Journal of
Nanoscience and Nanotechnology
Vol. 11, 3578–3585, 2011

Simple and Selective Sensing of Cysteine Using Gold Nanoparticles Modified by Ruthenium(II) Complexes

Mei-Jin Li[1,*], Chong-Qing Zhan[1], Ya-Mei Zheng[1], Guo-Nan Chen[1], and Xi Chen[1,2,*]

[1] *Key Laboratory of Analysis and Detection Technology for Food Safety, Ministry of Education, Department of Chemistry, Fuzhou University, Fuzhou 350002, China*
[2] *State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen 361005, China*

A simple and selective luminescence sensing method for the cysteine detection was developed based on gold nanoparticles modified by the new ruthenium(II) complexes. The intense emission of the modified ruthenium(II) complexes was quenched efficiently by gold nanoparticles due to the energy and charge transfer between the ruthenium(II) fluorophores and gold nanoparticles. Upon addition of cysteine, the emission of the ruthenium(II) complexes was enhanced significantly by the release of the ruthenium(II) complexes from the surface of the gold nanoparticles. Therefore, cysteine could be detected by this gold nanoparticles-ruthenium(II) complexes based probes. The synthesis of gold nanoparticles and the modification based fluorophores probed could be accomplished successfully within one step, which simplified the preparation of luminescence sensors. Moreover, since metal-to-ligand charge transfer transition (^{3}MLCT) emission band of the ruthenium(II) complexes was in the visible region, this approach was available for biomolecular sensing applications, and its relatively long life time made it suitable for the biological process studies.

Keywords: Cysteine, Gold Nanoparticles, Ruthenium(II) Complexes, Luminescence Sensor.

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1. INTRODUCTION

Recently, nanomaterials for optoelectronic sensor and light energy conversion applications have emerged as one of the exciting areas of research[1,2] and the applications of metal nanoparticles in bioanalysis have drawn great interest due to their high extinction and strong size- and distance-dependent optical properties.[3] One appealing feature of metal nanoparticles is their high extinction coefficients in the visible region, which thus enables them to function as efficient quenchers for most fluorophores for the design of biological sensors and optoelectronic devices. By taking advantage of this superquenching ability of metal nanoparticles (especially Au colloids), high performance fluorescence assay methods have been developed in recent years for optically sensing biologically important ions and molecules. Sensitizers for light-harvesting systems were reported by Prashant V. Kamat's group.[2] A photoactive molecule (pyrene) was bound to metal nanoparticles to enhance the photochemical activity which rendered the organic–inorganic hybrid nanoassemblies suitable for light-harvesting and optoelectronic applications. Similarly, based on the fluorescence quenching of fluorescein isothiocyanate caused by gold nanoparticles coated with a monoclonal antibody, a sensitive, highly specific immunoassay system for antigen detection was demonstrated by Ao et al.[4] Based on the thin films of gold nanoparticles capped with a 1-hexanethiol monolayer, a novel chemiresistor sensor was developed by Burkhard Raguse and co-workers.[5] It was used for the detection of organic analytes in high-conductivity aqueous electrolyte solution. Using both labeled and label-free methods, colorimetric uranium sensors based on uranyl (UO_2^{2+}) specific DNAzyme and gold nanoparticles were developed and demonstrated by Lee and co-workers.[6] Both sensors had low detection limits for UO_2^{2+} and excellent selectivity over other metal ions.

Amino acids are of particular importance as the fundamental unit of proteins and are essential for numerous processes, such as the formation and growth of new tissues, histamine, adrenaline, insulin, and urea in the body. Cysteine plays a critical role in a variety of important cellular functions, such as detoxification and metabolism. Low levels of cysteine are associated with slow growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, skin lesions and weakness. All indicate that the sensitive and selective detection of cysteine is important in biological and medical fields.[7] Currently,

*Authors to whom correspondence should be addressed.

 1533-4880/2011/11/3578/008 doi:10.1166/jnn.2011.3614

high-performance liquid chromatography (HPLC),[8] mass spectroscopy (MS),[9] electrochemical[10] and fluorescence analytical methods[11] have been applied to the determination of cysteine. However, these methods suffer from poor reproducibility, requirement of expensive and sophisticated instrumentation or complicated sample preparation. That most commonly used in fluorescence technique were organic fluorophores suffering from low brightness and poor photostability, which lead to reduced sensitivity and stability of the sensor. Recently, a variety of colorimetric sensors using Au nanoparticles for cysteine detection have been ingeniously designed due to the unique optical poperties of Au nanoparticles.[12] But most of colorimetric cysteine detection method using AuNPs alone has a high detection limit and low sensitivity. Chang et al. engineered a simple assay based on FRET and aggregation for the sensitive and selective determination of thiols using AuNPs modified by nile red.[13] Later, a gold nanoparticle-based fluorescent sensor using near-infrared organic fluorophore was development by Dong's groups.[14]

