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ARTICLE

DNA-scaffolded silver nanoclusters/Cu^{2+} ensemble: use as a turn-on fluorescent probe for histidine

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A new type of rapid, sensitive, and selective fluorescence turn-on assay was developed for detection of histidine using DNA-scaffolded silver nanoclusters/Cu^{2+} ensemble (DNA-AgNCs/Cu^{2+}). Cu^{2+} was first bound to the nucleic acid of the DNA-AgNCs forming DNA-AgNCs/Cu^{2+} ensemble and then liberated to solution via the highly specific interaction between the histidine and Cu^{2+} in the presence of histidine. The fluorescence of DNA-AgNCs was greatly quenched with the addition of Cu^{2+}, then the DNA-AgNCs/Cu^{2+} ensemble exhibited marvelous fluorescent enhancement in the presence of histidine, which showed the possibility for constructing the turn-on chemosensor of histidine. Compared to other methods, this approach promises high sensitivity, simplicity in design, and convenient operation, minimized organic solvents. The ultra-high selectivity demonstrated the feasibility of the assay for detecting histidine in sophisticated physical environment. The fitting range of the proposed assay is from 0 to 100 μM, with a detection limit of 1.4 μM (S/N=3) in artificial urine samples. The protocol was evaluated by analysis of artificial urine samples with good recoveries and showed great potential for practical application.

1 Introduction

L-Histidine plays a significant role in the growth and repair of tissues as well as in controlling the transmission of metal elements in biological bases.[1] Recent studies have shown that a deficiency of histidine in plasma may lead to an impaired nutritional state in patients with chronic kidney disease.[2] Hence, a variety of methods have been developed for quantifying histidine in biological fluids. Several methods have been developed for the determination of histidine, including liquid chromatography,[3] capillary electrophoresis,[4] electrochemistry method,[5] colorimetric method,[6] resonance light scattering technique (RLS),[7] surface-enhanced Raman scattering (SERS)[8] and fluorometry.[9] Alternatively, fluorescent method affords high sensitivity and specificity in the quantitative analysis of compounds having a suitable functional group, so much interest has been shown in preparing new fluorescent probes.[9b] Fluorescent sensors based on metalloporphyrin compounds have attracted widely interests in past few years.[10] However, the synthesis of the sensory molecule suffered from several drawbacks, such as usage of organic solvent, time-consuming multistep reactions, sophisticated purification process, labeling procedure sometimes. With the development of nanotechnology, more simple and rapid methods have been reported. He et al. developed a fluorescence turn-on assay for histidine based on Ni^{2+}-modified gold nanoclusters, offered high selectivity for histidine over other amino acids.[11]

The development of metal nanoclusters (NCs) based biosensors has become a hot spot of research due to metal nanoclusters' molecular-like properties such as fluorescence and unique charging properties.[12] The emergence of noble metal nanoclusters, as a novel type of robust and promising fluorescence material, offers great opportunity for many applications, such as biosensing[13] and biological imaging.[14] Particularly, DNA-scaffolded silver nanoclusters (DNA-AgNCs), first reported by Braun and co-workers in 1998, have attracted a great deal of attention in the field of bioassays.[15] The DNA-scaffolded silver nanoclusters (DNA-AgNCs) are an ideal alternative to organic dyes and quantum dots for biochemical applications because they display excellent photostability, subnanometer size, nontoxicity, biocompatibility.[16] They have been successfully applied to detect microRNAs,[17] metal ion,[18] adenosine and adenosine deaminase,[19] which demonstrate the great potential for biomolecular sensing.

