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A novel label-free colorimetric detection of L-histidine using Cu^{2+}-modulated G-quadruplex-based DNAzymes

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ABSTRACT

We proposed a colorimetric method for L-histidine detection based on Cu^{2+}-mediated DNAzyme and G-quadruplex-hemin complex catalyzed oxidation of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS). In this system, after the addition of L-histidine, the formation of G-quadruplex-hemin complex will be disturbed, thus the colorimetric signal intensity conversely corresponds to the concentration of histidine. In this assay, a lower detection limit of L-histidine (50 nM) is addressed comparing to previously reported colorimetric methods. The cost is extremely low as the proposed design is both label-free and enzyme-free. All the more vitally, the colorimetric detection procedure is substantially straightforward with no further modification processes. By and large, the sensor can provide a promising plan for the detection of L-histidine.

1. Introduction

Simple as its structure is, histidine, one of the 20 amino acids, functions as an indispensable element in series of important protein assembling inside our body. As previously reported, this semi-essential bioactive molecule contributes to some particularly key biological system including cell and tissue repair after accidental trauma [1,2], metallic–enzymatic reactions [3] and the intracellular homeostasis of some metal ions like copper. However, what makes His favored by physician-scientists is its function as a neurotransmitter or some other unknown part in the mysterious nervous system [4–6]. The typical level of L-histidine in body is 0.31–26.35 mg/mL by and large. Insufficiency of L-histidine leads to certain illnesses, while the profusion of L-histidine with more than 29.5 mg/mL results in histidinemia [7]. Besides, counting on the functions above, the pathophysiological processes of many sets of diseases, such as chronic kidney disease [8], Alzheimer's disease [9], pulmonary disease [10] and cancer [11] was reported to be associated with Histidine. Considering the importance of L-histidine, it would be attracting to develop a facile and low-cost sensing strategy for it.

Traditional analytical methods such as capillary electrophoresis [12–17], mass spectrometry [18] and high-performance liquid chromatography [19–24] have been the most common methods for L-histidine detection. However, these methods are usually low sensitive, complicated, dearly expensive, and time-consuming because of the involvement of the complex sample pretreatment procedures. In order to overcome these limitations, some ancient methods were improved, and many rapid and simple analytical strategies based on fluorescent [25–35], electrochemical voltammetry [36–38], nuclear magnetic resonance [39], spectroscopy [40] and colorimetric [41–45] have been established. Zhou et al. built up a classical portable chemical sensor using a combination of the personal glucose meters (PGM) with click chemistry for L-histidine detection with a great limit of 3.4 nM among many other chemical sensors [46]. Zhang's group explored a novel fluorescent sensor of Histidine and Cysteine by utilizing AMP-Tb as a fluorescent probe with enhanced fluorescence power corresponding to the concentration of Histidine and Cysteine respectively in the range from 0.2 to 150 μM and 0.5 to 200 μM, which not only shows its short range, but also its poor selectivity [47]. Liu's team synthesized histidine-Au nanoclusters providing a highly selective detection of histidine. In real sample detection they endeavor to apply to human blood plasma, however, it seems unpractical and not cost-effective in medical use with their constrained detection limit range [48]. Mobarraz et al. have synthesized L-cysteine capped-ZnS quantum dots, and they reported that histidine could effectively quench the quantum dots emission more than other amino acids which showed 4 times decreased than other amino acids [49]. However, in our test, the difference will be more obvious both in visual and in the database with more than 8 times. Most of the detection methods for L-histidine are costly, complicated and unwieldy optical detection scheme. As we all know, in optical detection strategy, the volume of the specimen assumes to be an essential part of sensitivity since the absence of the volume influences the

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coveted sensitivity. The constraint in detection limit is a noteworthy consideration of the optical detection strategy for the analysis of amino acids. Moreover, complication of labeling process and inconveniences of dealing with instrument are the real bottleneck of the optical strategy for detection. These shortcomings mentioned above in optical plan can be expelled by colorimetric method which is extinct with excellent convenience due to the unique advantage of not requiring confused instrumentations, and the detection of L-histidine with naked eye expected to be more suitable and practical in medical applications.

DNAzymes, a series of nucleotide sequences with specific catalytic activity, have been reported frequently applied in detection assays for such as ions and amino acids, due to the advantage in stability, high cost-effectiveness and modification convenience, by comparison with protein enzymes. The DNA probe in this assay has three main components and two catalytic functions, which respectively are DNA Cu^{2+} induced self-cleaving domain and G-rich sequence. The core of this assay mechanism is the step-by-step activation of the catalytic domains, that relates the colorimetric signal and the quantity of the L-histidine. The colorimetric signal of this assay is generated from the H_2O_2-mediated oxidation of ABTS catalyzed by the G-quadruplex–hemin complex, with a maximal absorption signal at a wavelength of 419 nm [7,50]. The G-quadruplex–hemin complex has a distinct HRP-mimicking catalysis activity, resulting from the ability to enhance the basicity of the bound porphyrin in the hemin, which is a metalloporphyrin, a peroxidase. In the absence of Cu^{2+}, the DNA probe presents strong intramolecular structure, much more stable than the G-quadruplex resulting from the folding of G-rich DNA sequence. The activation of the self-cleaving, which is induced by the metal ion, makes the formation of G-quadruplex possible. With the addition of the L-histidine which contains imidazole group easy to bind the Cu^{2+}, the L-histidine-Cu^{2+} is formed [51,52], leading to the inhibition of the formation of G-quadruplex, and then the correlation between the quantity of L-histidine and the absorbance is established.