In our study, one of the most versatile chromophoric systems, namely ruthenium(II) trisbipyridine derivation was selected. These complexes have been studied well due to their favorable photochemical and photophysical properties including low-lying metal-to-ligand charge transfer transition (MLCT), intense luminescence in the visible region of the spectrum, relatively long radiative lifetimes, tunable electronic structures, and thermal, chemical and photochemical stabilities.[15] Furthermore, these metal chromophores were widely used to photophysical processes including energy and electron transfer reactions in supramolecular inorganic assembly[16] and biological sensor[17] applications. Herein, a new luminescence sensor based on Au colloids modified by the ruthenium(II) polypyridine complex for the sensitive and selective detection of cysteine was reported. The synthesis of gold nanoparticles and the modification with the fluorophores could be accomplished successfully within one step, which simplified the preparation of luminescence sensors. Moreover, the ruthenium(II) complex fluorophore ensured the stability of the sensing method and its visible region from ^{3}MLCT emission band and relatively long life time made it suitable for biological applications.

2. EXPERIMENTAL DETAILS

2.1. Reagents and Instrumentation

$RuCl_3$, ditert-butyldicarbonate, chlorauric acid ($HAuCl_4$), sodium citrate, cysteine and tris(2-carboxyethyl)phosphine (TCEP) were purchased from Sigma-Aldrich. Other chemicals were analytical reagent graded and used as received. *cis*-$Ru(bpy)_2Cl_2 \cdot xH_2O$,[18] 4′-Methyl-2,2′-bipyridine-4-carbox-aldehyde,[19] $BocNH(CH_2)_6NH_2$[20] and $BocNH(CH_2)_{12}NH_2$[20] were synthesized according to the literature procedure. All solutions were prepared with deionized water (Milli-Q, Millipore). The pH of the phosphate buffer solution (PBS) was adjusted with concentrated NaOH or phosphoric acid. Emission spectra were recorded on a HITACHI F-4600 fluorescence spectrophotometer. A 1.00 cm path length rectangular quartz cell was used for all emission measurements. ^{1}H NMR spectra were recorded on a Bruker DPX-300 Fourier Transform NMR spectrometer with chemical shifts reported relative to tetramethylsilane. Positive-ion FAB and EI mass spectra were recorded on a Finnigan MAT95 mass spectrometer. Elemental analysis of the complexes was performed on a Carlo Erba 1106 elemental analyzer.

2.2. Synthesis of Ligands ($mbpyC_6NH_2$ and $mbpyC_{12}NH_2$)

$MbpyC_6NH_2$ was prepared by the reaction of $BocNH(CH_2)_6NH_2$ (72 mg) with 4′-methyl-2,2′-bipyridine-4-carboxaldehyde (60 mg) in dry acetonitrile, and the mixture was heated to reflux for overnight. The solvent was then removed *in vacuo*. The residue was dissolved in methanol, and excess $NaBH_4$ (45 mg) was added. The mixture was stirred at room temperature for 2 h, and the methanol was removed *in vacuo*, followed by an addition of 20 mL water, and extracted with CH_2Cl_2 three times. The organic phase was dried and the solvent was removed. The crude product was purified by column chromatography on silica gel eluting with $CHCl_3$-MeOH (50:1) to provide the white solid. The obtained solid was subsequently dissolved in 15 mL CH_2Cl_2, and 2 mL CF_3COOH was added. The mixture was stirred for an additional 3 h at room temperature. This was followed by washing with Na_2CO_3 solution (pH $\approx$ 9) and water. Chromatography on silica gel with CH_2Cl_2-MeOH as eluent gave ligand as a white solid (71 mg, yield 79%). Positive EI-MS: m/z 298 (M^+). ^{1}HNMR (300 MHz; $CDCl_3$; Me_4Si): 8.61 (d, $J = 4.9$ Hz, 1H, bpy), 8.53 (d, $J = 4.9$, 1H, bpy), 8.31 (d, $J = 1.5$, 1H, bpy), 8.23 (d, $J = 0.8$, 1H, bpy), 7.31(dd, $J = 4.9$, 1.5, 1H, bpy), 7.14 (dd, $J = 4.9$, 0.8, 1H, bpy), 3.89 (s, 2H, bpyCH_2N), 2.66 (m, 2H, CH_2), 2.63 (m, 2H, CH_2), 2.44 (s, 3H, CH_3), 1.48 (m, 4H, CH_2), 1.33 (m, 4H, CH_2).

