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Highly sensitive fluorescence detection of mercury ion based on Lys VI stabilized gold nanoclusters

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Au nanoclusters are presented as novel probes for sensitive detection of mercury ions. Au nanoclusters were synthesized by using Lys VI as a template via a convenient chemical reduction process. The as-prepared water-soluble AuNCs with average diameters of 1.6~1.8 nm exhibit unique fluorescence excitation at 400 nm, maximum emission at 625 nm, and can be quenched in the presence of mercury ions. Excellent linear relationships exist between the fluorescence intensity of the Au nanoclusters and Hg^{2+} concentrations in the range of 0.1 nM to 1 nM, 0.1 μm to 1 μM, or 2 μM to 8 μm, respectively. The detection limit for Hg^{2+} is 0.1 nM. The present nanosensor for Hg^{2+} detection possesses excellent biocompatibility, high selectivity, and good stability, which suggest its potential for diagnostic purposes.

Key words: Au nanoclusters; Lys VI; Mercury ions; High selectivity.

INTRODUCTION

It is known that Hg^{2+} is a highly toxic contaminant that exists in water, soil, and food. Mercury can accumulate in organisms and interact with the thiol groups in proteins to cause serious damage to brain, kidneys, central nervous system, immune system, endocrine system and constitute a serious threat to human health and natural environment [1-3]. Detecting Hg^{2+} is undoubtedly a significant step in environment and health monitoring. Although many methods for Hg^{2+} analysis have been well developed, existing methods require complex equipments and sophisticated operations. A variety of sensor platforms for Hg^{2+} detection have been developed using polymers, nanoparticles, grapheme oxide, carbon nanotubes, etc. [4-10]. However, the synthesis or surface modification of these materials is relatively complicated or time-consuming. Therefore, it is still very important to develop new materials, which require simple synthetic procedures for protein discrimination. Fluorescent metal nanoclusters have gained much attention, because of their burgeoning photophysical properties and potential applications in biosensing and bioimaging. Recently, Au metal clusters (Au NCs) have been employed as probes to detect Hg^{2+} based on the quenching of their fluorescence. Xie and his co-workers used native bovine serum albumin (BSA) to synthesize Au nanoclusters via one-pot chemical reduction and further used them as fluorescent probes for sensitive and selective Hg^{2+} and Cu^{2+} ions sensing [11, 12]. The limit of detection (LOD) was measured to be 10 nM and a remarkable selectivity toward Hg^{2+} could be obtained when conducting the assay in the presence of a chelating ligand, 2, 6-pyridinedicarboxylic acid (PDCA)[13]. Although the above-mentioned methods proved to be efficient, there still remains a challenge for facile one-step synthesis of fluorescent AuNCs, which could be used to detect trace Hg^{2+} below 1 nM with excellent linear relationship. Here we demonstrate the synthesis of Lys VI stabilized AuNCs with unique fluorescence in the red region of the visible spectrum and further use these clusters as sensitive and selective probes for label-free Hg^{2+}.

EXPERIMENTAL

Chemicals and Materials

Lysozyme (chicken eggwhite) was purchased from Sigma-Aldrich. $HAuCl_4$, NaOH, $CaCl_2$, $MnCl_2$, $FeCl_3$, $CrCl_3$, $NiCl_2$, $CoCl_2$, $CuCl_2$, $HgCl_2$, were obtained from Guangzhou Qiyun

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Biotechnology Co. LTD. Milli-Q ultrapure water (Milli-pore, Hamburg, Germany) was used in all experiments.

Synthesis of Lys VI-AuNCs

The AuNCs were synthesized by chemical reduction of $HAuCl_4$ with Lys VI. A solution of Lys VI (5 mL, 25 mg/mL) was added to an equal volume of 10 mM $HAuCl_4$. The mixture was vigorously stirred for 2 min, the reaction pH was adjusted to 12.0 with NaOH. The resulting solution was incubated overnight at 37 °C.

Characterization of Lys VI-AuNCs

TEM and high resolution TEM (HRTEM) measurements were carried out by using a FEI Tecnai G20 instrument with 200 kV accelerating voltage. The absorption and fluorescence spectra of the AuNCs were obtained using a double-beam UV-visible spectrophotometer (UV-2550, Shimadzu, Japan) and a Hitachi F-7000 fluorometer (Hitachi, Japan), respectively.

