Analytica Chimica Acta 1008 (2018) 90–95

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Nickel-mediated allosteric manipulation of G-quadruplex DNAzyme for highly selective detection of histidine

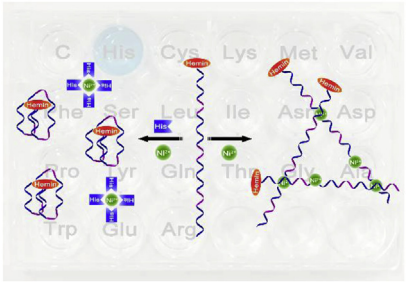
Zijun Li [a], Jian Zhao [a], Zhaoyin Wang [a, **], Zhihui Dai [a, b, *]

[a] Jiangsu Collaborative Innovation Center of Biomedical Functional Materials and Jiangsu Key Laboratory of Biofunctional Materials, School of Chemistry and Materials Science, Nanjing Normal University, Nanjing, 210023, PR China
[b] Nanjing Normal University Center for Analysis and Testing, Nanjing, 210023, PR China

HIGHLIGHTS

- Nickel-mediated allostery of G-quadruplex is reported for the first time.
- Activity of G-quadruplex DNAzyme is manipulated by nickel-mediated allostery and nickel-histidine affinity pair.
- Histidine in biological fluids can be facilely and directly distinguished in the absence of additional reagents.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 17 October 2017
Received in revised form
30 November 2017
Accepted 11 December 2017
Available online 3 January 2018

Keywords:
Nickel-mediated allosteric manipulation
G-quadruplex DNAzyme
High selectivity
Histidine quantification

ABSTRACT

Since abnormal metabolism of histidine (His) is defined as an indicator of several diseases, detection of His in biological fluids becomes increasingly urgent to us. However, due to similar structures and properties of different amino acids, selective quantification of His is difficulty, and typically needs the participation of special reagents. In this work, we report for the first time that nickel ions (Ni^{2+}) can induce the allostery of G-quadruplex, and is thus able to manipulate the activity of G-quadruplex DNAzyme. Experimental results indicate the interaction between Ni^{2+} and guanine is critical to the allostery. In comparison with Ni^{2+}-guanine interaction, Ni^{2+}-His interaction exhibits higher affinity. Therefore, a colorimetric His biosensor is fabricated, and His can be facilely discriminated by naked eyes. Relying on the high activity of DNAzyme, His in a range of 50 nM–400 μM is determined with this method, and low detection limit (36 nM) is obtained. More importantly, His can be directly distinguished in the absence of other toxic reagents. In addition, the amount of His in serum is also measured, suggesting the applicability of this biosensor in real sample detection. Overall, this work provides an alternative way to design G-quadruplex DNAzyme-based analytical approaches.

1. Introduction

Since specific DNA structures are the basis of molecular recognition and signal transduction, metal ions-mediated DNA allostery plays an important role in designing analytical protocols [1,2]. According to metal ions-induced mismatches, such as T-Hg^{2+}-T and C-

* Corresponding author. Jiangsu Collaborative Innovation Center of Biomedical Functional Materials and Jiangsu Key Laboratory of Biofunctional Materials, School of Chemistry and Materials Science, Nanjing Normal University, Nanjing, 210023, PR China.
** Corresponding author.
E-mail address: daizhihuii@njnu.edu.cn (Z. Dai).

https://doi.org/10.1016/j.aca.2017.12.040
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Ag^+-C [3,4], single-stranded DNA may form metal ions-bridged duplex structures, which are key principles of various Hg^{2+} and Ag^+ biosensors [5–7]. In addition, with the help of metal ions, diverse DNA structures can be constructed, broadening the detection realm from small molecules to whole cells [8–12]. Besides as building blocks, DNA can also be used as catalytic oligonucleotides that are so-called DNAzyme [13–15]. Structures of DNAzymes are fundamental to their activities, thus metal ions can manipulate activities of DNAzymes by allosteric regulation. Obviously, to design more advanced biosensors, surveying metal ions-mediated allosteric manipulation of DNAzyme is of great value.