Chemosensing ensemble is a novel type of "off/on" fluorescent chemosensor for analyte: the fluorescent indicator is bound through noncovalent interactions to the receptor, which quenches its emission; then, the added analyte displaces the indicator to release into the solution for displaying its full fluorescence.[20] This method does not require the establishing of any covalent linking between the fluorophore and the receptor, which eliminated complicated labeling, laborious purification and toxic organic solvent. In our previous study, we have developed a "turn-off" fluorescent sensor for Cu^{2+} utilizing DNA-AgNCs, which is based on the interaction between Cu^{2+} and nucleic acids of DNA, and therefore the fluorescence of as-prepared surrounding AgNCs was quenched via metal–metal interplay.[18] As copper was a well-known metal ion to form

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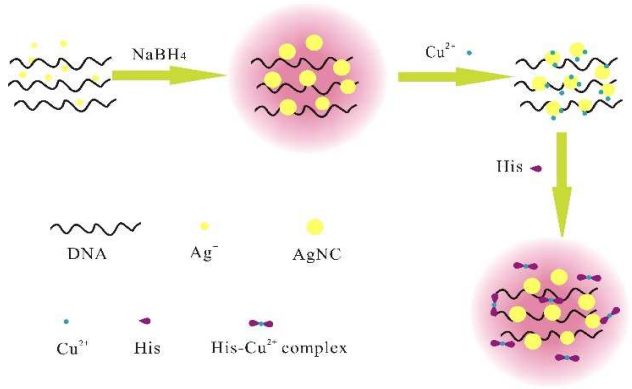
stable complexes with histidine,[6] we herein describe our ongoing efforts in developing a simple method for highly selective and sensitive detection of histidine (Scheme 1). We first synthesized highly fluorescent DNA-AgNCs. The fluorescence of DNA-AgNCs was firstly quenched with addition of Cu^{2+}. Then, in the presence of histidine, Cu^{2+} was snatched from the DNA-AgNCs/Cu^{2+} ensemble, leading to the fluorescence recovery of the DNA-AgNCs. Compared to previous fluorescence-base methods, the major advantage of the proposed method is to simplify the process of preparation. DNA-scaffolded silver nanoclusters/Cu^{2+} ensemble can be obtained within 2 hours in a low-cost way, without any chemical modification and purification procedures. This architecture ensured non-toxic, cost-effective, label-free and sensitive detection of histidine. Using this label-free approach avoids the need to design complicated fluorescent chemosensors and the use of organic solvent; this is a green chemical assay. The developed DNA-AgNCs/Cu^{2+} ensemble acts as fluorescent indicator in a "turn-on" mode and the interaction between Cu^{2+} and imidazole residue offers high selectivity for histidine over other amino acids. Moreover, the novel probe was developed for fluorescent detection of histidine with a rare low detection down to 1.4 μM (S/N=3) and furthermore for assessing the feasibility of the proposed method, combined with the artificial urine sample.



Scheme 1. Schematic illustration of our proposed fluorescent probe based on DNA-AgNCs/Cu^{2+} ensemble for highly selective and sensitive detection of histidine.

2 Experimental

2.1 Reagents and Materials. All chemicals used were obtained from commercial sources and directly used without additional purification. Cytosine-(C)-rich ssDNA (DNA scaffold) was purchased from Sangon Inc. (Shanghai, China)with the sequence: 5'-ATCCTCCCACCGGGCCTCCCACCATAAAAACCCTTAA TCCCC-3'. Silver nitrates ($AgNO_3$) were purchased from Shanghai Chemical Reagent Co., Ltd, Sodium borohydride ($NaBH_4$), $CuCl_2$, Histidine (His), Leucine (Leu), Alanine (Ala), Phenylalanine (Phe), Glycine (Gly), Glutamic (Glu), Lysine (Lys), Tyrosine (Tyr), Tryptophan (Trp), Serine (Ser), Valine (Val), Proline (Pro), Asparticacid (Asp), Isoleucine (Ile), Methionine (Met), Ornithin (Orn), Arginine (Arg), were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The artificial urine solution contained 1.1 mM lactic acid, 2.0 mM citric acid, 25 mM sodium bicarbonate, 170 mM urea, 2.5 mM calcium chloride, 90 mM sodium chloride, 2.0 mM magnesium sulfate, 10 mM sodium sulfate, 7.0 mM potassium dihydrogen phosphate, 7.0 mM dipotassium hydrogen phosphate, and 25 mM ammonium chloride and all mixed in Milli-Q water, and the pH of the solution was adjusted to 6.0 by the addition of 1.0 M hydrochloric acid.