$MbpyC_{12}NH_2$ was prepared using a procedure similar to that for $mbpyC_6NH_2$ except $BocNH(CH_2)_{12}NH_2$ (90 mg) was used instead of $BocNH(CH_2)_6NH_2$. Chromatography on silica gel with CH_2Cl_2-MeOH as eluent gave ligand as a white solid (75 mg, yield 65%). Positive EI-MS: m/z 383 (M^+). ^{1}HNMR (300 MHz, $CDCl_3$): 8.62 (d, $J = 4.9$ Hz, 1H, bpy), 8.53 (d, $J = 4.9$, 1H, bpy), 8.31 (d, $J = 1.5$, 1H, bpy), 8.22 (d, $J = 0.8$, 1H, bpy), 7.32 (dd, $J = 4.9$, 1.5, 1H, bpy), 7.14 (dd, $J = 4.9$, 0.8, 1H, bpy), 3.90 (s, 2H, bpyCH_2N), 2.67 (m, 4H, CH_2), 2.44 (s, 3H, CH_3), 1.48 (m, 4H, CH_2), 1.26 (m, 16H, CH_2).

2.3. Synthesis of Ruthenium(II) Complexes (Ru-C_6 and Ru-C_{12})

Ru-C_6 was prepared by the modification of a literature method for $[Ru(bpy)_2(phen)]^{2+}$.[21] $MbpyC_6NH_2$ (36 mg) was added to a solution of *cis*-$Ru(bpy)_2Cl_2 \cdot xH_2O$ (50 mg) in absolute ethanol (50 mL), and the mixture was heated to reflux under N_2 for 5 h, during which the purple black solution turned red brown. After the removal of the solvent under reduced pressure, the residue was chromatographed on neutral Al_2O_3 with CH_2Cl_2-MeOH as eluent to give the second band as the yellow solid (55 mg, yield 73%). Positive FAB-MS: m/z 747 ($[M\text{-}Cl]^+$), 712 ($[M\text{-}2Cl]^+$). ^{1}HNMR (300 MHz, CD_3OD): 8.70 (m, 5H, bpy), 8.58 (s, 1H, bpy), 8.12 (m, 4H, bpy), 7.82 (m, 4H, bpy), 7.70 (d, $J = 5.8$, 1H, bpy), 7.61 (d, $J = 5.8$, 1H, bpy), 7.47 (m, 5H, bpy), 7.34 (d, $J = 5.8$, 1H, bpy), 3.97 (s, 2H, CH_2N), 2.87 (m, 2H, CH_2), 2.67 (m, 2H, CH_2), 2.59 (s, 3H, CH_3), 1.42 (m, 4H, CH_2), 1.29 (m, 4H, CH_2). Anal. Found (%): C, 57.25; H, 5.66; N, 13.83. Calc. for $C_{38}H_{42}Cl_2N_8Ru \cdot H_2O$: C, 56.99; H, 5.54; N, 13.99.

Ru-C_{12} was prepared using a procedure similar to that for Ru-C_6 except $mbpyC_{12}NH_2$ (42 mg) was used instead of $mbpyC_6NH_2$. Chromatography on neutral Al_2O_3 with CH_2Cl_2-MeOH as eluent gave the second band as the yellow solid (60 mg, yield 71%). Positive FAB-MS: m/z 796 ($[M\text{-}2Cl]^+$). ^{1}HNMR (300 MHz, CD_3OD): 8.69 (m, 5H, bpy), 8.57 (s, 1H, bpy), 8.11 (m, 4H, bpy), 7.81 (m, 4H, bpy), 7.69 (d, $J = 5.8$, 1H, bpy), 7.60 (d, $J = 5.8$, 1H, bpy), 7.47 (m, 5H, bpy), 7.33 (d, $J = 5.8$, 1H, bpy), 3.96 (s, 2H, CH_2N), 2.88 (m, 2H, CH_2), 2.64 (m, 2H, CH_2), 2.58 (s, 3H, CH_3), 1.60 (m, 4H, CH_2), 1.31 (m, 16H, CH_2). Anal. Found (%): C, 59.84; H, 6.32; N, 12.80. Calc. for $C_{44}H_{54}Cl_2N_8Ru \cdot H_2O$: C, 59.72; H, 6.38; N, 12.66.

2.4. Synthesis of Gold Nanaoparticles

Gold colloids were prepared by a citrate reduction of $HAuCl_4 \cdot 4H_2O$ following a procedure from the literature.[22] All glassware used in the following experiments was cleaned in a freshly prepared bath with 3:1 HCl/HNO_3 and rinsed thoroughly in H_2O prior to use. The gold colloids were prepared through a chemical reduction by sodium citrate according to the reference. Briefly, $HAuCl_4 \cdot 4H_2O$ (33.98 mg) was dissolved in 50 mL water and then the solution was heated to boiling. A 5 mL aqueous solution of sodium citrate (114.10 mg) was added, and the mixture was boiling for 15 min. The solution was then left to cool down naturally to room temperature. Finally, a dark-red gold colloid (6.0×10^{-9} M) was obtained. The average diameter of these gold colloids was about 16 nm.

