Talanta 107 (2013) 402–407

Contents lists available at SciVerse ScienceDirect

Talanta

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

Label-free dsDNA-Cu NPs-based fluorescent probe for highly sensitive detection of L-histidine

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ARTICLE INFO

Article history:
Received 12 October 2012
Received in revised form
21 January 2013
Accepted 23 January 2013
Available online 30 January 2013

Keywords:
Copper nanoparticles
L-histidine
Label-free
Biosensor

ABSTRACT

A new and facile strategy using double-stranded DNA-copper nanoparticles (dsDNA-Cu NPs) as fluorescence reporters for the highly sensitive and selective detection of L-histidine was demonstrated. In the dsDNA-Cu NPs-based sensing system, the fluorescence was quenched considerably upon the addition of L-histidine. Under the optimized experimental conditions, the probe exhibits excellent performance (e.g., a satisfactory detection limit of 5 μM and high specificity). Our in situ method requires no covalent attachment of DNA to a fluorophore, which could significantly reduce the cost and simplify the procedure for L-histidine detection. Moreover, the proposed sensing system could be applicable for the detection of target biomolecule in complex biological samples. These striking properties make it an attractive platform for the direct detection of L-histidine.

1. Introduction

In recent years, noble metal nanostructures, such as copper nanoparticles (Cu NPs), silver nanoclusters and gold nanodots, have attracted immense interest in bioimaging and biosensing due to their unique optical, electronic, and catalytic properties [1–5]. For example, Shiang et al. have developed a gold nanodots-based luminescence probe for the detection of human immunoglobulin G [1]. Dickson reported a bright, near-IR-emitting silver nanocluster created in single-stranded DNA consisting of 12 cytosine bases that shows very high emission rates and excellent photostability [2]. Compared with other nanomaterials, copper and copper oxide nanoparticles received increasing attention due to the useful applications in photovoltaic cells, optical and biological sensors, conductive materials, and coating formulations [6].

There are a number of approaches for the synthesis of Cu NPs with high efficiency [7–10], for example, using mercaptocarboxylic acids with different carbon chain lengths [7], bovine serum albumin (BSA) [8], and poly (allylamine) [9] as stabilizers and capping agents. Recently, oligonucleotide-templated copper nanoparticles have drawn increasing attention due to their ease of synthesis, good water solubility, low toxicity, biocompatibility, and excellent stability. Coupling of the excellent photoluminescence features with the functionality of the host DNA matrix, the DNA-based fluorescent nanomaterials possess great potential for chemical and biological sensing. Rotaru et al. reported that double-stranded DNA (dsDNA) can act as an efficient template for the formation of Cu NPs at a low concentration of $CuSO_4$, and the formed Cu NPs have excellent fluorescence features [11]. Later, using the intelligent Cu NPs fluorescence probe, Jia et al. successfully distinguished match and mismatch sequences with 15-mer probe DNA in solution [12]. Chen et al. developed a novel, simple, and label-free method for the detection of Pb^{2+} using dsDNA-Cu NPs [13]. The Cu NPs exhibit fascinating features, while the use of dsDNA-Cu NPs in biological analysis still remains at a very early stage. It would be interesting to explore the design and construction of the biosensing system using dsDNA-Cu NPs as fluorescence probe for the detection of other targets.

L-histidine, one of the 20 natural amino acids, plays an important role as a neurotransmitter or neuromodulator in the mammalian central-nervous system. It could also control the transport of metals in biologically important bases [14] and minimize internal bleeding from microtrauma [15]. Thus, the determination of L-histidine in biological fluids may be of great importance. In the past few years, many L-histidine assay methods have been reported, such as capillary electrophoresis [16,17], fluorimetric [18], and colorimetric methods [19,20], etc. Very recently, DNAzyme-based sensing platform has also been used for the detection of L-histidine [21]. Considering the importance of L-histidine, it would still be attracting to develop a simple and cost-effective sensing strategy for it. Interestingly, we found that L-histidine could quench the fluorescence of dsDNA-Cu NPs. Herein, a new and facile strategy using dsDNA-Cu NPs as fluorescence probe for the highly sensitive and selective detection of L-histidine was demonstrated.