2.2 Instrumentation. Fluorescence spectra were measured in a fluorescence microplate reader (infinite M200 pro, TECAN, Switzerland) using a black 384 well microplate (Fluotrac 200, Greiner, Germany). The excitation wavelength used was 560 nm for the emission spectra.

2.3 Preparation of DNA-scaffolded silver nanoclusters (DNA-AgNCs). The synthesis of DNA-AgNCs was according to the reported method in our previously study[18]. Briefly, 3 μM DNA scaffold or control DNA and 20 μM $AgNO_3$ were sequentially added and mixed with sodium phosphate buffer (20 mM, pH 6.6), and the reaction mixture was incubated at room temperature, in the dark, for 20 minutes. 20 μM $NaBH_4$ was added and the reaction mixture was incubated at room temperature, in the dark, for one hour. Following reduction of Ag^+ ions, highly fluorescent DNA-AgNCs were produced with fluorescence emission at 632 nm.

2.4 Assays for histidine using the DNA-AgNCs/Cu^{2+} ensemble. The DNA-AgNCs/Cu^{2+} ensemble was prepared by mixing 70 μL of DNA-AgNCs and 10 μL of 100 μM Cu^{2+} in a plastic tube. For the fluorescent "on" detection of histidine, 10 μL histidine or control samples or Mill-Q water (as blank sample) was added to the DNA-AgNCs/Cu^{2+} ensemble. The final volume was 100 μL. 80μL mixture solution was injected into 384 well microplate and incubated for 20 min at room temperature. After that the fluorescence spectra of mixture was measured in a fluorescence microplate reader (excited at 560 nm).

3 Results and discussion

3.1 Characterization of DNA-AgNCs. DNA-scaffolded silver nanoclusters (DNA-AgNCs) were synthesized by reducing $AgNO_3$ with $NaBH_4$ in the presence of the cytosine-(C)-rich ssDNA with the sequence: 5'-ATCCTCCCACCGGGCCTCCCACCATAAAAACCCTTAA TCCCC-3'. The absorption and fluorescence emission were measured to confirm the formation of DNA-AgNCs (Fig. S1A). As shown in Fig. S1A, the as-prepared DNA-AgNCs exhibit strong fluorescence emission at 632 nm with excitation at 560 nm. A typical transmission electron microscopy (TEM) image in Fig. S1B shows that the DNA-AgNCs are mono-dispersed and uniform.

3.2 DNA-AgNCs/Cu^{2+} ensemble for detection of histidine. The fluorescence turn on strategy is based on the analyte competing for a Cu^{2+}: The DNA-AgNCs solution can be highly fluorescent, however, in the presence of Cu^{2+}, the fluorescence of the DNA-AgNCs was found to be quenched by Cu^{2+}. This phenomenon could be ascribed to the interaction between Cu^{2+} and nucleic acids of DNA. As we know, many divalent metal ions are fluorescence quenchers and work via numerous mechanisms, including ground-state complexation, collisional conversion of electronic to kinetic energy, heavy atom effects, magnetic perturbations, charge transfer phenomena, electronic energy transfer, and fluorescence resonance energy transfer[11, 21]. Among them, Cu^{2+} is a highly efficient fluorescent quencher due to its paramagnetic properties via electron or energy transfer, and Cu^{2+} could quench the fluorescence of DNA-AgNCs via metal–metal interplay[18]. When histidine was present in the complex system, Cu^{2+} interacted with the imidazole residue of histidine, which is different from other amino acids.

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The fluorescence of released DNA-AgNCs was extremely enhanced, which can be used as a selective "turn on" signal for histidine assay.