Histidine (His) is one of essential amino acids (AAs), and abnormal metabolism of His has been defined as an indicator of several diseases. The decrease of His will cause Friedreich ataxia, Parkinson's disease, and epilepsy [16], while the increase of His may lead to symptoms of intoxication [17]. Accordingly, detection of His in biological fluids has become increasingly urgent, and a number of bioanalytical methods have been developed. Currently, His biosensors are designed mainly using fluorescent probe [18], His aptamer [19], Cu^{2+}-modulated silver nanoclusters [20], and Ni^{2+}-based nanomaterials [21,22]. Although the aforementioned approaches have their own merits, these assays suffer from complicated operation and high cost. Recently, Liu and coworkers proposed two attractive strategies for the detection of His based on Cu^{2+}-regulated guanine (G)-quadruplex complexes [23,24]. However, Cu^{2+} can bind to His and cysteine (Cys), simultaneously. Therefore, in these two and some other studies [25], to realize selective detection of His, *N*-Ethylmaleimide, a toxic and unstable reagent, has to be employed, which inevitably limits applications of these biosensors. Because AAs have very similar structures, and normally coexist in biological samples, selective detection of an individual AA with simple methods is still a great challenge.

Taking the versatility of metal ions-DNA interactions into account, we believe Ni^{2+} may mediate allosteric manipulation of G-quadruplex DNAzyme, which is promising to create a facile His biosensor with improved selectivity. First, G-quadruplex DNAzyme is constructed using G-rich DNA. Ni^{2+} is able to interact with G (particularly the N7 of the G residue), and introduce a purine-like Ni^{2+} base pair in DNA [26]. Second, G-quadruplex DNAzyme is low cost, high stability, and ease of use [27,28]. Activity of G-quadruplex DNAzyme can be conveniently characterized with colorimetric methods [29]. Third, in comparison with other AAs, His can selectively bind to Ni^{2+}. The selectivity of Ni^{2+}-His affinity pair has been repeatedly confirmed in various biological applications, especially in isolation and purification of peptides and proteins with His tail [30,31]. As expected, our experimental results identify for the first time that Ni^{2+} can destruct the G-quadruplex structure, and thus depress the peroxidase-mimicking activity of G-quadruplex DNAzyme. More importantly, Ni^{2+}-mediated allosteric manipulation can be impeded by His, resulting in recovery of activity. On a basis of these principles, a novel His biosensor with outstanding selectivity is designed.

2. Materials and methods

2.1. Chemicals and materials

All kinds of amino acids were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Nickel acetate tetrahydrate ($Ni(Ac)_2 \cdot 4H_2O$), thioflavin T (ThT), and 3,3′,5,5′-Tetramethylbenzidine (TMB)-H_2O_2 solution were obtained from Sigma-Aldrich Co., Ltd. (Shanghai, China). Tris(hydroxymethyl)aminomethane (Tris) was obtained from Alfa Aesar Co. Ltd. (Shanghai, China). Single-stranded DNA probe (ssDNA probe) was purchased from Promega Biotech Co., Ltd. (Beijing, China). Hemin modified G-rich DNA (hemin-DNA: 5′-hemin-GGGAAAGGGAAAGGGAAAGGG-3′) and poly(24G) (5′-GGGGGGGGGGGGGGGGGGGGGGGG-3′) were obtained and purified with HPLC by TaKaRa Co., Ltd. (Dalian, China). The oligonucleotide was dissolved in the 10 mM Tris-HCl buffer solution (pH 8.0). All other chemicals used in this work were of analytical grade and directly used without additional purification. All solutions were prepared with ultrapure water (18.2 MΩ cm) from a Milli-Q purification system (Bedford, MA).

2.2. Instrumentation

The UV-vis tests were performed on a UV-vis absorption spectrometer (Cary 50, Varian, USA). The fluorescence measurements were carried out on an Fluoromax-4 spectrometer (Horiba, France). Circular Dichroism (CD) measurements were conducted on a Chirascan (Applied Photophysics, Britain).

2.3. UV-vis detection of His

10 μL of 100 μM Ni^{2+} and 10 μL of His at different concentrations were first mixed and reacted for 30 min. Then, 10 μL of 10 μM hemin-DNA was added into the mixture. Above mixed solutions were incubated at 90 °C for 5 min, then cooled to room temperature slowly. After that, 170 μL of TMB-H_2O_2 solution was added, and the mixture was kept for 15 min. Afterwards, 200 μL of 2 M H_2SO_4 was added into the above solution to stop reaction. Then, the mixed solution was diluted with 1000 μL of 10 mM Tris-HCl buffer (pH 8.0), and was ready for Uv-vis measurement.