2.5. Modification of Gold Nanoparticles

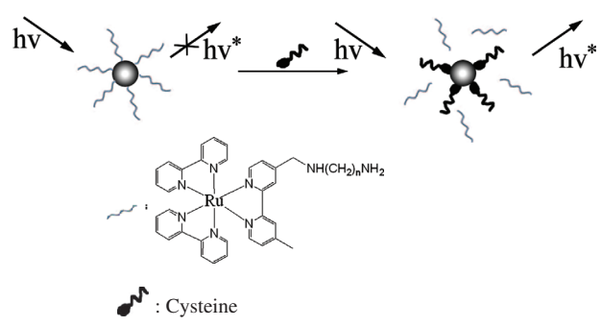
A range of concentrations ($0 \sim 6.0 \times 10^{-9}$ M) of as-prepared gold colloids were mixed with 2.4×10^{-6} M Ru-C_6 in PBS buffer solution (10 mM, pH 7.0) for 10 min to obtain Ru-C_6-Au composites. By monitoring the emission intensity of the final composites, the optimum concentration of gold colloids for the formation of the Ru-C_6-Au composite was found to be 3.0×10^{-9} M, corresponding to 2.4×10^{-6} M for Ru-C_6. And Ru-C_{12}-Au composite was obtained in the same way.

2.6. Detection of Cysteine

For the cysteine detection, various concentrations of cysteine prepared in PBS buffer solution (10 mM, pH 7.0) were then added to the Ru-C_6-Au composite and allowed to stir for 10 min before the emission measurements. The urine sample was reduced by tris(2-carboxyethyl)phosphine (TCEP) in aqueous solution, then methanol was added to removed the protein. On the other hand, a control experiment with sodium chloride indicated a negligible effect of salinity on the detection of cysteine. The emission spectra were recorded in the wavelength range from 540 to 750 nm upon excitation at 450 nm, using 5 nm(ex)/10 nm(em) slit widths, at the scanning speed of 240 nm/min. All experiments were performed at room temperature.

3. RESULTS AND DISCUSSION

Two ruthenium(II) polypyridine complexes containing amino group with different alkyl chains were synthesized and characterized (as shown in Scheme 1). Cysteine sensing by gold colloids functionalized with ruthenium(II) complexes was studied and the effect of the linker length on the sensing system was investigated as well. The design process of our luminescence sensor was schematically presented in Scheme 1. The Ru-Au composite was formed, when gold colloids were added into the ruthenium(II) complex solution, since amino groups were known to have a strong interaction with gold surface.[23] In this Ru-Au composite, the luminescence of the ruthenium(II) complexes turned weak, due to the highly efficient energy and charge transfer between the ruthenium(II) complexes and gold colloids. After the addition of cysteine, the ruthenium(II) complexes would be moved away from the surfaces of gold



Scheme 1. Schematic representations of luminescence sensing for cysteine detection.

colloids due to the higher affinity of the thiol group of cysteine to gold colloids. As a result, the concentration of free ruthenium(II) complexes (not absorbed to gold colloids) in the solution increased correspondingly, and the light of the system was switched on. The increase of the luminescence intensity was found to be dependent on the amount of the added cysteine. Therefore, the selective detection of cysteine could be realized by this sensitive and simple approach.

3.1. Photophysical Properties of the New Ruthenium(II) Complexes

The newly synthesized ruthenium(II) complexes have a good solubility in water. The photophysical data for two ruthenium(II) complexes were collected in Table I. The electronic absorption spectra of complexes in water were mainly dominated by an intense high-energy absorption band at *ca*. 280 nm, and low-energy bands at *ca*. 424–446 nm. With reference to previous studies on the related ruthenium(II) polypyridine systems,[24] the higher energy absorption was assigned as intraligand (IL) transition, while the low-energy bands were assigned to the metal-to-ligand charge transfer (MLCT, $d\pi(Ru) \rightarrow \pi^*(bpy)$) transitions, which were absent in the electronic absorption spectra of the free ligands. The ruthenium(II) complexes were found to emit strongly with emission maxima at *ca*. 607 nm at room temperature in aqueous solution, assigned as derived from a triplet MLCT state, similar to that observed in other related ruthenium(II) diimine systems.[24]