To demonstrate the feasibility of the proposed strategy, Cu^{2+} was initially added to the prepared DNA-AgNCs, the fluorescence of solution was fully quenched as shown in Fig. 1 (b). When histidine was added into the mixture solution, a marvelous fluorescent enhancement was observed (see curve c and d). These observations support the feasibility of this method for determination of histidine. The corresponding photographs of the solutions illuminated by a UV lamp with excitation at 365 nm were shown in the inset of Fig. 1. The prepared DNA-AgNCs turned from pink-red to colourless after adding Cu^{2+}. However, the pink-red was restored quickly when the mixture solution in the presence of histidine. These observations strongly support the sensing mechanism of ourmethod, and it provides an additional simple approach for detecting histidine.

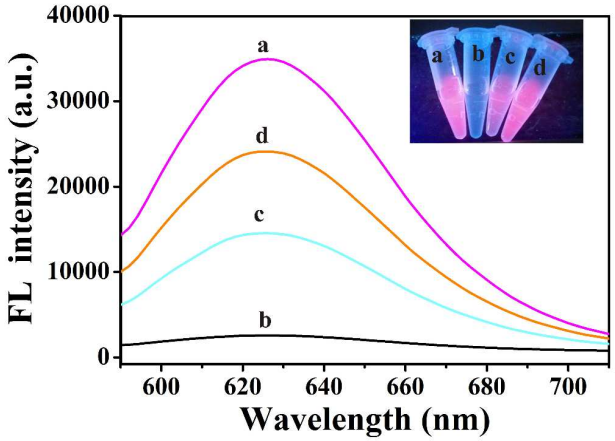

Fig. 1. (a) Fluorescence spectrum of DNA-AgNCs; (b) Fluorescence spectrum of DNA-AgNCs in the presence of 10 μM Cu^{2+}; (c) and (d) Fluorescence responses of DNA-AgNCs in the presence of 50μM and 100 μM histidine; The inset shows the corresponding photograph of solutions of DNA-AgNCs (a), DNA-AgNCs-Cu^{2+} (b), DNA-AgNCs-Cu^{2+}-histidine (c) and (d) illuminated by a UV lamp with excitation at 365 nm.

In order to obtain a low backgroud signal, it was necessary to determine the optimise the concentration of Cu^{2+}. As shown in Fig. S2, the fluorescence of DNA-AgNCs was fully quenched when the concentration of Cu^{2+} was 10 μM in the system. Unless noted otherwise, the following experiments were all carried out under the optimal condition.

3.3 Selectivity of the proposed strategy for histidine. A major shortcoming associated with some conventional methods for quantitation of histidine was the serious interferences arising from other natural amino acids because of their similar properties and structure. Under optimum conditions, the effects of 16 natural amino acids or potential coexisting biomolecules on the response from the DNA-AgNCs/Cu^{2+} are summarized in Fig. 2A. Compared to the response from 50 μM histidine, no obvious signals from these tested amino acids or biomolecules are observed, demonstrating the good selectivity of the proposed method for histidine over other natural amino acids and biomolecules. This selective dissociation of Cu^{2+} from the decorated DNA-AgNCs arises because of the imidazole side chain moiety contained in histidine, which is the most distinct difference in the structure between histidine and other natural amino acids. In order to further demonstrate the excellent selectivity of our proposed strategy, the competition experiments were conducted in the presence of mixture of other amino acids. Significantly, the fluorescence intensity change of histidine in the mixture of 5-folded other amino acids was less than 5%, relative to histidine alone at same concentration (Fig. 2B). The result suggested that the coexistence of other amino acids in the system of DNA-AgNCs/Cu^{2+} did not affect the detection of histidine. Therefore, the proposed method is practical for the determination of histidine in the mixture of amino acids found in protein without separation.