2.4. CD assay

Sample preparation for CD assay was the same with that for Uv-vis detection, while higher concentrations of DNA and Ni^{2+} were needed. The final concentrations of hemin-DNA, Ni^{2+} and His were 10 μM, 300 μM and 1.2 mM, respectively.

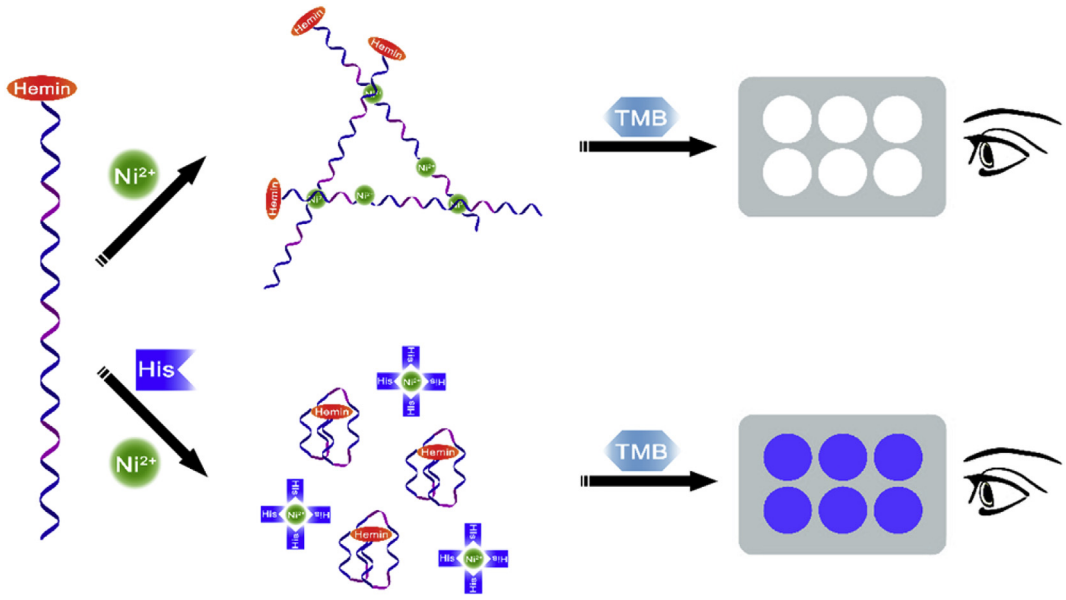
2.5. Fluorescent characterization

ThT and ssDNA probe were used in this work to survey Ni^{2+}-mediated allostery. For ThT assay, 10 μL of 50 μM ThT was added into related solutions, and the mixed solutions were kept for 30 min. After that, the mixed solutions were diluted with 300 μL of 10 mM Tris-HCl buffer (pH 8.0) for fluorescent measurement. For ssDNA probe assay, 10 μL of 100 μM Ni^{2+}, 10 μL of 10 μM poly(24G), and 10 μL of 10 μM ssDNA probe were mixed and incubated at 90 °C for 5 min. After that, the mixed solution was cooled to room temperature for fluorescent measurement.

3. Results and discussion

3.1. Sensing mechanism of a colorimetric His biosensor

Sensing mechanism of the proposed His biosensor has been illustrated in Scheme 1. The sensing system consists two components, *i.e.*, hemin-modified G-rich oligonucleotides (hemin-DNA) and Ni^{2+}. In our previous work, we demonstrated that hemin-DNA can construct G-quadruplex DNAzyme in one step without the participation of metal ions [32]. In this study, hemin-DNA is further used to investigate Ni^{2+}-mediated allosteric manipulation. Ni^{2+} plays different roles in different cases. In the absence of His, G-quadruplex structure is destroyed by Ni^{2+}-G interaction, thus inhibiting peroxidase-mimicking activity of G-quadruplex DNAzyme. However, in the presence of His, His presents stronger binding affinity to Ni^{2+}, which protects the intact structure of G-quadruplex DNAzyme. Due to the high peroxidase-mimicking



Scheme 1. Illustration of a Colorimetric His Biosensor based on Ni^{2+}-Mediated Allosteric Manipulation of G-quadruplex DNAzyme.

activity of G-quadruplex DNAzyme, the color of 3,3′,5,5′-tetramethylbenzidine (TMB)-H_2O_2 solution changes from colorless to blue. Therefore, concentration of His ([His]) can be measured with a colorimetric method, even can be distinguished by naked eyes.

