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ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY C · AUGUST 2007

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Fluorescent Conjugated Polymer-Stabilized Gold Nanoparticles for Sensitive and Selective Detection of Cysteine

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Received: May 21, 2007; In Final Form: June 25, 2007

We report a new fluorescent detection method for cysteine based on one-step prepared fluorescent conjugated polymer-stabilized gold nanoparticles. The as-prepared fluorescent conjugated polymer-stabilized gold nanoparticles fluoresce weakly due to the fluorescence resonance energy transfer between the fluorophore and the gold nanoparticles. Upon the addition of cysteine, a thiol-containing amino acid, the fluorescence of the colloidal solution increases significantly, indicating that cysteine can modulate the energy transfer between fluorophore and gold. This phenomenon then allows for sensitive detection of cysteine with a limit of detection (LOD) of 25 nM. The linear range of determination of cysteine is from 5×10^{-8} to 4×10^{-6} M. None of the other amino acids found in proteins interferes with the determination. Moreover, due to the excellent protecting ability of the fluorescent conjugated polymers, the synthesis of metal nanoparticles and modifying with fluorophores can be accomplished within one step, which makes our method much simpler than conventional methods. We also expect that it will be possible to detect other biologically important analytes based on the fluorescent conjugated polymer-stabilized metal nanoparticles.

1. Introduction

Recently, the applications of metal nanoparticles (NPs) in bioanalysis have drawn great interest due to their special optical and electrical properties.[1−4] One feature that makes them particularly appealing is that metal NPs possess high extinction coefficients in the visible region, which thus enables them to function as efficient quenchers for most fluorophores.[5−6] By taking advantage of this superquenching ability of metal NPs (especially Au NPs), many high-performance fluorescence assay methods have been developed in recent years for optically sensing biologically important ions and molecules. For example, Chang and co-workers reported the determination of thiols[7] and Hg ions[8] using organic dye (Nile red and Rhodamine B, respectively) adsorbed Au NPs based on the fluorescence resonance energy transfer between Au NPs and small dye molecules. Similarly, based on the fluorescence quenching of fluorescein isothiocyanate caused by gold nanoparticles coated with a monoclonal antibody, Ao et al.[9] demonstrated a sensitive, highly specific immunoassay system for antigen detection, with the limit of detection about 0.17 nM. In one recent work, He et al.[10] developed a homogeneous assay method to detect the Cu^{2+} ion based on the modulation of the photoluminescent quenching efficiency between perylene bisimide chromophore and Au NPs in the presence of Cu^{2+} ions. However, almost all the reported methods are based on the use of small molecule fluorophores, and one obvious disadvantage of such systems is the inefficient quenching of small molecule fluorophores, which will thus limit the more general application of metal NPs in sensor approaches.[11] In addition, most of these methods involved modification steps to functionalize the pre-synthesized metal NPs with photoactive fluorophores, which undoubtedly led to complications as well as being time-consuming. Therefore, it is necessary and important to make further efforts to develop alternative methods with a wider applicability and more simplicity for the applications of metal NPs in sensor approaches.

Herein, we report a new fluorescent sensor based on water-soluble conjugated polymer-stabilized Au NPs for sensitive and selective detection of cysteine. Cysteine plays a critical role in a variety of important cellular functions, such as detoxification and metabolism, and cysteine deficiency is involved in many syndromes, such as edema, liver damage, and skin lesions, which thus make the detection of cysteine very important.[12−14] In the present work, a water-soluble anionic fluorescent conjugated polymer (the structure is shown in Scheme 1, denoted as PFS) was chosen as the fluorophore. As compared to the conventional small molecule fluorophores, conjugated polymers exhibit exceptional fluorescence quenching or energy transfer efficiencies and, therefore, are of particular interest as optical platforms in highly sensitive chemical and biological sensors.[15−18] Moreover, owing to the excellent stabilizing ability of conjugated polymers toward nanoparticles, the synthesis of metal NPs and the modification with fluorophores could be accomplished within one step, which then makes our method much simpler than the conventional methods where additional steps to functionalize the pre-synthesized NPs with fluorophores could not be avoided.[8,19,20]

