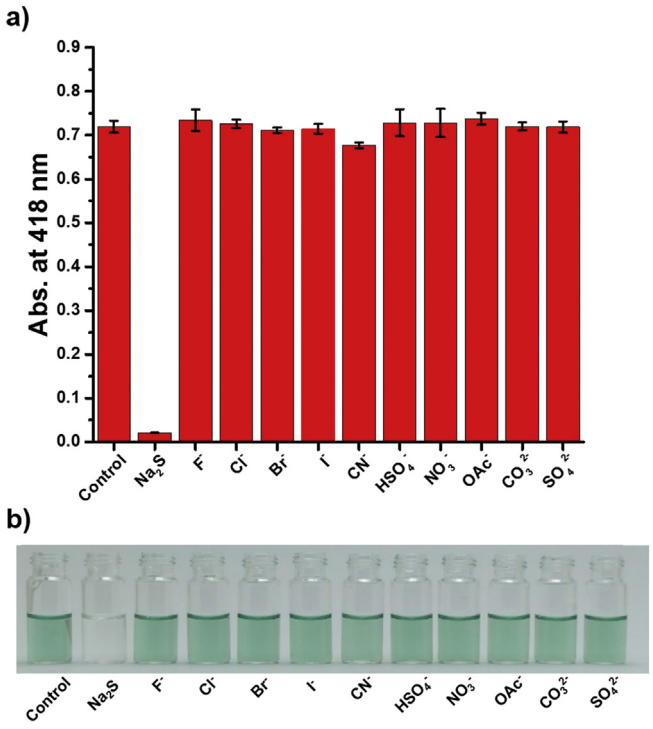


Fig. 3. a) Plot of the absorbance at 418 nm obtained 10 min after the addition of H_2O_2 (1 mM) to the HEPES buffer (20 mM, pH 8.0) containing KCl (10 mM), Triton X-100 (0.05% (w/v)), $ABTS^{2-}$ (2 mM), DNAzyme (0.2 μM) with various anions (50 μM); b) The color of the $ABTS^{2-}$ (2 mM), H_2O_2 (1 mM) reaction mixture in the presence of different anions (50 μM). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hydrogen sulfide concentration in drinking water, as stipulated by the World Health Organization limit (within ~15 μM) [33].

3.2. Mechanistic studies of hydrogen sulfide detection using DNAzyme

The proposed mechanism for hydrogen sulfide detection (Scheme 1) is supported by UV-Vis absorption spectroscopy and circular dichroism (CD) measurements. As shown in Fig. 2(a), the addition of sodium sulfide to PS2.M-hemin DNAzyme led to a decrease in the absorbance at 402 nm, and a similar pattern was observed in the case of the addition of sodium sulfide to hemin alone (Fig. 4). Furthermore, the CD spectra (Fig. 2(b)) of PS2.M-hemin DNAzyme in the absence and presence of sodium sulfide are not changed significantly. These spectroscopic results imply that hydrogen sulfide directly interacts with the hemin of the PS2.M-hemin DNAzyme and did not induce significant conformational changes to the G-quadruplex structure.

3.3. Selectivity tests for hydrogen sulfide detection

To test the selectivity for hydrogen sulfide, we investigated the changes of G-quadruplex DNAzyme activity by other anions. The absorbance of the $ABTS^{2-}$-H_2O_2 (1 mM) reaction mixture with G-quadruplex DNAzyme was recorded in the presence of different physiologically and environmentally important anions (50 μM). As shown in Fig. 3(a), of the various tested anions, only hydrogen sulfide inactivated the peroxidase-like activities of the PS2.M-hemin DNAzyme. As a result, $ABTS^{2-}$ is not oxidized to $ABTS^{\bullet+}$, allowing the colorimetric detection of hydrogen sulfide by the naked eye and selectively (Fig. 3(b)).

Interestingly, cyanide (CN^-) did not affect the PS2.M-hemin DNAzyme activity even though it can inhibit horseradish peroxidase (HRP) activity, in some cases more effectively than hydrogen sulfide [34]. To evaluate the reason of this phenomena, hemin was titrated with sodium sulfide and sodium cyanide respectively (Fig. 4). As a result, the titration curves was very similar with activity of DNAzyme in the presence of hydrogen sulfide and cyanide respectively and saturation point of cyanide was much higher than sulfide (Fig. S4 and S5). This results imply that hydrogen sulfide more efficiently blocks the active site (hemin) in the G-quadruplex DNAzyme than cyanide. Also, selectivity test of G-quadruplex DNAzyme over His and Cys known as binding groups to hemin in peroxidase active sites was performed. As a result, His nearly did not inhibit the peroxidase like activity of G-quadruplex DNAzyme, whereas Cys inhibited the activity moderately (Fig. S6).

3.4. Interference effects of other anions

Interference of other anions on hydrogen sulfide sensing was assessed by measuring the changes in hydrogen sulfide-induced PS2.M-hemin DNAzyme peroxidase activity in the presence of the