3.2. Ni^{2+}-mediated allosteric manipulation of G-quadruplex DNAzyme

Circular dichroism (CD) is a powerful tool to survey the effect of metal ions on DNA structures [33]. As shown in Fig. 1A, hemin-DNA reveal a positive peak at 215 nm and a positive peak at 295 nm, which is a typical CD curve of antiparallel G-quadruplex structure [34]. After the addition of Ni^{2+}, the peak at 295 nm disappears, and the peak at 215 nm greatly decreases, indicating occurrence of Ni^{2+}-mediated allostery. Interestingly, in the presence of His, typical peaks reemerge. This result indicates Ni^{2+}-His interaction causes the recovery of G-quadruplex structure. Some fluorescent molecules may distinguish G-quadruplex structure in accompany with enhancement of fluorescence. Among them, thioflavin T (ThT) is a recently reported one [35,36], and G-quadruplex/ThT complex has been employed in designing analytical methods [37,38]. In this study, ThT is utilized to confirm Ni^{2+}-mediated allosteric manipulation. In comparison with ThT, fluorescence of G-quadruplex/ThT complex is significantly enhanced (curve a and b in Fig. 1B). However, in the presence of Ni^{2+}, G-quadruplex structure is deconstructed, and only about 10% fluorescence is persisted (curve c in Fig. 1B). Once His is added, fluorescence is dramatically recovered (curve d in Fig. 1B). We speculate the Ni^{2+}-G interaction impedes the formation of hydrogen bonds between G residue. Without the formation of hydrogen bonds, G-tetrad cannot be constructed, leading to allostery of G-quadruplex structure. Owing to higher affinity to His, Ni^{2+} preferentially bind to His instead of G residue. Thus, G-quadruplex structure can be preserved in the presence of His. According to our preliminary results (Fig. S1), beside of G-quadruplex structure, single-stranded DNA containing a G-rich sequence (poly(24G)) is also affected by Ni^{2+}-G interaction. More investigations on this issue are ongoing in our lab.

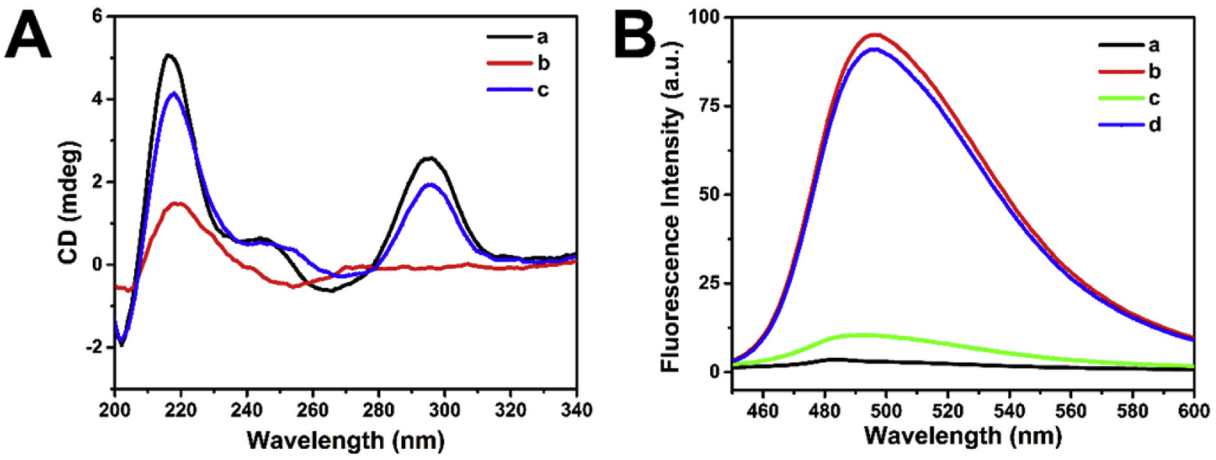


Fig. 1. (A) CD spectra of (a) hemin-DNA, (b) hemin-DNA and Ni^{2+}, and (c) hemin-DNA, Ni^{2+} and His. (B) Fluorescence spectra of (a) ThT, (b) ThT and hemin-DNA, (c) ThT, hemin-DNA and Ni^{2+}, and (d) ThT, hemin-DNA, Ni^{2+} and His.