The principle of the fluorescent sensor designed is schematically presented in Scheme 1. As shown, in the PFS−Au NPs composites, PFS fluoresces weakly due to the occurrence of a highly efficient fluorescence resonance energy transfer (FRET) between PFS and Au NPs. After the addition of cysteine, PFS will be moved away from the surfaces of Au NPs because of

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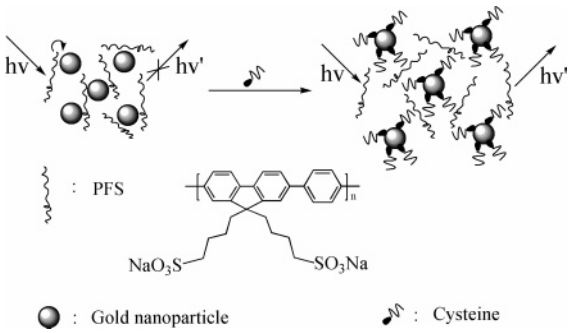
10.1021/jp073913p CCC: $37.00
Published on Web 08/22/2007

SCHEME 1: Schematic Representations of Fluorescent Sensors for Cysteine Detection



the strong interactions between the thiol group of cysteine and gold.[21] As a result, the fluorescence of PFS can recover correspondingly, and the extent of the recovery should depend on the amount of cysteine added. Therefore, the detection of cysteine can be realized by a sensitive and simple approach.

2. Experimental Procedures

2.1. Materials. Poly(9,9-bis(4′-sulfnoatobutyl)fluorene-*co*-alt-1,4-phenylene) sodium salt (PFS) was synthesized according to the literature.[22] $HAuCl_4 \cdot 3H_2O$ was purchased from Beijing Chemical Company. L-Cysteine was purchased from Shanghai CapitalBio Corporation. 2-Mercaptoethanol, thioglycolic acid, and sodium borohydride (98%) were purchased from Sigma. All other reagents were of analytical reagent grade and used as received. The water used was purified through a Millipore system.

2.2. Methods. All glassware used in the following experiments was cleaned in a bath of freshly prepared 3:1 HCl/HNO_3 and rinsed thoroughly in H_2O prior to use. The stock solution of PFS was prepared in deionized water, and the concentration was calculated based on the molecular weight of the repeating units, 556.62. PFS-stabilized Au NPs were then synthesized by a one-step reduction in homogeneous solution at ambient conditions, modeled after the method of Brust et al.[23] Briefly, a range of volumes (0.1, 0.2, 0.5, and 1.0 mL) of 0.24 mM PFS solution was mixed with 50 mL of 0.24 mM $HAuCl_4$ under vigorous stirring. After 5 min, 0.48 mL of a freshly prepared 0.25 M aqueous sodium borohydride solution was added. The reaction was allowed to proceed for another 30 min and then stored at 4 °C until needed. The size and morphology of Au NPs were characterized with transmission electron microscopy (TEM). By monitoring the emission of the final colloid solutions,[7] the optimum concentration of PFS needed to stabilize Au NPs was found to be 9.6×10^{-7} M, corresponding to 0.24 mM Au. Thus, under the optimum conditions, no redundant PFS existed in the system. On the other hand, a control experiment indicated a negligible effect of the products from the reacted sodium borohydride to the detection of cysteine. Therefore, no more separation steps were necessary to purify the as-prepared nanocomposites.

For the cysteine detection, 1.25 mL of the as-prepared PFS−Au NPs solution was first diluted to 5.0 mL by deionized water. Subsequent titration experiments were then carried out by adding aliquot amounts of a 1.2×10^{-5} M freshly prepared cysteine solution. The solution was incubated for 5 min before the fluorescence measurements. The preliminary investigation showed that the interaction could reach equilibrium within 5 min. The emission spectra were recorded in the wavelength of 390−550 nm upon excitation at 370 nm, using 10 nm/10 nm slit widths. Each spectrum was the average of three scans. The pH of the previous aqueous solution in the cysteine detection was 6.0, which was detected by a pH 211 Microprocessor pH meter (Hanna Instruments). All experiments were performed at room temperature (293 K).