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http://dx.doi.org/10.1016/j.talanta.2013.01.038

2. Methodology and experimental details

2.1. Chemicals and materials

Oligonucleotides (ssDNA 5'ATA CGC TCA TAC GTT CAT CAC G3'; cDNA 5'CGT GAT GAA CGT ATG AGC GTA T 3') were synthesized by invitrogen Bio Inc. (Shanghai, China). Amino acids were purchased from Sigma. All other chemicals were bought from Beijing Dinguo Biotechnology Co. Ltd. (Beijing, China) and used without further purification. All stock and buffer solutions were prepared using Millipore water (18 MΩ cm).

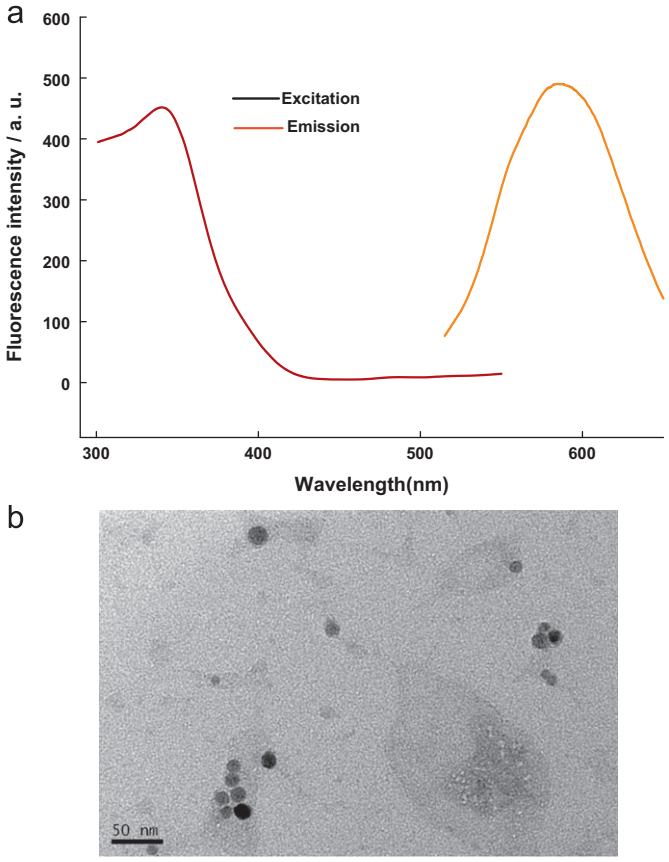
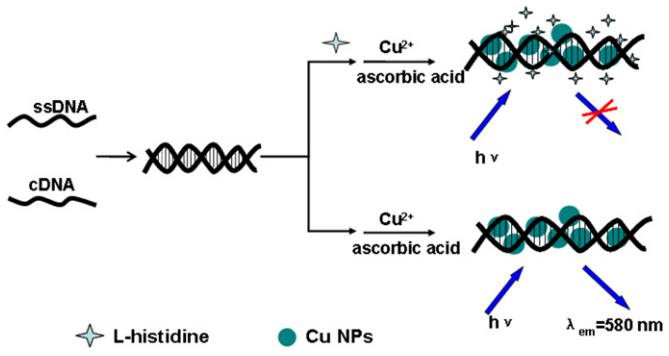


Fig. 1. (a) Excitation (λ_{ex}=340 nm) and emission (λ_{em}=580 nm) spectra of copper nanoparticles and (b) transmission electron microscopy (TEM) image of copper nanoparticles. Cu^{2+} concentration: 500 μM, ascorbic acid concentration: 2 mM, DNA concentration: 500 nM, buffer: 10 mM MOPS, pH 7.5, 150 mM NaCl, and 1 mM $MgCl_2$.



Scheme 1. Schematic illustration of the strategy for analysis of L-histidine based on the dsDNA-Cu NPs.