3.3. Colorimetric quantification of His via Ni^{2+}-mediated manipulation of G-quadruplex DNAzyme

As structures are the basis of DNAzyme activities, peroxidase-mimicking activity of G-quadruplex DNAzyme can be regulated by Ni^{2+}-mediated allostery and Ni^{2+}-His affinity pair. Because of oxidation of G-quadruplex DNAzyme, the color of TMB-H_2O_2 solution changes from colorless to blue. After adding acid solution, a steady yellow solution is obtained [39–41]. Therefore, activity of G-quadruplex DNAzyme can be reflected by the intensity of solution color. As shown in Fig. 2A, His itself does not affect the peroxidase-mimicking activity of G-quadruplex DNAzyme, while the activity can be greatly reduced by Ni^{2+}, resulting in low absorption intensity at 450 nm ($A_{450\ nm}$). In the presence of His, Ni^{2+}-His affinity pair is formed, facilitating the recovery of peroxidase-mimicking activity (Fig. 2B). As a result, $A_{450\ nm}$ remarkably increases. According to these results, a colorimetric His biosensor can be fabricated, in which the G-quadruplex DNAzyme is formed using hemin-DNA. It is noteworthy that hemin-DNA is chosen in this work for two reasons. On one hand, it has been demonstrated that the activity of G-quadruplex DNAzyme formed by hemin-DNA is higher than traditional G-quadruplex DNAzyme, while its background noise is much lower. High activity and low background noise are helpful to improve the performances of G-quadruplex DNAzyme-based biosensor. On the other hand, hemin-DNA can form G-quadruplex DNAzyme without the participation of any metal ions, which is of excessive importance in a biosensor that employs a metal-mediated procedure.

Since quantification of His is on a basis of Ni^{2+}-mediated allosteric manipulation, concentration of Ni^{2+} ($[Ni^{2+}]$) is essential to analytical performances. Low $[Ni^{2+}]$ may narrow the detection range, while high $[Ni^{2+}]$ may raise the detection limit. To pursue the optimal performances, effect of $[Ni^{2+}]$ on activity of G-quadruplex DNAzyme is first studied. As the increase of $[Ni^{2+}]$ from 1 nM to 1 mM, $A_{450\ nm}$ gradually decreases (Fig. 2C). In the range of 100 nM–500 μM, a linear relationship is observed between the $A_{450\ nm}$ and $[Ni^{2+}]$ (Fig. 2D). If the $[Ni^{2+}]$ is higher than 500 μM, a plateau is obtained. In this analytical system, 100 μM Ni^{2+} can be efficiently bounded by His. Therefore, $[Ni^{2+}]$ is fixed at 100 μM is chosen as the optimal concentration in His detection. In this condition, $A_{450\ nm}$