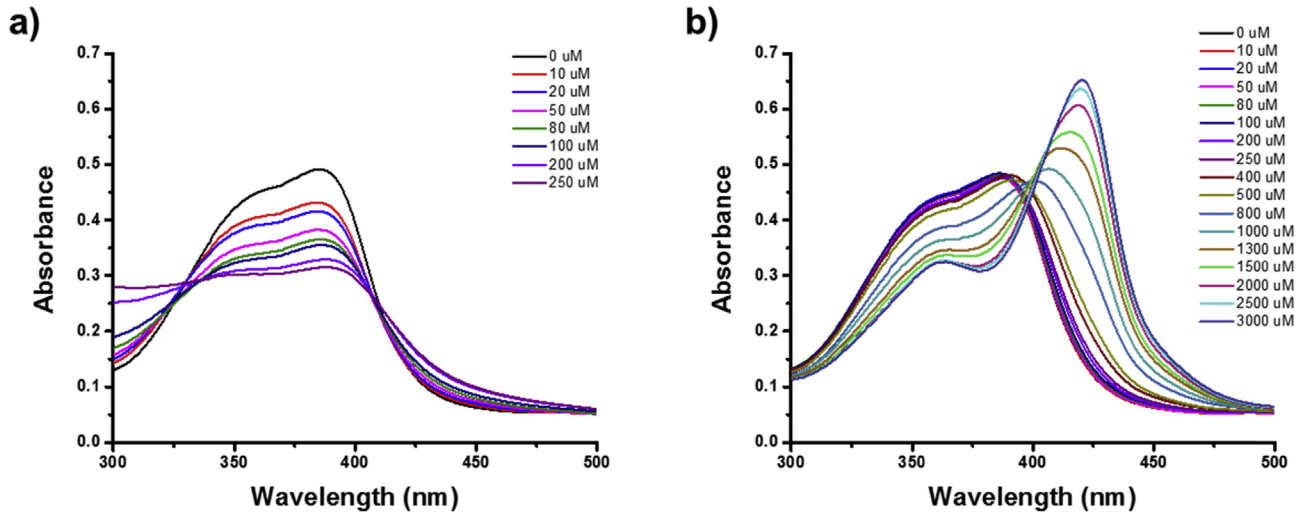


Fig. 4. The UV-Vis spectra of hemin (10 μM) after the addition of various concentration of anion in HEPES buffer (20 mM, pH 8.0). a) Na_2S (0–250 μM), b) NaCN (0–3000 μM).

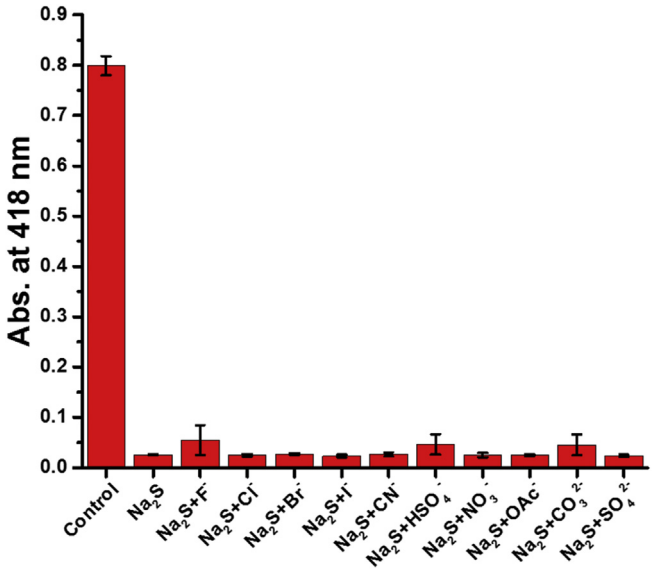


Fig. 5. Absorbance at 418 nm obtained 10 min after the addition of H_2O_2 (1 mM) to the HEPES buffer (20 mM, pH 8.0) containing KCl (10 mM), Triton X-100 (0.05% (w/v)), $ABTS^{2-}$ (2 mM), DNAzyme (0.2 μM), and Na_2S (50 μM) in the absence and presence of anions (500 μM).

Table 1
Measurement of hydrogen sulfide concentrations in tap water samples.

Sample	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
Tap water	20.0	20.5	102.7	5.1
	15.0	14.4	96.0	3.9
	5.0	4.5	91.0	6.6

interference anions (10 equiv.). As shown in Fig. 5, the selectivity for hydrogen sulfide was not affected by the presence of other common interfering anions, which may be the reason why hydrogen sulfide effectively inactivated the PS2.M-hemin DNAzyme activity more efficiently than other anions.

3.5. Determination of concentrations of hydrogen sulfide in tap water samples

To demonstrate the ability of the developed colorimetric sensor system to analyze hydrogen sulfide in a practical sample, the concentrations of hydrogen sulfide in tap water samples were determined. The tap water real samples were prepared according to a previous literature method [35]. Table 1 lists the results of the determination of concentrations of hydrogen sulfide by UV-Vis absorption. From Table 1, the recoveries were found to be 91.0–102.7% with relative RSDs ranging from 3.9% to 6.6%. These results indicate that our sensor system has good precision and accuracy for the detection of hydrogen sulfide in tap water samples.

4. Conclusions

In summary, we have developed a simple colorimetric hydrogen sulfide sensor using a G-quadruplex DNAzyme. Unlike previous conformational change modulated G-quadruplex DNAzyme-based sensors, our sensor uses direct inhibition of active site in G-quadruplex DNAzyme by hydrogen sulfide without any conformational changes to the G-quadruplex. Hydrogen sulfide caused inactivation of the peroxidase-like activities of the G-quadruplex DNAzyme by directly blocking the active site, allowing the label-free colorimetric detection of hydrogen sulfide with the naked eye by using $ABTS^{2-}$ as a peroxidase substrate. This sensor system was highly selective for hydrogen sulfide over other anions, and it allowed colorimetric assay of hydrogen sulfide to 410 nM. Moreover, the sensor system was used to determine the hydrogen sulfide concentrations of tap water samples. These results demonstrate that this sensor system has potential applications in environmental systems for hydrogen sulfide detection.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dyepig.2016.11.050.

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