2.2. Procedure for L-histidine detection

All the detection procedures were performed in buffer A (10 mM MOPS, pH 7.5, 150 mM NaCl, and 1 mM $MgCl_2$). In a typical procedure at the optimized conditions, 5 μL ssDNA (10 μM) and 5 μL cDNA (10 μM) were mixed together in buffer A. After incubation for 10 min, 30 μL of various concentrations of L-histidine was added into the solution, and was allowed to react for 10 min. Then, the desired amount of ascorbic acid (2 mM) and $CuSO_4$ (500 μM) was added to give a final volume of 100 μL. The fluorescence emission of the system was then recorded after it being incubated for 10 min.

2.3. Apparatus

Fluorescence measurements were carried out on an F-4500 fluorescence spectrometer (Hitachi, Japan) with both excitation

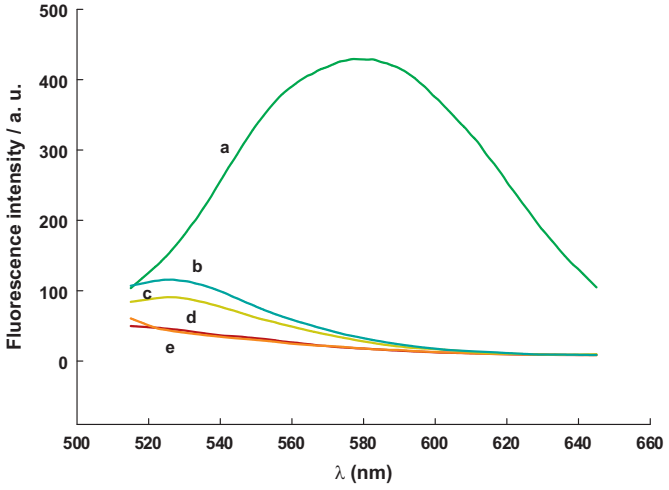


Fig. 2. Fluorescence spectra obtained in different solution: (a) ssDNA strand+cDNA strand+Cu^{2+}+ascorbic acid; (b) ssDNA strand+cDNA strand+L-histidine+Cu^{2+}+ascorbic acid; (c) ssDNA strand+cDNA strand+ascorbic acid; (d) cDNA strand+Cu^{2+}+ascorbic acid; and (e) ssDNA strand+Cu^{2+}+ascorbic acid. The L-histidine concentration: 4 mM, Cu^{2+} concentration: 500 μM, and ascorbic acid concentration: 2 mM.

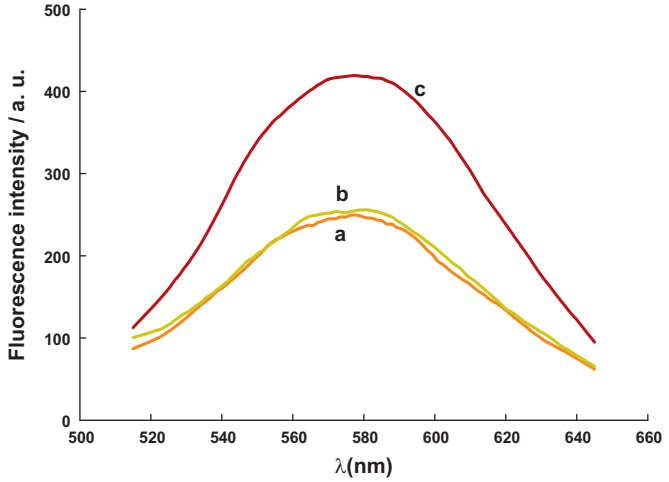


Fig. 3. Fluorescence spectra of dsDNA-CuNPs via a pre-addition (a) and post-addition approach (b) for the addition of L-histidine into the sensing system; curve c represents the fluorescence spectra of dsDNA-CuNPs in the absence of target. The L-histidine concentration: 250 μM, ascorbic acid concentration: 2 mM, and Cu^{2+} concentration: 500 μM.

and emission slits set at 10.0 nm. A quartz fluorescence cell with an optical path length of 1.0 cm was used. Excitation wavelength was set at 340 nm, and the emission spectra from 515 nm to 645 nm were collected. All measurements were carried out at room temperature unless stated otherwise. Transmission electron miroscope (TEM) analysis was performed with a JSM-5600LV microscope (JEOL, Japan).