Hg^{2+} Detection Using Lys VI-AuNCs

Hg^{2+} (1×10^{-10} to 1×10^{-2} M) and other metal ions (10^{-4} M) were separately added to an equal volume of the Lys VI-AuNCs. After 30 min, the mixed solutions were transferred separately into a 1 mL quartz cuvette. Their fluorescence spectra were recorded by operating the fluorescence spectrophotometer at an excitation wavelength of 400 nm.

RESULTS AND DISCUSSION

Figure 1 displays the HRTEM images of the as-prepared, well monodispersed Lys VI-AuNCs. The close-up in the top inset shows lattice planes separated by 0.235 nm, corresponding to the (111) lattice spacing of the face-centered cubic Au (0.23 nm) [14]. The average diameter of the AuNCs is 1.6~1.8 nm. The mean diameter of Lys VI-AuNCs was determined as 1.6~1.8 nm, as judged from over 100 individual particles.

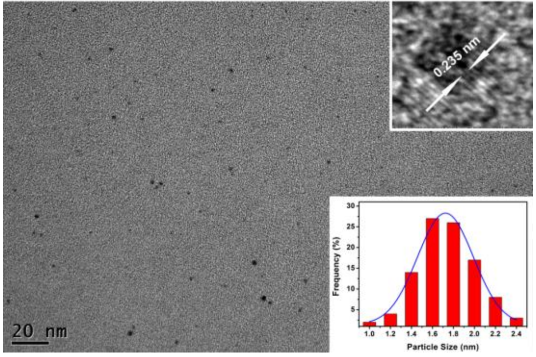

Fig. 1. Typical TEM image of the as-prepared Lys VI-AuNCs. The insets show the HRTEM image (top) and the size distribution histogram (bottom).

The as-prepared Lys VI-AuNCs showed a broad absorption band and absence of localized surface plasmon resonance bands, suggesting the formation of the Au NCs (Figure 2). In addition, the fluorescence emission (the excitation wavelength is 400 nm and the emission peak is 625 nm) was also investigated to confirm the formation of Au NCs.

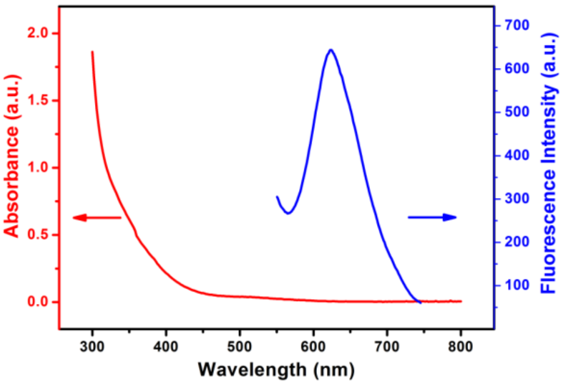

Fig. 2. UV-vis absorption spectrum and fluorescence emission spectrum of the as-prepared Lys VI-AuNCs.

Before applying the Lys VI-AuNCs for detection of mercury ions, the effect of reaction time between AuNCs and Hg^{2+} on the fluorescent AuNCs was investigated. As shown in Figure 3, the fluorescence intensity of the Lys VI-AuNCs decreases rapidly at the beginning (0~20min) after the addition of Hg^{2+}, and further maintains the same value. Thus, 30 min was selected as the reaction time. In our experiments, fluorescence detection was carried out 30 min after Hg^{2+} addition.

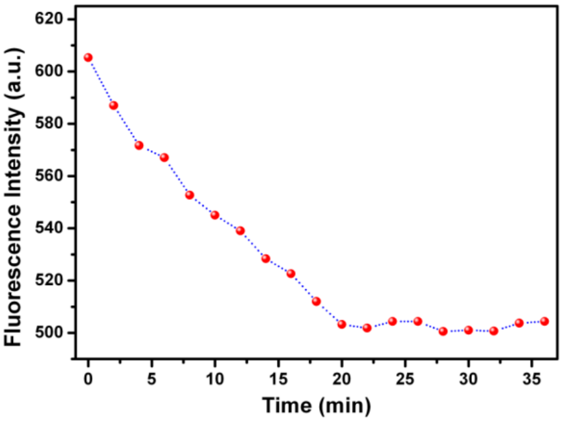

Fig. 3. The relation between fluorescence intensity and reaction time.