3.2. Characterization of the Gold Nanoparticles

Figure 1 shows the electronic absorption spectra of the colloidal solutions of 16-nm gold nanoparticles before and after Ru-C_{12} capping. Upon the addition of the ruthenium(II) complex, the absorption band at 525 nm decreased while new band appeared at longer wavelengths, suggesting that gold nanoparticles were ready to bind the new ruthenium(II) complex and aggregated subsequently. TEM images (Fig. 2) clearly show the aggregate formation of Ru-C_{12}-Au. The results showed that conjugation of the new ruthenium(II) complexes with gold nanoparticles facilitated aggregation of the gold nanoparticles due to the cancelation of negative charges on the surface of the gold nanoparticles. After addition of cysteine to the Ru-C_{12}-Au composite solutions, the absorption band at 525 nm decreased further and new band at longer wavelengths

Table I. Photophysical data of the new ruthenium(II) complexes.

Complex	Solvent	λ_{abs}/nm(ε/dm$^3 \cdot$mol$^{-1} \cdot$cm^{-1})	λ_{em}/nm
Ru-C_6	H_2O	280 (94,230), 425 (14,650), 446 (16,780)	607
Ru-C_{12}	H_2O	280 (103,390), 424 (16,130), 445 (18,900)	607

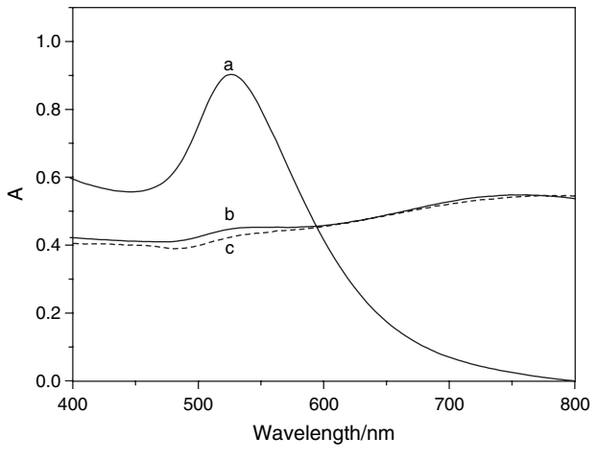


Fig. 1. The electronic absorption spectra of the gold nanoparticles (a), gold nanoparticles modified by the ruthenium(II) complex (b) and gold nanoparticles modified by the ruthenium(II) complex after addition of cysteine (c).

became broader (Fig. 1(c)). These suggested that addition of cysteine caused not only release of the ruthenium(II) chromophore but also aggregation of gold nanoparticles as shown in TEM images (Fig. 2(c)).

3.3. Emission Quenching of the Ruthenium(II) Complexes by Au Colloids

The emission spectrum of Ru-C_6 in PBS buffer was shown in Figure 3(a). Upon addition of Au colloids to the Ru-C_6 buffer solution, up to ca. 90% of the luminescence intensity was quenched (Fig. 3(c)). This result could be explained that the formation of Ru-Au composites caused by the absorption of Ru-C_6 complexes on the Au colloids through amino group resulted in the decrease of free Ru-C_6 complexes (not absorbed to Au colloids) in the solution.

Another fluorophore Ru-C_{12} was also used to modify the Au colloids. The quenching effect of two ruthenium(II) complexes was shown in Figure 4. The quenching efficiency of Ru-C_6 (curve C_6) emission was found to be slightly higher than that of Ru-C_{12} (curve C_{12}) when the concentration of Au colloids quencher was lower than 1.25×10^{-9} M. Upon addition of the same concentration of Au colloids (4.5×10^{-10} M) into Ru-C_6 (2.4×10^{-6} M) and Ru-C_{12} (2.4×10^{-6} M) solution, the emission intensity of solution was quenched by 56% and 42% respectively. When the quencher concentration was higher than 1.25×10^{-9} M, the quenching effect for both of the ruthenium(II) complexes was the same, and the quenching efficiency reached ca. 90%. This emission quenching suggested a highly efficient energy and charge transfer between the ruthenium(II) complexes and Au colloids which was considered to be a major deactivation contribution to the excited fluorophores on Au colloids, as reported previously.[25] Additionally, the close proximity