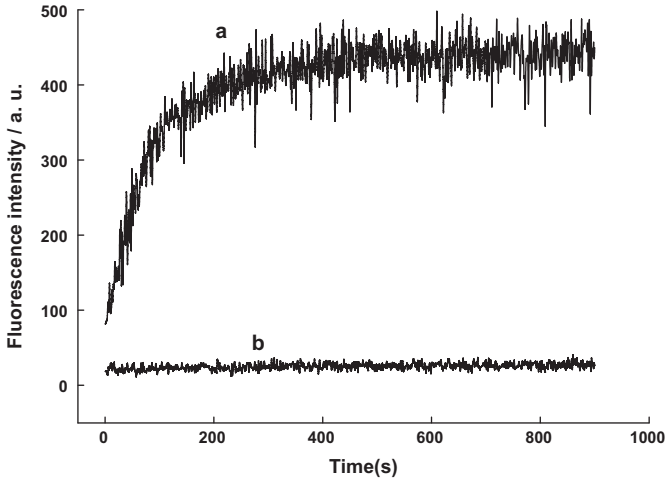


Fig. 4. Time-dependent fluorescence responses of the sensing system in the absence (a) and presence of target (b). The L-histidine concentration: 4 mM, Cu^{2+} concentration: 500 μM and ascorbic acid concentration: 2 mM.

3. Results and discussion

3.1. Probe design

We prepared dsDNA-Cu NPs in an aqueous phase at room temperature according to the previously reported method [11], and the obtained fluorescent Cu NPs showed excitation and emission peaks at 340 nm and 580 nm, respectively (Fig. 1a). The prepared Cu NPs were characterized by using fluorescence spectroscopy and transmission electron microscopy (Fig. 1b). Our new sensing strategy for L-histidine is based on fluorescence quenching of dsDNA-Cu NPs by L-histidine. As shown in Scheme 1, the probe contains two nucleic acid strands (ssDNA strand and cDNA strand). The ssDNA strand is predesigned entirely complementary to the cDNA strand. At a low concentration of $CuSO_4$, the dsDNA could act as an efficient template for the formation of Cu NPs with strong fluorescence emission (Fig. 2, curve a). However, upon the addition of L-histidine into the dsDNA-Cu NPs sensing system, its fluorescence was quenched