As shown in Figure 4, the relation between fluorescence intensity and Hg^{2+} concentration is complicated. It can be divided into four sections. In the first section, the fluorescence intensity of the Lys VI-AuNCs displayed a gradual decrease at 625 nm with increasing Hg^{2+} concentration. The fluorescence quenching of AuNCs in the presence of Hg^{2+} is presented in Figure 4 (a), which exhibits a good linear relationship in the range of 0.1 nM to

1 nM.

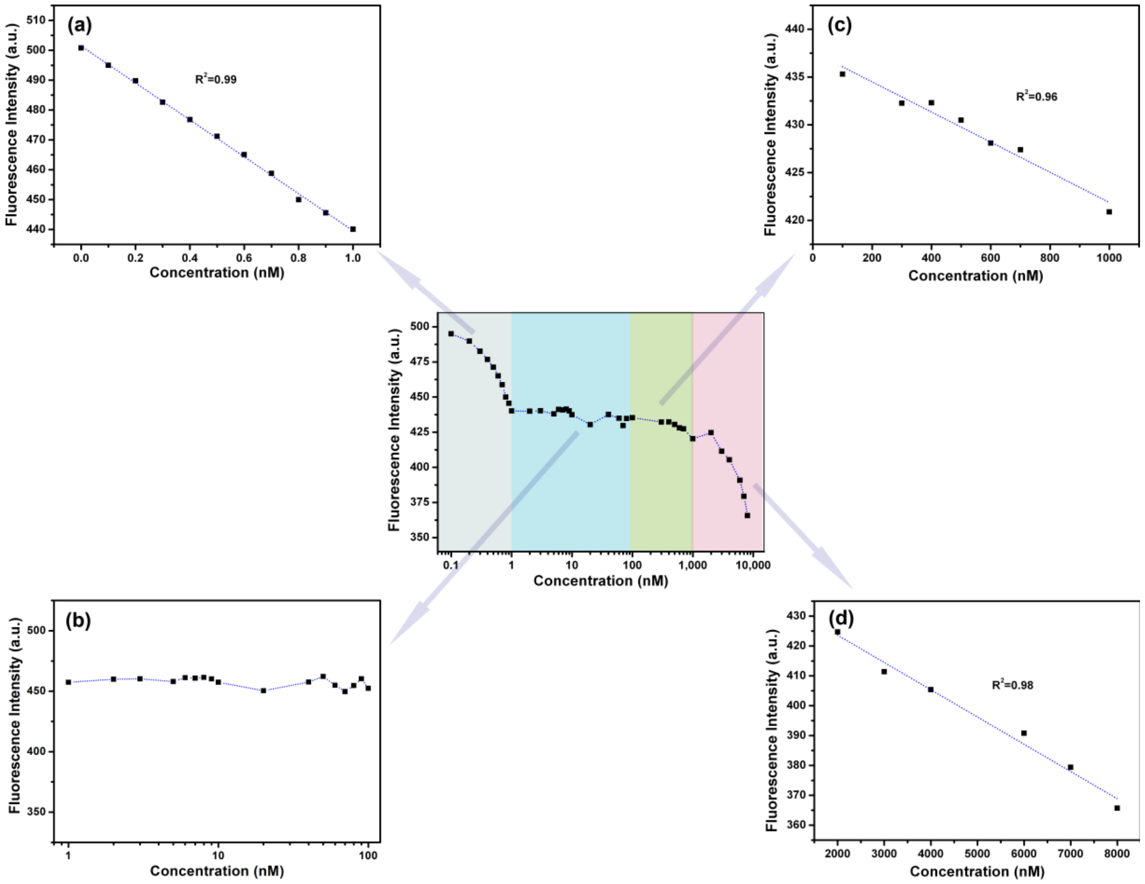

Fig. 4. Fluorescence changes of the Ag NCs in the presence of different concentrations of Hg^{2+}.