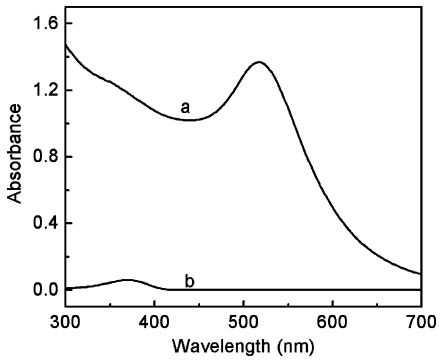


Figure 1. UV−vis absorption spectra of as-prepared PFS-stabilized Au NPs solution (curve a) and PFS aqueous solution (curve b, 9.6×10^{-7} M).

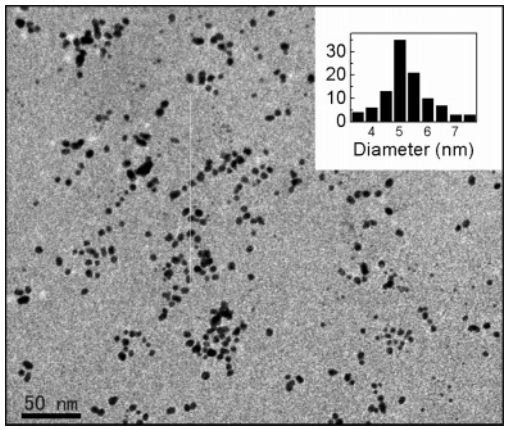


Figure 2. Typical TEM image and corresponding particle size distribution histogram (the inset) of PFS-stabilized Au NPs.

2.3. Instrumentation. Absorption measurements were performed with a Cary 500 UV−vis-NIR spectrometer (Varian). Fluorescence measurements were carried out on a LS-55 Luminescence Spectrometer (PerkinElmer). A 1.00 cm path length rectangular quartz cell was used for these measurements. TEM measurements were made on a JEOL 2000 transmission electron microscope operating at 200 kV. The sample for TEM characterization was prepared by placing a drop of the colloidal solution on the carbon-coated copper grid and drying at room temperature.

3. Results and Discussion

3.1. Preparation and Characterization of PFS-Stabilized Au NPs. Monodisperse PFS-stabilized Au NPs were prepared by the reduction of $AuCl_4^-$ in the presence of PFS with $NaBH_4$ as the reducing agent. The as-prepared colloidal solution was wine-red, and the formation of NPs was examined using the UV−vis spectrum, which showed a characteristic surface plasmon band at 517 nm (curve a in Figure 1). In addition, the absorption spectrum of as-prepared PFS-stabilized Au NPs showed a weak shoulder peak around 370 nm, which corresponded to the absorption of the PFS molecule (curve b in Figure 1).

TEM was applied to further characterize the morphology and size of as-prepared Au NPs. Figure 2 shows a typical TEM image and the corresponding particle size distribution histogram. As shown in Figure 2, the nanoparticles are generally spherical

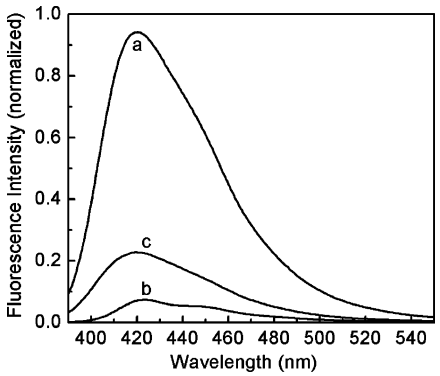


Figure 3. Fluorescence emission spectra of solutions containing PFS (curve a) and PFS−Au NPs (curve b) in the absence and presence of 5.0×10^{-6} M cysteine (curve c). Concentrations of PFS and Au NPs are 2.4×10^{-7} and 1.5×10^{-8} M, respectively.

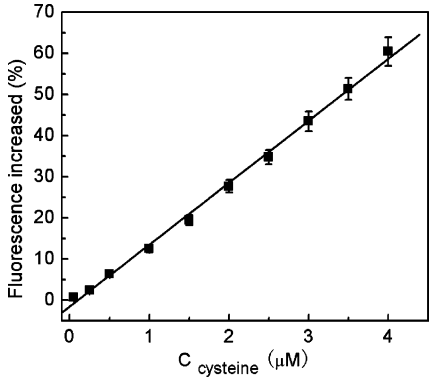


Figure 4. Linear plots of the fluorescence intensity of PFS−Au NPs increased (%) at 420 nm vs the added cysteine concentration. Error bars represent the standard deviation of three measurements.

and well-separated from each other. The size distribution histogram shown in the inset of Figure 2 reveals a diameter of 5.1 ± 0.8 nm, as judged from more than 100 individual particles.