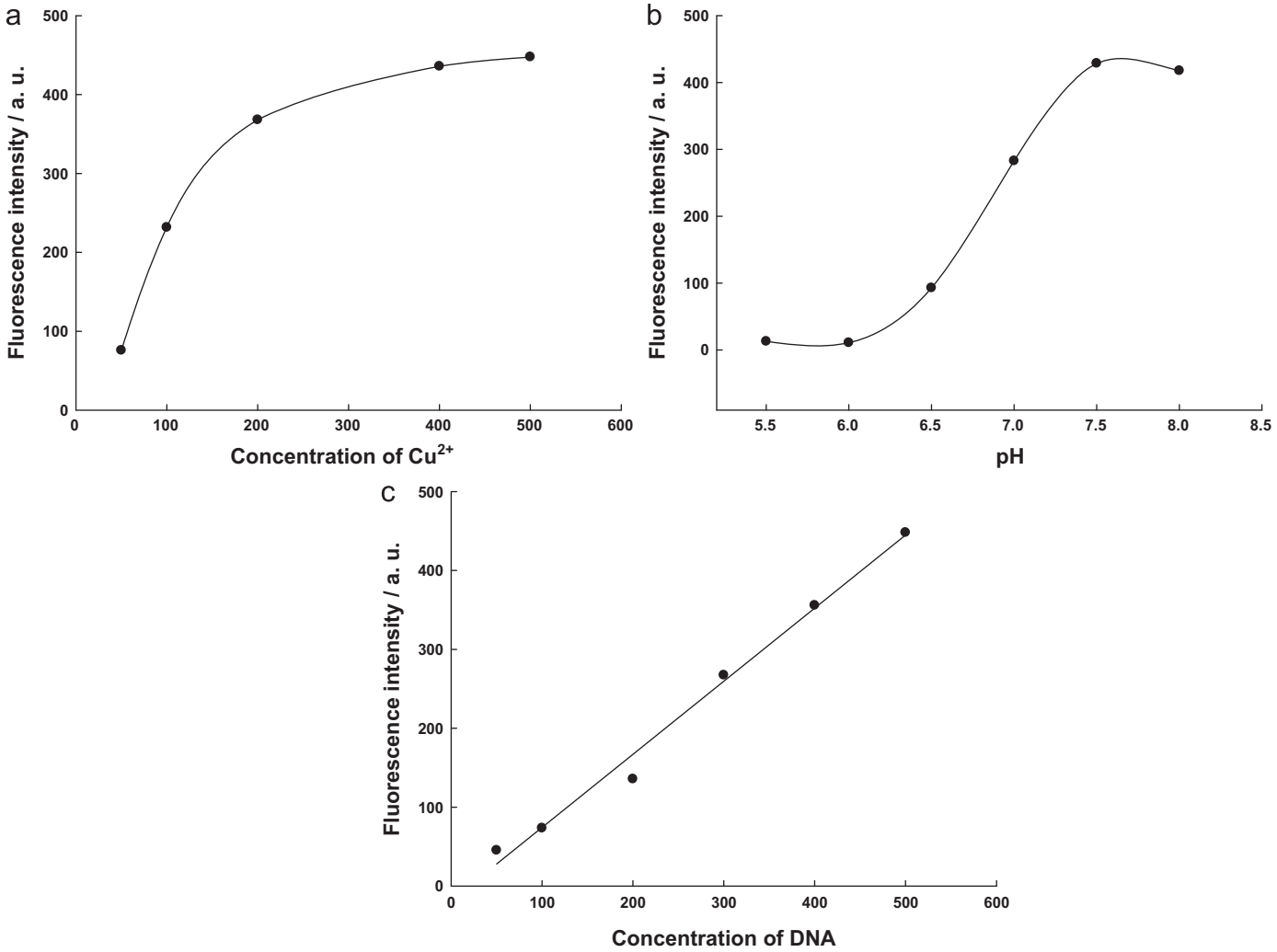


Fig. 5. (a) Effects of pH on the fluorescence intensity of the dsDNA-CuNPs in the absence of target; (b) impact of concentration of Cu^{2+} on the fluorescence of the dsDNA-Cu NPs; and (c) impact of concentration of DNA on the fluorescence of the dsDNA-Cu NPs. Ascorbic acid concentration: 2 mM.

considerably (Fig. 2, curve b). The proposed probe avoids complicated operation procedures and requires no covalent attachment of DNA to a fluorophore, and is thus a simple and inexpensive method for the detection of L-histidine. Utilizing the present sensing platform, we accomplished the detection of target amino acid with high sensitivity and selectivity.

To demonstrate the feasibility of our new strategy in constructing dsDNA-Cu NPs sensing platform, control experiments were carried out under the same conditions. In the presence of dsDNA, Cu NPs would be formed with the reduction of Cu^{2+} by ascorbic acid. In a control experiment, we test the feasibility of our sensing platform in the absence of Cu^{2+}. The experimental results are shown in Fig. 2. It was observed that when there is no Cu^{2+} added into the sensing system, no obvious fluorescent signal was detected, which could be attributed to the absence of formation of Cu NPs (Fig. 2, curve c). Rotaru et al. [11] reported that dsDNA-Cu NPs might be formed in these steps: the reaction of reducing copper (II) to copper (I) followed by the disproportionation of copper (I) into copper (II) and copper (0), the latter being clustered on dsDNA producing stable nanoparticles. In another control experiment, by using cDNA strand (Fig. 2, curve d) and ssDNA strand (Fig. 2, curve e), no obvious fluorescent signal was observed. These results demonstrated that the single-stranded DNA is not an effective template for the formation of Cu NPs. Only dsDNA could act as an efficient template for the formation of Cu NPs. It was noted that, at a fixed 250 μM L-histidine concentration, the fluorescence of dsDNA-CuNPs could be quenched considerably, no matter via a pre-addition or a post-addition approach for L-histidine (Fig. 3).