When Hg^{2+} concentrations increased to 1~100 nM, a strange phenomenon was observed. The change of fluorescence intensity is not obvious and the related reason is not found (Figure 4 (b)). However, when Hg^{2+} concentrations increased to 100~1000 nM, the fluorescence intensity versus Hg^{2+} concentrations was linear again (Figure 4 (c)). In addition, the fluorescence intensity decreased as Hg^{2+} concentrations continually increased (Figure 4 (d)). The Hg^{2+}-induced fluorescence quenching of the Lys VI-AuNCs is probably related to the formation of metallophilic bonding between $Hg^{2+}(4f^{14}5d^{10})$ and Au^{+} $(4f^{14}5d^{10})$.

To further investigate the detection selectivity for mercury ions of Lys VI-AuNCs, the changes in fluorescence of the Lys VI-AuNCs that were induced with 50 μM of the following metal ions (Ca^{2+}, Mn^{2+}, Fe^{3+}, Cr^{3+}, Ni^{2+}, Co^{2+} and Cu^{2+}) were detected. As shown in Figure 5, all of the other ions including Cu^{2+} did not quench the fluorescence of Au clusters, while only Hg^{2+} could effectively quench the fluorescence. These results indicate that the Lys VI-AuNCs display excellent selectivity toward mercury ions.

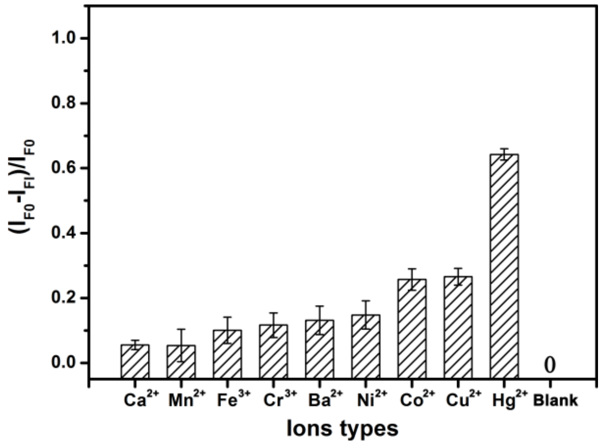

Fig. 5. Fluorescence changes of Lys VI-AuNCs in the presence of different metal ions. IF_0 and IF_I correspond to the fluorescence intensity of Lys VI-AuNCs in the absence and in the presence of metal ions.

CONCLUSIONS

In summary, we describe here a Lys VI-directed, facile one-pot method for the synthesis of water-soluble fluorescent AuNCs. The approach is simple and more environmentally friendly than previously reported etching-based or organic phase-based strategies. The as-prepared water-soluble AuNCs with average diameters of

1.6~1.8 nm exhibit a unique fluorescence excitation at 400 nm, a maximum emission at 625 nm, and can be quenched in the presence of Hg^{2+}. Excellent linear relationships exist between the fluorescence intensity of the Au nanoclusters and the concentrations of Hg^{2+} in the range of 0.1 nM to 1 nM, 0.1 μM to 1 μM, or 2 μM to 8 μM, respectively. The detection limit for Hg^{2+} is 0.1 nM. The present nanosensor for Hg^{2+} detection possesses excellent biocompatibility, high selectivity, and good stability, which suggest its potential for diagnostic purposes.

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ВИСОКО-ЧУВСТВИТЕЛНО ОТКРИВАНЕ НА ЖИВАЧНИ ЙОНИ НА БАЗАТА НА Lys VI – СТАБИЛИЗИРАНИ ЗЛАТНИ НАНО-КЛЪСТЕРИ

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(Резюме)

Представени са златни нано-клъстери(AuNCs) като нови датчици за чувствително откриване на живачни йони. Те се синтезират с използването на Lys VI като основа чрез удобна химична редукция.   as a template via a convenient chemical reduction process. Така приготвените водо-разтворими златни нано-клъстерисъс среден диаметър 1.6~1.8 nm показватуникално флуоресцентно възбуждане при 400 nm с максимална емисия при 625 nm и могат да бъдат закалени в присъствие на живачни йони. Съществува отлична линейна зависимост между интензивността на флуоресценцията на AuNCsи концентрацията на Hg^{2+}ш интервала от 0.1 nMдо 1 nM и съответно от0.1 μmдо 1 μM, или от 2 μMдо 8 μm. Границата на откриване за Hg^{2+}е 0.1 nM. Представеният наносензор за откриване на Hg^{2+}притежава от;ична биологична съвместимост, висока селективност и добра стабилност, което внушава неговия потенциал за диагностични цели.