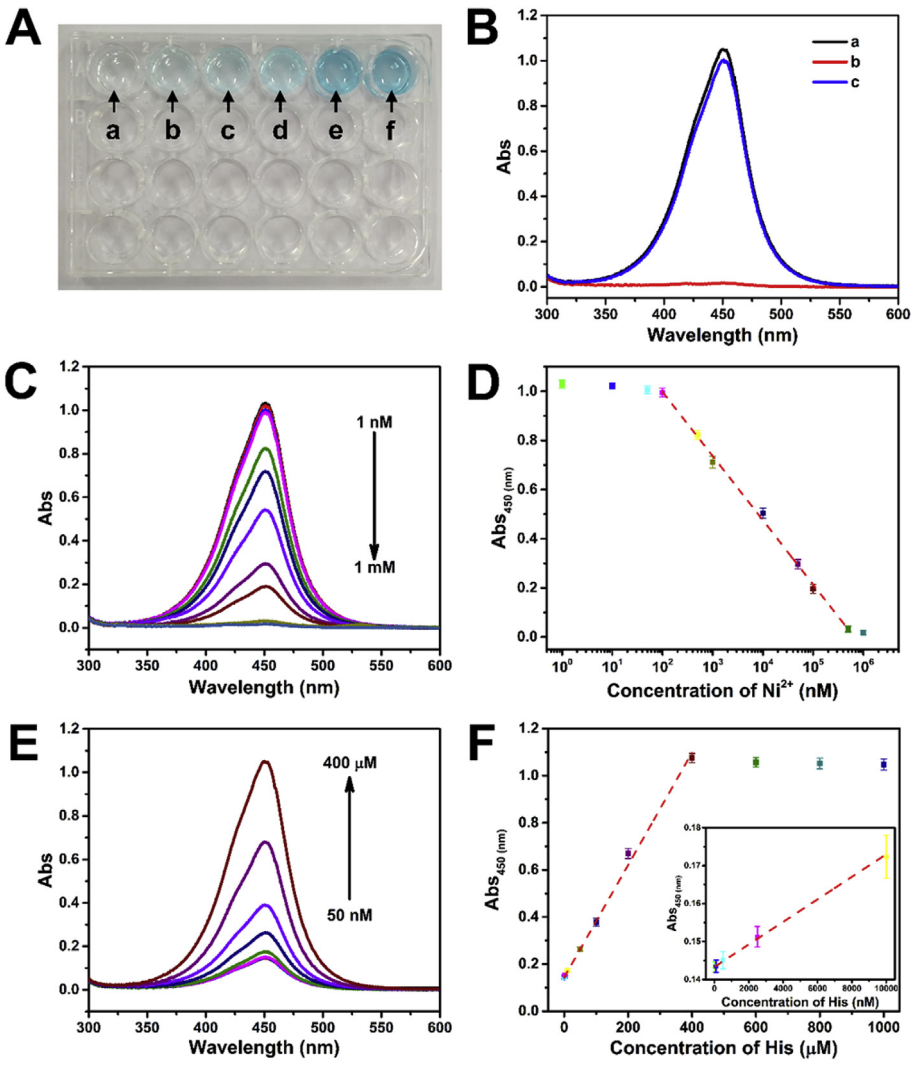


Fig. 2. (A) Picture of TMB-H_2O_2 solution with (a) hemin-DNA and Ni^{2+}, (b) hemin-DNA, Ni^{2+} and 5 nM His, (c) hemin-DNA, Ni^{2+} and 50 nM His, (d) hemin-DNA, Ni^{2+} and 500 nM His, (e) hemin-DNA, Ni^{2+} and 400 μM His, and (f) hemin-DNA and 400 μM His. The $[Ni^{2+}]$ in this assay is 100 μM. (B) Uv-vis absorption spectra of (a) hemin-DNA, (b) hemin-DNA and Ni^{2+}, and (c) hemin-DNA, Ni^{2+} and His. (C) Uv-vis absorption spectra of G-quadruplex DNAzyme in the presence of different $[Ni^{2+}]$. (D) $A_{450\ nm}$ of G-quadruplex DNAzyme as a function of $[Ni^{2+}]$. $[Ni^{2+}]$ is in a range of 1 nM to 1 mM. (E) Uv-vis absorption spectra of G-quadruplex DNAzyme in the presence of Ni^{2+} and different [His]. (F) $A_{450\ nm}$ of Ni^{2+}-treated G-quadruplex DNAzyme as a function of [His]. The $[Ni^{2+}]$ in this assay is 100 μM.