After the start of the reaction, reduction of Cu^{2+} by ascorbic acid was completed in just a few minutes (Fig. 4). At high concentration of L-histidine, low fluorescence intensity was observed for the dsDNA-CuNPs, which confirmed that L-histidine shows a strong quenching effect on the dsDNA-CuNPs. The luminescence quenching in the presence of L-histidine may be attributed to the complexation between L-histidine and the Cu^{2+} ion, since L-histidine contains a high-affinity site for Cu^{2+} ion [22,23]. It is a challenge for us to fully understand the quenching mechanism of L-histidine on dsDNA-Cu NPs due to the high complexity of the dsDNA-Cu NPs system and the lack of adequate understanding of the chemical properties. Further detailed investigations on the underlying mechanism are still under way. Anyway, considering the fluorescence characteristics of dsDNA-Cu NPs and the quenching effects of L-histidine, a novel label-free sensor for L-histidine detection has been proposed in the presented study.

3.2. Optimization of the detection conditions

In order to achieve the system's best sensing performance, the reaction pH, concentration of Cu^{2+}, and concentration of DNA were optimized. As shown in Fig. 5, under acidic conditions, no obvious fluorescence intensity was detected, while striking fluorescence signal was observed in alkaline pH. Over the pH range from 5.5 to 8.5, it can be found that the optimal fluorescence signal of dsDNA-Cu NPs was provided at pH 7.5 (Fig. 5a). It was reported that a higher metal ion/base ratio is necessary to form Cu nanoparticles effectively. At very low metal ion concentration, Cu^{2+} ions prefer to interact with the backbone phosphate negative groups via nonspecific electrostatic attraction [12,24,25]. With increasing concentration, the Cu^{2+} ions begin to bind to DNA bases, with much higher affinity than to the phosphate groups, and are further reduced by ascorbic acid to form luminescent Cu nanoparticles [12]. As shown in Fig. 5b, we found that the fluorescence intensity increased with the increasing concentration of Cu^{2+}. Our results obtained were in fair agreement with these reports. Moreover, the fluorescence intensity was proportional to the concentration of DNA (Fig. 5c). Experimental results showed that the pH 7.4, concentration of 500 nM Cu^{2+} and 500 nM DNA could provide the optimal performance for the sensing system.

3.3. Assay performance of the sensing system

To test whether the proposed biosensor could be used for L-histidine quantitation, various concentrations of L-histidine were used under the optimized conditions. As shown in Fig. 6a, a dramatic decrease in the fluorescence intensity was observed with increasing concentrations of L-histidine. An approximately 19-fold fluorescence decrease was observed. Fig. 6b depicts the relationship between the fluorescence intensity and concentration of L-histidine. This new system was very sensitive to L-histidine, with a detection limit of 5 μM determined by 3δ/slope (δ, standard deviation of the blank samples). The detection limit is at least 2 orders of magnitude lower than that of the HRP mimicking DNAzyme-based sensor [19]. To test the repeatability of the present biosensing platform, a series of three repetitive measurements of target samples were carried out. The relative standard deviations achieved for the target sample at 10 μM, 100 μM, or 4 mM were not more than 5.0%.

Furthermore, the selectivity of this method for L-histidine was investigated by testing its fluorescence response to other amino