2. Experimental

2.1. Reagents and Methods

L-Histidine was purchased from Sigma-Aldrich life science & technology Co. Ltd. (Wuxi, China). L-Arginine, glycine, and L-alanine were purchased from Yuanye Bio-technology Co., Ltd. (Shanghai, China). The HPLC-purified DNA probe (agc ttc ttt cta ata cgg tgg gta ggg cgg gtt ggg cta ccc acc tgg gcc tct ttc ttt tta aga aag aac) was obtained from Sangon Biotechnology Co., Ltd. (Shanghai, China). Hepes free acid (HEPES), Sodium chloride (NaCl), Tris [Tris (hydroxymethyl) aminomethane], hydrochloric acid (HCl), Copper sulfate($CuSO_4$), Hydrogen peroxide (H_2O_2) were procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hemin, 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) were purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). All other reagents were of analytical-reagent grade. Ultra-pure water was used for all the experiments, and all the DNA sequences were prepared in TE buffer and stored at −20 °C.

2.2. Apparatus

The absorbance was recorded on an Enspire® multimode plate readers (Perkin Elmer, USA) using a 96-well plate. The absorption spectra of the solution were measured at wavelengths from 400 to 470 nm. The absorbance was obtained at 420 nm.

2.3. Investigation of Feasibility

In order to test the feasibility of this detection method, two samples were prepared. For sample 1, we prepared two mixtures (A and B). In mixture A (35 μL), 400 nM DNA probe, 37.5 mM NaCl and 1.25 mM HEPES (pH = 7.0) were added into 27.2 μL purified water, then heated at 80 °C for 2 min and cooled to room temperature for 30 min. As to mixture B (35 μL), 4 mM L-Histidine, 400 μM Cu^{2+} were added into 31.6 μL tris buffer and incubated at 25 °C for about 5–10 min. Then, mixture A and B were blended together at 25 °C for 15–20 min, and 1 μL of 100 μM hemin was subsequently added. 30 min later, 15 μL for 20 mM H_2O_2 and 20 mM ABTS were added. After 20 min, the absorbance range from 400 to 470 nm was measured. For sample 2, L-histidine was not added.

2.4. Optimization of Reaction Conditions

Theoretically many potential factors may have influence on the detection assay in different procedures, such as the formation of Cu^{2+}-L-his complex, the combining between Cu^{2+} and D-DNAzyme and the self-cleaving process, and the G-rich sequences release and the G-quadruplex formation, etc. Therefore, we select the concentration of DNA probe and Cu^{2+} to be evaluated to reach the optimal experimental condition.

2.5. L-histidine Detection Assays

In this assay, we prepare thirteen samples, of different concentration of L-his ranging from 0.65 to 80 μM under an optimized condition. Each sample is a blend of Mixture A and Mixture B. Mixture A contains the reaction buffer (28.6 μL H_2O, 1.25 mM HEPES, 37.5 mM NaCl), 200 nM DNA probe, and mixture B, a reaction buffer (50 mM Tris-HCl, 500 μM Cu^{2+}, pH = 7.5) and a series amount of L-histidine. Then 1 μL of 100 μM hemin, 15 μL of 20 mM H_2O_2, and 15 μL of 20 mM ABTS were added. Absorbance at 420 nm and absorption spectra from 400 to 470 nm were recorded.

2.6. Selectivity Assay

To test the selectivity of this assay, a range of amino acids were applied. For this test, mixture A remained the same as mentioned above. While mixture B contained different types of amino acid, including L-histidine, L-alanine, L-arginine, and glycine. Then, 1 μL of 100 μM hemin, 15 μL of 20 mM H_2O_2 and 15 μL of 20 mM ABTS were subsequently added.

2.7. Preparation of Real Samples

L-histidine detection was assayed under the optimal experimental conditions. Human urine samples (1%) and different L-histidine concentrations (in the working range of this method) were thoroughly mixed, and the reaction was permitted to take place at room temperature for 3 min. The mixture was diluted 100 times before the subsequent colorimetric detection procedures. No other pretreatments were performed for the detection of the real samples. While mixture A was prepared according to that for the investigation of feasibility, mixture B was in 10 mM Tris-HCl (pH = 7.5), 400 μM/L Cu^{2+}, and different concentrations of L-histidine. Subsequent procedures and absorbance measurements were carried out following the L-histidine assay. Recoveries of L-histidine from the urine samples were calculated by the regression equation using the absorbance and the concentration of L-histidine.

3. Results and Discussion

3.1. Mechanism

Principle of the proposed detection method is illustrated in Fig. 1; this unimolecular probe is a combination of a DNA-cleaving DNAzyme (D-DNAzyme) and an HRP-mimicking DNAzyme (H-DNAzyme) that includes three main components. Domain I is the substrate of DNA-