It is noteworthy that the PFS-stabilized Au NPs prepared as reported here showed remarkable stability in aqueous solution for months under ambient conditions, despite weak interactions between PFS and gold. Being a fluorescent polyelectrolyte, PFS played dual roles in the present work. Besides functioning as a photoactive fluorophore, it also acted as a good protective agent for colloids because it is capable of combining steric and electrostatic stabilization, which then resulted in electrosteric stabilization.[24,25] Therefore, the choice of fluorescent conjugated polymers in the present work enabled the synthesis of metal NPs and the modification with fluorophores being accomplished within one step. On the contrary, using the corresponding monomer does not stabilize colloids well. This is also one advantage of the present strategy of fabricating fluorescent sensors ultilizing conjugated polymers.

3.2. Interaction between Cysteine and PFS-Stabilized Au NPs. Figure 3 shows the fluorescence emission spectra of a solution containing PFS (curve a) and PFS−Au NPs (curve b) in the absence and presence of cysteine (curve c). As shown, the water-soluble conjugated polymer, PFS, exhibits a strong emission in aqueous solution with the emission maximum at 420 nm and two shoulders around 450 and 475 nm upon excitation at 370 nm (curve a in Figure 3), which is characteristic of polyfluorenes.[22] The emission of PFS was quenched dramatically in the PFS−Au NPs composites (curve b in Figure 3), which suggests a highly efficient energy transfer between PFS and Au as reported previously.[11] However, when cysteine was added to the solution, the fluorescence intensity was found to be enhanced, as shown in Figure 1, curve c. This indicates that the presence of cysteine could modulate the emission behavior of PFS−Au NPs in the solution. Since cysteine possesses a thiol group, due to the strong affinity of thiol to gold, cysteine molecules could be favorably capped on the surface of Au NPs, which resulted in moving PFS away from the gold surface. As a result, the energy transfer between PFS and gold was weakened, and the emission of PFS was then turned on. To further verify the previous mechanism, the responses of the PFS−Au NPs solution to some small thiol molecules, such as 2-mercaptoethanol and thioglycolic acid, were then examined. The results indicated that both molecules could increase the fluorescence intensity of the solution. Contrarily, no increase was observed when ethanol and acetic acid were added to the solution. This suggested that the thiol group of cysteine played an important role in the system. Furthermore, control experiments indicated a negligible effect of cysteine on the emission of PFS alone, which further supported that the increased fluorescence did originate from the modulation of the energy transfer between PFS and gold.

Recently, several groups reported that in the presence of cysteine, gold nanoparticles or nanorods could self-assemble to form a network structure.[26−28] However, in the present system, no aggregation of Au NPs was observed under our experimental conditions, which was evidenced by UV−vis absorption spectroscopy (not shown). The spectroscopic results showed that neither an obvious red shift of the plasmon band of gold nor the appearance of a new absorption band in the longer wavelength was observed in the presence of up to 4 μM cysteine. This stability against aggregation is likely to be due to the steric effects contributed from the polymer, PFS.

3.3. Determination of Cysteine. Fluorescent conjugated polymers are particularly attractive in sensor design because their optical and electrical properties can be greatly modified by very minor perturbations of the environmental stimuli and there exist amplification actions by a collective response in macromolecules.[29,30] Since the previous studies indicated that thiol-containing cysteine could modulate the fluorescence emission of PFS-stabilized Au NPs, we then expect as-prepared PFS-stabilized Au NPs can be used to quantitatively detect cysteine, a biologically important analyte. As expected, our further experiment showed that the increase of fluorescence intensity could quantitatively reflect the amount of cysteine added. As shown in Figure 4, a good linear relationship between the increase of fluorescence intensity at 420 nm and the concentration of cysteine is obtained in the range of 5×10^{-8} to 4×10^{-6} M. The limit of detection (LOD) at an S/N ratio of 3 for cysteine is 25 nM, which can be comparable to the most sensitive method reported for cysteine detection.[7,26−28,31,32] Also, the LOD for cysteine is much lower than those in plasma (micromolar), which suggests that the present approach has great potential for diagnostic purposes. In addition, three separate measurements have been conducted, which revealed good reproducibility.