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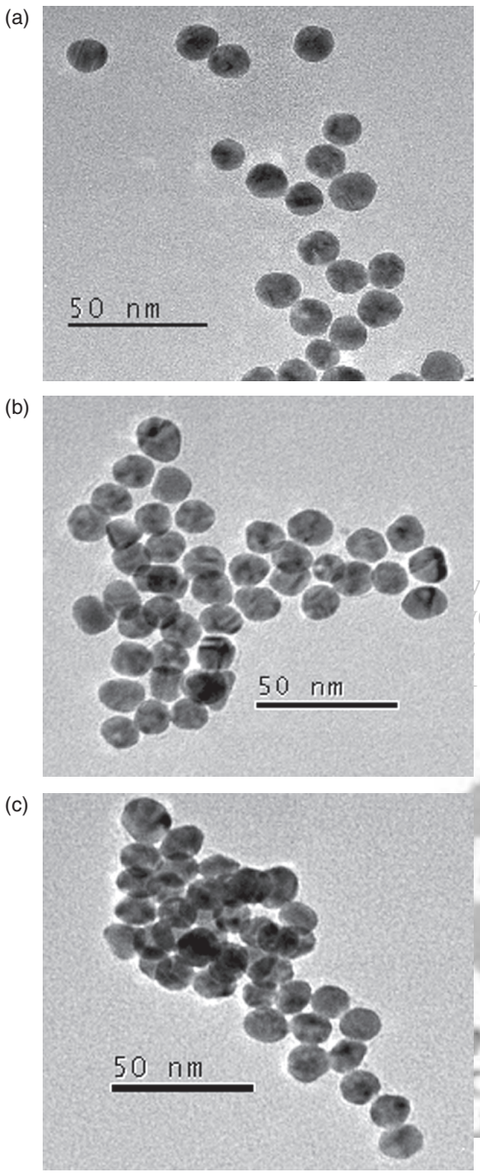

Fig. 2. TEM images of the gold nanoparticles (a), gold nanoparticles modified by the ruthenium(II) complex (b) and gold nanoparticles modified by the ruthenium(II) complex after addition of cysteine (c).

of the ruthenium(II) complex fluorophores on the surface of Au colloids often resulted in fluorophore aggregation effect,[26] which enhanced the interaction between the ruthenium(II) complex fluorophores and Au colloids. And the aggregated ruthenium(II) complex fluorophores would lead to mental-enhanced self-quenching between the neighbor fluorophores.[26] In Ru-Au composites, the ruthenium(II) complex not only functioned as a photoactive fluorophore, but also acted as a good protective agent for colloids because it was capable of combining the steric and electrostatic stabilization.[27] This was also one advantage of the present strategy of fabricating luminescent sensors utilizing ruthenium(II) complex.

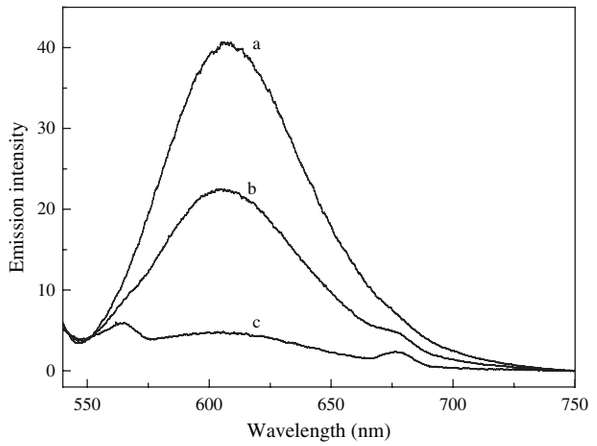

Fig. 3. Emission spectra of solutions containing Ru-C_6 in PBS buffer (curve a) and Ru-C_6-Au complex (curve c) in the absence and presence of 1.5×10^{-5} M cysteine (curve b). Concentrations of Ru-C_6 and Au colloids are 1.2×10^{-6} M and 1.5×10^{-9} M, respectively. Excitation: 450 nm.

3.4. Interaction Between Cysteine and Ru-Au Composite

The luminescence intensity increased upon addition of cysteine to Ru-C_6-Au composite aqueous solution, as shown in Figure 3(b). There was no shift of the emission wavelength (about 607 nm) but the emission intensity was enhanced with the increase of the cysteine concentration in the solution. This phenomenon indicated that the presence of cysteine could modulate the emission behaviors of Ru-C_6-Au composite in the solution. Due to the stronger affinity of thiol to gold colloids than amino groups,[25] cysteine molecules could be favorably capped on the surface of Au colloids, which resulted in moving Ru-C_6 away from the surface of Au colloids and increasing the concentration of free Ru-C_6 fluorophores in the solution. After the release of Ru-C_6 complex from Au colloids, the energy