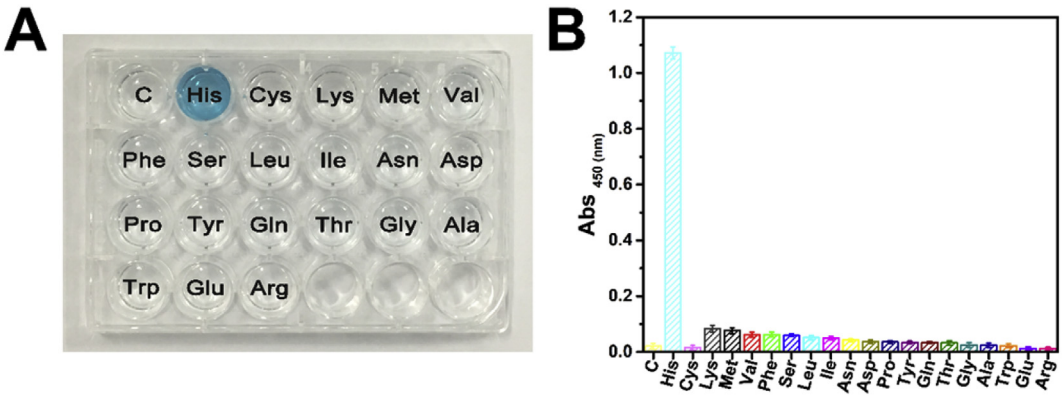


Fig. 3. (A) Picture of TMB-H_2O_2 solution with G-quadruplex DNAzyme in the presence of Ni^{2+} and different AAs. (B) Bar graph of $A_{450\ nm}$ of G-quadruplex DNAzyme in the presence of Ni^{2+} and different AAs. C represents the absence of AAs.

Table 1
Detection of His in serum samples with the proposed biosensor.

Sample	His added (μM)	His found (μM)	Recovery rate (%)	RSD (n = 3)
Sample 1	0	15.6	Not applicable	3.5
Sample 2	5	20.4	96	4.8
Sample 3	50	66.8	102.4	3.2
Sample 4	100	117.5	101.9	2.7

increases with the enlarging of [His] (Fig. 2E). And a linear dependence is obtained in the range of 50 nM–400 μM (Fig. 2F). The regression equation is $A_{450\ nm}$ = 2.38[His] (μM) + 0.144 (R = 0.997), and the calculated detection limit is 36 nM (S/N = 3). In comparison with other colorimetric His biosensors, detection limit and linear range of our biosensor are better or at least comparable (Table S1). In this detection condition, one Ni^{2+} can interact with four His, which is corresponding to the fact that Ni^{2+} can four-, five- and six-coordinate with other molecules [42,43]. To be noted, as a well-performed colorimetric biosensor, His can be distinguished by naked eyes if the [His] is higher than 5 nM (Fig. 2A).

3.4. Selectivity of this biosensor

To investigate its selectivity, this biosensor is challenged with other 19 kinds of AAs. Except His, other AAs including Cys cannot recover the activity of G-quadruplex DNAzyme, and cannot cause the increase of $A_{450\ nm}$ (Fig. 3A and B). Besides, this method is not affected by typical biological thiols such as glutathione and homocysteine (Fig. S2). The excellent selectivity should be attributed to high specificity of Ni^{2+}-His interaction and stability of DNAzyme. In detail, different from some other metal ions that can interact more than one AA, Ni^{2+} only reveals a strong affinity for His. Meanwhile, in comparison with metallic nanomaterials used in His biosensor, G-quadruplex DNAzyme is more stable. Therefore, without the help of additional reagents, this biosensor can directly distinguish His from other AAs, which is desirable in related biosensors.

3.5. Analysis of His in real samples

Encouraged by the performances of this biosensor, we attempt to evaluate the amount of His in biological fluids. According to the results of this biosensor, [His] in serum is 15.6 μM, which is in accord with data from previous studies [44,45]. Meanwhile, by accomplishing recovery experiments, high recovery percentage and low relative standard deviation (RSD) are obtained (see Table 1), indicating satisfactory accuracy and applicability of this biosensor in real sample detection. Considering His as a health indicator, this method may be valuable in clinical test.

4. Conclusion

Herein, Ni^{2+}-mediated allostery of G-quadruplex DNAzyme is reported. In combination with Ni^{2+}-His affinity pair, peroxidase-mimicking activity of G-quadruplex DNAzyme is precisely inhibited and recovered. Relying on these principles and using hemin-DNA to form metal-free G-quadruplex DNAzyme, a novel biosensor is fabricated, and His in a linear range of 50 nM–400 μM can be quantified. More importantly, this bioanalytical system can directly distinguish His from other kinds of AAs including Cys, suggesting its rare selectivity. In addition, this biosensor exhibits the capability of detecting His in biological fluids. Overall, this work provides a novel way to manipulate activity of G-quadruplex DNAzyme, and a new perspective of designing DNA-based biosensors.

Acknowledgments

This work was supported by the National Natural Science Foundation of China for Projects 21625502, 21475062 and 21505077 and PAPD.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.aca.2017.12.040.

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