The low LOD value obtained here should be ascribed to the key component of the assay, PFS. As compared to small molecule counterparts, the electronic structure of the conjugated polymers coordinates the action of a large number of absorbing units. The perturbation can migrate along the polymer backbone before transferring to the chromophore reporter and results in an amplification of signals.[11] Therefore, the optical properties of conjugated polymers possess particularly high sensitivity to the environmental stimuli and are especially attractive in sensor design. In the present work, the conjugated polymers acted as

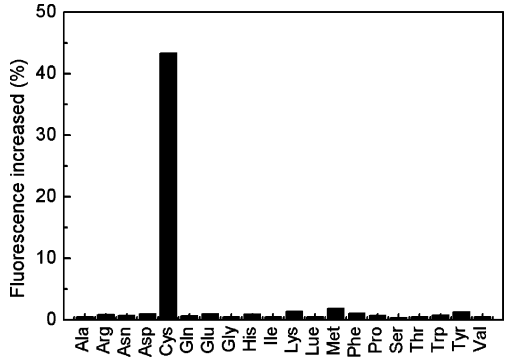


Figure 5. Fluorescence intensity of PFS−Au NPs increased (%) at 420 nm upon the addition of 3.0×10^{-6} M cysteine and other α-amino acids.

an amplifier for the modulated energy transfer between PFS and Au, thus allowing detection limits for cysteine in the nanomolar range.

It is noteworthy that the control over the concentration of PFS in the sensor system is particularly essential. Either redundant or insufficient amounts of PFS present in the solution were found to deteriorate the system's sensitivity. For instance, when using much more PFS than needed to stabilize Au NPs, the background signal will be higher due to the strong emission from free fluorophores, which can affect the sensitivity for cysteine detection greatly. Also, if insufficient amounts of PFS were presented, not only would the stabilization of as-prepared Au colloids be distinctly influenced, also the LOD value for cysteine detection would be higher. Thus, a series of experiments with varied PFS/Au ratios has been carried out to ensure the critical concentration of PFS needed. By monitoring the emission of the final colloid solutions,[7] the optimum concentration of PFS needed to stabilize Au NPs was found to be 9.6×10^{-7} M, corresponding to 0.24 mM Au. The presented dynamic range and LOD value were all obtained under optimal conditions.

One of the significant features of the present system is its ability to detect cysteine in the presence of various other α-amino acids. The fluorescence change upon the addition of 20 α-amino acids at the same concentration is presented in Figure 5. As shown, the change of cysteine was strikingly larger than the other amino acids, indicating that the thiol functionality in cysteine is essential for the increased fluorescence. In addition, among the other 19 amino acids, methionine had a relatively higher fluorescence increase, which was mainly because of the weak interaction between the $S-CH_3$ group and the gold nanoparticles. However, the fluorescence increases in the presence of 19 other amino acids are all less than 5% relative to cysteine at the same concentration (3.0×10^{-6} M). Therefore, the proposed method is practical for the determination of cysteine in the mixture of amino acids found in proteins. Although not investigated here, the results presented in this study indicate the diagnostic potential of PFS-stabilized Au NPs for the analysis of cysteine in biological samples.

4. Conclusion

In conclusion, we have demonstrated a new homogeneous assay method for highly sensitive and selective detection of cysteine by taking advantage of the fluorescence quenching of conjugated polymers by metal NPs. These novel fluorescent sensors function by the combination of highly efficient energy transfer between Au NPs and conjugated polymers and the strong affinity of thiol to gold. The method allows the selective analysis of cysteine with a LOD as low as 25 nM. In addition, due to the excellent protecting ability of the fluorescent conjugated polymers, the synthesis of metal NPs and the modification with fluorophores can be accomplished within one step successfully, which simplifies the preparation of fluorescent sensors greatly. Furthermore, the present strategy is not restricted to either conjugated polymers or the analytes we have investigated herein. In fact, the assay method described in this work can be conveniently generalized to other conjugated polymer systems. Also, we expect it will be possible to detect other biologically important analytes based on fluorescent conjugated polymer-stabilized metal NPs.

Acknowledgment. This work was supported by the National Natural Science Foundation of China (20427003 and 20575064).

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