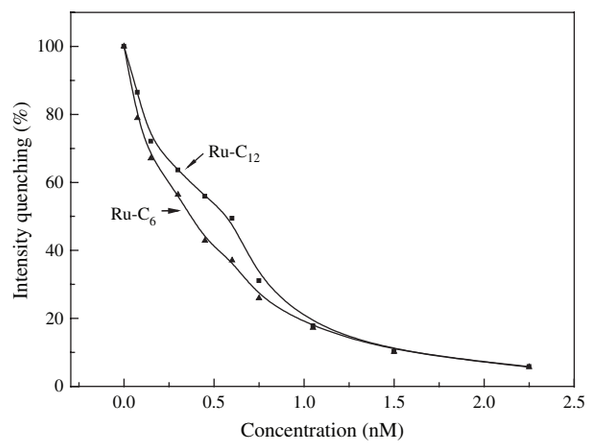

Fig. 4. Emission quenching percentage of Ru-C_{12} (2.4×10^{-6} M) and Ru-C_6 (2.4×10^{-6} M) versus the added Au concentration. Excitation: 450 nm, emission: 607 nm.

and charge transfer between Ru-C_6 and Au colloids was weakened, and the emission intensity of the system was increased. In order to improve the system sensitivity for cysteine detection, the background of the luminescence of Ru-C_6-Au composite was needed to minimize. By monitoring the quenching emission of the final Ru-C_6-Au composite, the optimum concentration of Au colloids for the formation of the Ru-C_6-Au composite was 3.0×10^{-9} M, corresponding to 2.4×10^{-6} M for Ru-C_6 complex. In addition, the control experiment with sodium chloride indicated a negligible effect of cysteine on the emission of Ru-C_6 in PBS buffer, and ionic intension did not influence the luminescence enhancement after an addition of cysteine (Fig. 5(c)). The result further supported that the increase of the luminescence intensity did originate from the modulation of the energy and charge transfer between Ru-C_6 and Au colloids.

The effect of the added cysteine on the luminescence intensity of Ru-Au composites was also studied. From results as shown in Figure 5, the luminescence intensity of Ru-C_{12}-Au was enhanced more strongly than that of Ru-C_6-Au composite upon addition of cysteine. Addition of the 7.5×10^{-6} M cysteine, the emission intensity of Ru-C_{12}-Au composite and Ru-C_6-Au composite increased by 28% and 12%, respectively. With the 37.5×10^{-6} M cysteine, this luminescence restoration percentage for Ru-C_{12}-Au would be up to 47%, which higher than that of Ru-C_6-Au composite by 17%. It could be considered that the interaction between gold nanoparticles and the ruthenium(II) complexes through amino group might be additive to the electrostatic effect between the ruthenium(II) chromophore unit and the rest of negative citrate ion on the gold surface. The longer alkyl chain of Ru-C_{12} made the ruthenium(II) complex unit far away from the gold nanoparticle surface, which resulted in a weaker electrostatic effect between them than that of Ru-C_6. Also, the density of the ruthenium(II) chromophore with longer linker on the gold nanoparticle surface was higher than that of chromophore with shorter linker. Therefore, the replacement of Ru-C_{12} by cysteine seemed easier and more binding Ru-C_{12} complex fluorophores could actually be removed from gold surface, which resulted in the stronger enhancement of the luminescence intensity of the system.

It was noteworthy that the control over the concentration of ruthenium(II) complex in the sensing system was particularly essential. Either redundant or insufficient amount of ruthenium(II) complex fluorophores in the solution was found to deteriorate the sensing sensitivity. For instance, when more ruthenium(II) complexes were used than that is needed to stabilize Au colloids, the background signal became higher due to the strong emission from free fluorophores, which would affect the sensitivity for the cysteine detection greatly. Also, if insufficient amount of the ruthenium(II) complexes was applied, the stabilization of as-prepared Au colloids was influenced distinctly, and the limit of detection (LOD) of cysteine became higher. Thus, the best Ru/Au ratio and their concentrations should be ensured prior to the cysteine determination. By monitoring the luminescence characteristics of the final Ru-Au composite solutions, the optimum concentration of Ru-C_{12} complex for stabilizing Au colloids was found to be 2.4×10^{-6} M, corresponding to 3.0×10^{-9} M for Au colloids.

3.5. Determination of Cysteine

We studied the selectivity of the luminescent sensor. Contrast experiments were carried out to detect cysteine in the presence of various other amino acids. The changes of luminescence upon addition of various amino acids at the same concentration to Ru-C_{12}-Au composite were presented in Figure 6. It was clear that the increase of the