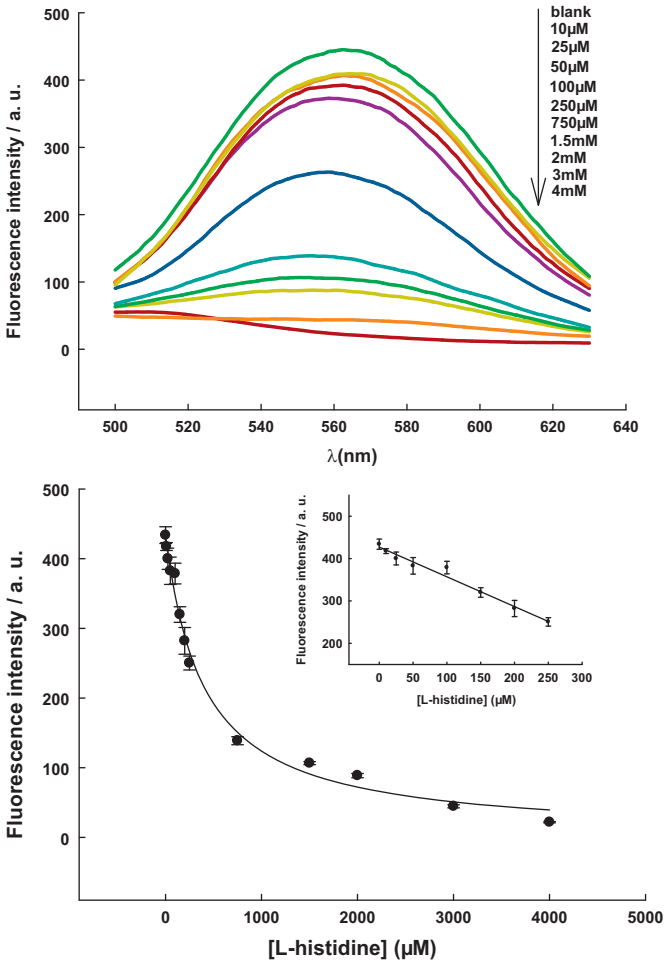


Fig. 6. (a) Fluorescence spectra of assay systems at various concentrations of L-histidine corresponding to data in the graph. (b) The relationship of the fluorescence enhancement with the target concentration. The inset shows the responses of sensing system to L-histidine at low concentration. The error bars indicated the standard deviations of three experiments.

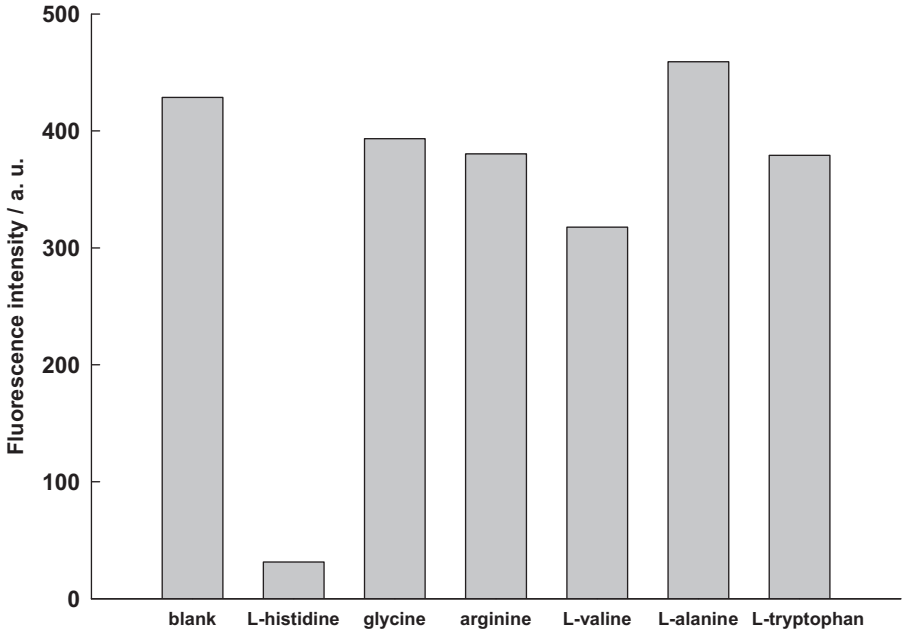


Fig. 7. Selectivity of the assay for L-histidine over other potential interferences (amino acid concentration: 4 mM).

Table 1
Recovery of L-histidine assay at different concentrations in sample A.

Sample A	Added (μM)	Found (μM)	Recovery (%)
1	100	97.5[a] ± 3.0[b]	97.5
2	150	158.6[a] ± 7.2[b]	105.7
3	200	200.4[a] ± 5.8[b]	100.2

Sample A including the presence of two potential interferences (500 μM glycine and 500 μM L-valine).