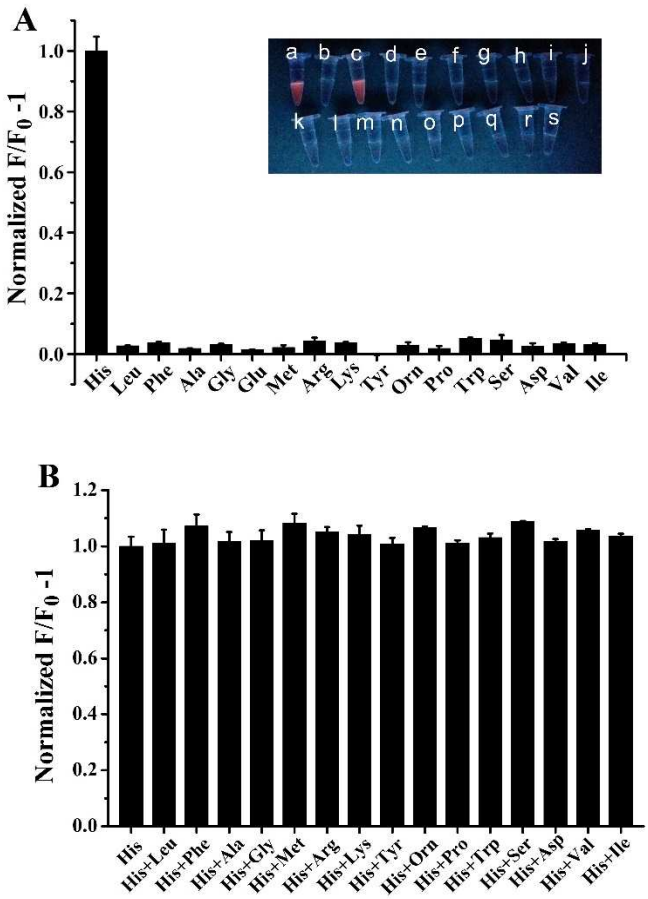

Fig. 2. (A) Fluorescence responses of DNA-AgNCs/Cu^{2+} probe to histidine and various amino acids. The inset shows the corresponding photograph of solutions of DNA-AgNCs (a), DNA-AgNCs-Cu^{2+} (b), DNA-AgNCs/Cu^{2+} in the presence of 50 μM histidine (c) and DNA-AgNCs/Cu^{2+} in the presence of 50 μM other amine acids (d-s); (B) Bars represent the fluorescence responses of the DNA-AgNCs/Cu^{2+} ensemble probe to 50 μM histidine, 50 μM histidine in the presence of a mixture of 250 μM other amino acids is also presented, using the fluorescence enhancement at 632 nm to monitor the responses, respectively.

In order to test the feasibility of our proposed method in real samples, we studied the possible applicability of the DNA-AgNCs/Cu^{2+} ensemble for the direct measuring of histidine in artificial urine. Urine is a variable fluid, both between individuals and in the same individual over time, thus we prepared a stable artificial urine according to the reported literature,[22] which is a suitable replacement for normal urine for use in a wide range of experiments. As shown in Fig. 3A, the fluorescence of DNA-AgNCs/Cu^{2+} ensemble was restored

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gradually in the presence of the increasing concentration of histidine. The characteristics of calibration curve and detection limit of the fluorescent sensor were investigated. From Fig 3B, it can be seen that the fluorescent enhancement constant (F/F_0-1) is sensitive to the concentration of histidine, the fitting range is from 0 to 100 μM with a Boltzmann sigmoidal: Y=-2.983+15.333/{1+exp [(29.37-x)/20.02]}, where *Y* stands for F/F_0-1 and *X* is the concentration of histidine (regression coefficient R^2=0.9986). The limit of detection of histidine using DNA-AgNCs/Cu^{2+} ensemble reached to 1.4 μM (S/N=3). As shown in the inset of Fig. 3B, the linear range of the calibration curve was obtained from 10 to 40 μM, with a linear equation: Y=-1.358+0.2049*X. To ascertain the information, the recovery of added known amount histidine to the artificial urine samples was studied. A summary of the spiked concentration for each water sample and the corresponding analysis results were shown in Table S1. The concentrations of histidine in the spiked artificial urine samples determined by the developed method were in good agreement with those of histidine added. The quantitative recoveries ranged from 98.6% to 100.4%, which indicated that the present method has a promise in practical application with great accuracy and reliability.