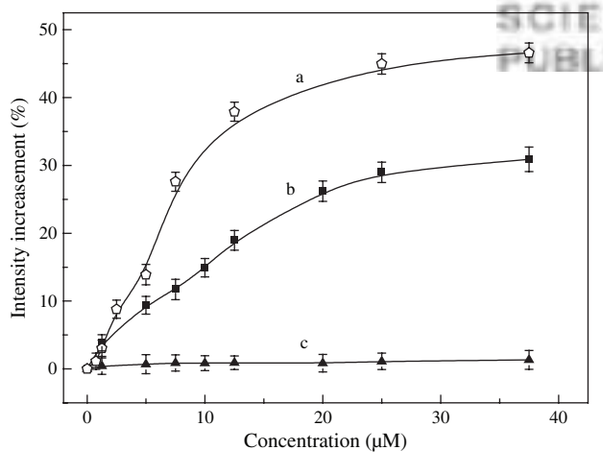

Fig. 5. Luminescence intensity increase of Ru-C_{12}-Au composite (a) (Ru-C_{12}: 1.2×10^{-6} M, Au colloids: 1.5×10^{-9} M) and Ru-C_6-Au composite (b) (Ru-C_6: 1.2×10^{-6} M, Au colloids: 1.5×10^{-9} M) in PBS buffer as a function of added cysteine concentration, and (c) fluorescence intensity increase of Ru-C_{12}-Au composite in PBS buffer as a function of added sodium chloride concentration. Excitation: 450 nm, emission: 607 nm.

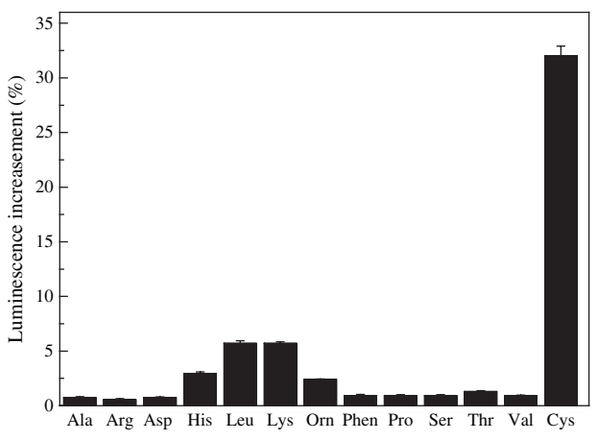

Fig. 6. Luminescence intensity increase (%) of Ru-C_{12}-Au composite upon the addition of 1.0×10^{-5} M cysteine and other amino acids. Excitation: 450 nm, emission: 607 nm.

RESEARCH ARTICLE

luminescence intensity upon addition of cysteine was significantly higher compared to other amino acids, indicating that the thiol functionality in cysteine played a key role in the luminescence enhancement. These results also indicated that the proposed approach was with potential applications in the determination of cysteine in the mixture of amino acids found in proteins.

Photoactive ruthenium(II) complexes were particularly attractive in the design of sensors because of their stable optical and electrical properties and tunable electronic structures. Our previous studies proved that cysteine could modulate the emission of the ruthenium(II) complex-Au composite, therefore, we expect as-prepared Ru-C_{12}-Au composite could be used to determine cysteine quantitatively. As expected, our further experimental results indicated that the increase of the luminescence intensity could quantitatively reflect the amount of cysteine added. As shown in Figure 5, a good linear relationship between the luminescence intensity of Ru-C_{12}-Au composite at 607 nm and the concentration of cysteine was obtained in the range of 7.5×10^{-7} to 1×10^{-5} M, the LOD of cysteien was 7.5×10^{-7} M, which was lower than that of 12.5×10^{-7} M in Ru-C_6-Au composite sensing system. Using the standard addition method, the tCys concentration in a reduced human urine sample was measured as 204 μM with a RSD of 3.8%. The result was within the normal range for healthy individuals. Therefore, the method could prove to be feasible and reproducible for the detection of cysteine in biological samples.

RESEARCH ARTICLE

4. CONCLUSION

In conclusion, a new method was demonstrated for preparation of luminescent cysteine sensing probes based on the luminescence quenching of the ruthenium(II) complex by gold colloids, which was realized by the combination of the highly efficient energy and charge transfer between Au colloids and ruthenium(II) complex and the strong affinity of thiol to gold nanoparticles. The method allowed the selective analysis of cysteine with a LOD as low as 7.5×10^{-7} M. In the study, two ruthenium(II) complexes with different alkyl lengths were used to tune the distance between fluorophores and gold colloids, which could affect the quenching efficiency and cysteine determination including linear range and LOD. In addition, the synthesis of gold colloids and the modification with fluorophores could be accomplished successfully within one step, which simplified the preparation of luminescence sensors. The ruthenium(II) complex fluorophore ensured the stability of the sensing method and its visible region from ^{3}MLCT emission band and relatively long life time made it suitable for biological applications.

Acknowledgment: This work was supported by the National Scientific Foundation of China (NSFC No. 20801014, No. 20735002), Scientific Foundation of Fujian Province (No. 2009J05026) and the start-up funding from the Fuzhou University (No. 826505).

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Received: 12 April 2010. Accepted: 7 July 2010.