[a] Mean values of three determinations.
[b] Standard deviation.

acids. Under the same conditions as L-histidine, only L-histidine caused a dramatic fluorescence decrease (Fig. 7). Meanwhile, the quantitation of L-histidine has also been carried out in standards in the presence of two potential interferences (500 μM glycine and 500 μM L-valine). The results showed good agreement with the added and determined concentrations of L-histidine (Table 1). These results indicated that the proposed method shows good selectivity for the L-histidine detection.

To test the feasibility of the practical application of the sensing system, we further conducted its L-histidine detection in urine samples, one of the most challenging media containing a variety of proteins and other potential interferents. In order to avoid the interference of background fluorescence signal of urine, varying amounts of L-histidine were added to the diluted 0.1% urine samples. As shown in Fig. 8, the L-histidine titration curve in the urine samples was similar to that in the buffer solution, with a linear concentration range from 10 μM to 4 mM for L-histidine. These results confirmed that the proposed sensing system was applicable for practical L-histidine detection in real samples with other potentially competing coexisting species.

4. Conclusion

In summary, we reported a dsDNA-Cu NPs-based assay for the first time to detect L-histidine. Our proposed method avoids the modification of fluorophore on the DNA, which could significantly

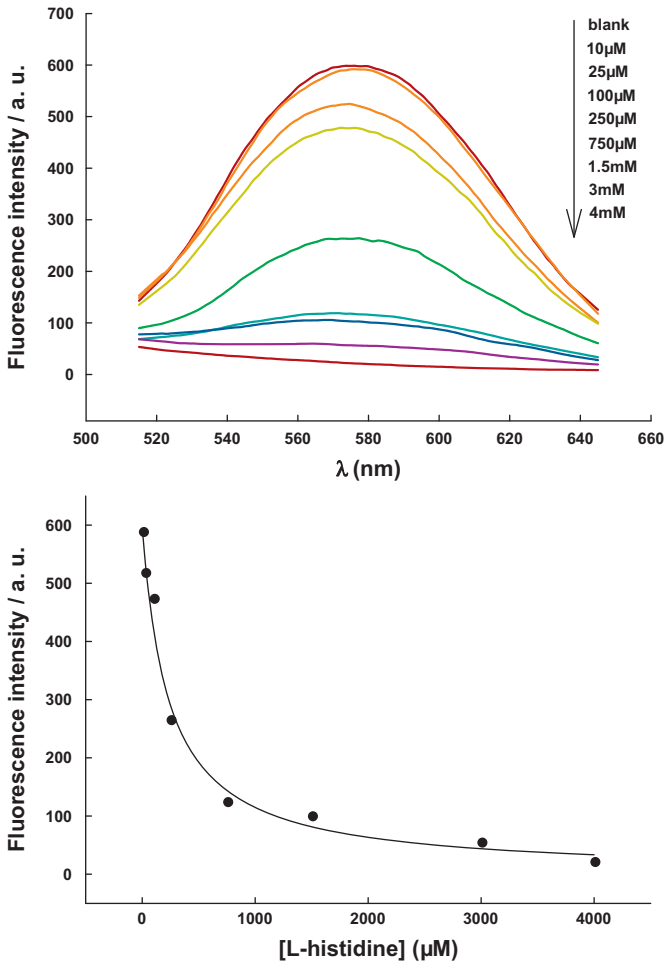


Fig. 8. (a) Fluorescence spectra of assay systems at various concentrations of L-histidine. (b) The relationship of the fluorescence enhancement with the target concentration in urine samples.

reduce the cost and simplify the procedure for L-histidine detection. When L-histidine is present, the fluorescence was quenched considerably. The proposed method enables L-histidine detection with high sensitivity, simple procedure, and short assay time. Compared with conventional assays, this method would be a useful alternative, and also be a potential tool for further application in the detection of L-histidine in complex environmental or biological samples.

Acknowledgments

This work was supported by the National Key Scientific Program of China (2011CB911000), NSFC (Grants 20975034, 21177036, and 21205068), the Foundation for Innovative Research Groups of NSFC (Grant 21221003), the National Key Natural Science Foundation of China (21135001), the National Instrumentation Program (2011YQ030124), the Ministry of Education of China (20100161110011), and the Hunan Provincial Natural Science Foundation (Grant 11JJ1002).

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