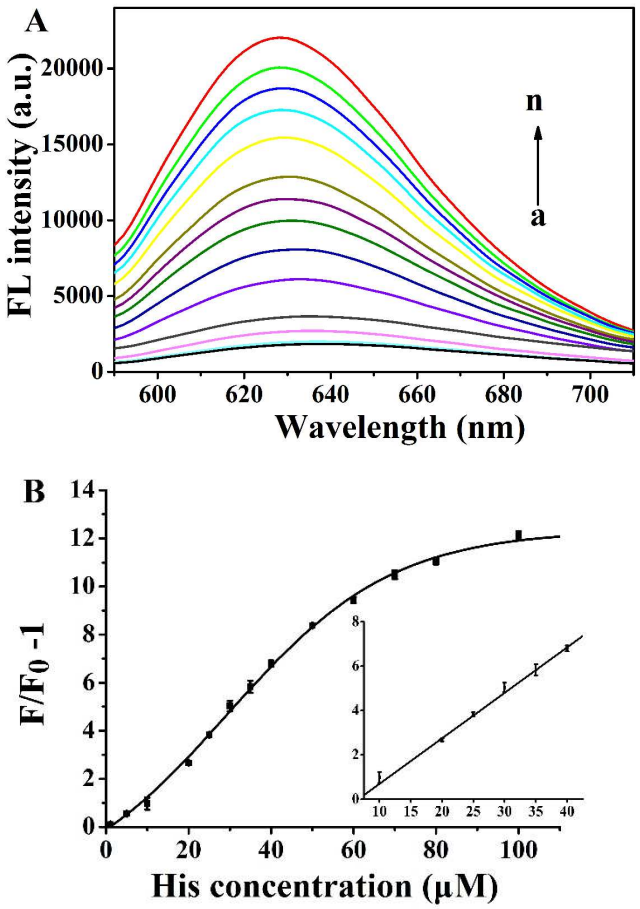

Fig.3. (A) Fluorescence responses of DNA-AgNCs/Cu^{2+} ensemble to histidine. The fluorescence emission spectra are shown for various histidine concentrations. a-n represents different concentrations of 0, 1, 5, 10, 20, 25, 30, 35, 40, 50, 60, 70, 80 and 100μM; (b) Plot of fluorescence responses of DNA-AgNCs/Cu^{2+} ensemble solution to the various concentrations of histidine indicated, the inset shows the linear range of the calibration curve.

4 Conclusions

In summary, we have demonstrated a new type of rapid, highly sensitive, and selective fluorescence turn-on assay for detection of histidine using a DNA-AgNCs/Cu^{2+} ensemble. This assay is based on the highly specific interaction between histidine and Cu^{2+}, and the strong fluorescence DNA-AgNCs probe in a competition assay format. The resulting high sensitivity and selectivity for histidine was achieved. The system is simple in design and fast in operation and is more convenient and promising than other methods. The novel strategy eliminated the need of organic solvents, enzymatic reactions, separation processes, chemical modifications, and sophisticated instrumentations. In addition, the detection and discrimination process can be seen with the naked eye under a hand-held UV lamp. The detection limit of this method is lower or at least comparable to the previous fluorescence-based methods. Importantly, the protocol offers high selectivity for the determination of histidine among amino acids found in proteins, as well as detection in artificial urine samples. Thus, the assay showed great potential for practical application as a disease-associated biomarker and would be needed to satisfy the great demand of amino acid determination in fields such as food processing, biochemistry, pharmaceuticals, and clinical analysis. Significantly, as many biomolecules could form stable complexes with metal ions selectively, these findings offer a potential approach to the detection of a wild spectrum of analytes.

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Notes and references

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