Fluorescence and Electrochemiluminescence of Luminol-Reduced Gold Nanoparticles: Photostability and Platform Effect

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Water-soluble gold nanoparticles (AuNPs) capped with both fluorescent (FL) 3-aminophthalate (APA) and electrochemiluminescent (ECL) luminol molecules were described in our previous work. The synthetic and characteristic efforts of these functionalized AuNPs (lumAuNPs) were subsequently followed by investigations of their FL and ECL properties, as reported in the present work. It was observed that the FL intensity of a single gold nanoparticle was 70 times brighter than that of one free APA molecule, even though 91% of the FL emission of APA molecules on the surfaces of AuNPs was inhibited by gold cores through both intra- and interparticle quenching effects. Moreover, the photobleaching of surface-bound APA molecules was found to be dramatically inhibited compared with that of free ones in carbonate buffer. The improvement of photostability was attributed to the reactive AuNPs which acted as radical scavengers to protect the surface-bound APA molecules from oxidation by carbonate radicals. Furthermore, as-prepared lumAuNPs could react with cysteine to produce strong electrochemiluminescence, which was enhanced by 20-fold compared with that in the absence of cysteine. The experimental results suggested that luminol and cysteine were coadsorbed on the gold nanoparticle platform via Au−N and Au−S interactions, respectively. The shorter distance between reactant molecules by overcoming the electrostatic repulsion, that is, platform effect, was proposed to be responsible for the ECL enhancement. Combined with the biocompatibility of gold cores, the brighter FL emission, enhanced photostability, and stronger ECL intensity may make as-prepared lumAuNPs promising FL and ECL biomarkers for their applications in biosensors and bioimaging.

1. Introduction

Functionalized nanomaterials are now of intense research interest due to their wide applications in many areas from biosensors and drug delivery to the design of nanostructures.[1−4] By virtue of binding functional ligands, for example, fluorescent (FL) and electrochemiluminescent (ECL) molecules, to the surfaces of gold nanoparticles (AuNPs), functionalized AuNPs became one of the most attractive improvements in the development of optical probes due to their high sensitivity and ease of use.[4−7] In most of these functionalized AuNPs, ligands were bound to the AuNPs via some postmodification methods including electrostatic adsorption,[6,7] ligand-exchange,[8,9] and covalent coupling.[10,11] However, these methods suffered from several disadvantages[12] such as complex experimental procedures,[13] low coupling reaction efficiency and surface coverage,[11] and pH dependence for electrostatic adsorption.[14] It is still of great significance to explore convenient methods for the attachments of FL and/or ECL molecules to AuNPs. Moreover, gold cores in the functionalized AuNPs not only acted as carriers by use of the large surface area and excellent biocompatibility[6,7] but also affected the FL or ECL behaviors of the ligands themselves.[11,15] Investigations on the properties of functionalized AuNPs might offer information about the interactions between gold cores and surface-bound ligands and improve ligand properties, which was thus an important subject for both fundamental interest and practical applications.

Organic fluorophore functionalized AuNPs are one kind of widely used material for the development of convenient, chemically stable, and biocompatible FL markers.[4,5] Most of the previous research focused on the effects of the gold cores on the FL intensity of surface-bound ligands, such as quenching[13,14] and enhancement[16−18] effects. However, little attention has been paid to the effects of metal nanoparticles on the photobleaching of FL ligands. Photobleaching of organic fluorophores was considered to be the major limitation of their applications in biosensor and the real-time imaging.[19] In recent studies on optoelectronic devices, the photostability of conducting polymers was reported to be improved by doping the polymer with very small amounts of gold nanomaterials.[20,21] Moreover, Geddes and

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co-workers systematically studied the effects of various silver nanostructures on the FL intensity and photostability of several fluorophores in a series of publications, which they called metal-enhanced fluorescence. Related work on this topic was well summarized by the group in a recent review.[22] However, no similar effects were reported for the gold nanostructures. Besides, both the polymer photostability[20,21] and metal-enhanced fluorescence[22] were studied in a condition where fluorophores were not bound to the surfaces of the metal nanomaterials but simply mixed with each other.

Electrochemiluminescence is another useful optical property for the design of biomarkers. The attachment of ECL molecules to the surfaces of AuNPs by electrostatic adsorption[6,7] and/or covalent coupling[23] is a common method to fabricate ECL probes for biosensor applications. Luminol is one of the most popular ECL reagents with a rather high light efficiency.[24−26] Recently, Roux and co-workers[11] attached luminol to the surfaces of AuNPs via an amide-forming reaction. ECL ligand functionalized AuNPs were thus obtained, and gold cores were found to facilitate the ECL reaction of luminol. However, this work only studied the ECL property of luminol bound to AuNPs via postmodification in the presence of H_2O_2.

In our previous work, a convenient one-spot synthesis of water-soluble, luminol-capped AuNPs (lumAuNPs) with ECL activity was described.[27] The lumAuNPs were prepared by the direct reduction of $HAuCl_4$ by luminol, which was oxidized to 3-aminophthalate (APA), an organic fluorescent compound. According to the detailed surface analysis, it was validated that luminol and APA coexisted on the surfaces of AuNPs.[27] The as-prepared lumAuNPs might have ECL and FL properties due to the surface-bound luminol and APA ligands. The synthetic and characteristic efforts were subsequently followed by the investigations of their FL and ECL properties, as reported in the present work. Both the intra- and interparticle quenching effects of AuNPs on the FL intensity of surface-bound APA molecules were studied. It was also found that the photostability of lumAuNPs was greatly improved compared with that of the free APA molecules in carbonate buffer. Although the electrochemiluminescence of lumAuNPs assembled on gold electrodes had been studied in the presence of H_2O_2,[27] it was found that lumAuNPs dispersed in a solution containing cysteine could produce strong electrochemiluminescence in this work. The enhancement mechanisms of both the photostability and ECL intensity are proposed based on several control experiments.

2. Experimental Section

2.1. Chemical and Solutions. A 0.01 mol/L stock solution of luminol was prepared by dissolving luminol (Sigma) in 0.1 mol/L NaOH solution without further purification. Working solutions of luminol were prepared by diluting the stock solution. Fresh APA solution was prepared daily by dissolving 3-aminophthalic acid hydrochloride (ACROS) in redistilled water for the FL measurements. $HAuCl_4 \cdot 4H_2O$ was obtained from Shanghai Reagent (Shanghai, China). A 1.0 g/L $HAuCl_4$ stock solution was prepared by dissolving 1 g of $HAuCl_4$ in 1 L of redistilled water and stored at 4 °C. Nitrogen and oxygen (99.999%) were used in the atmosphere experiments. All other reagents were of analytical grade and used as received. Redistilled water was used throughout.

2.2. Synthesis of lumAuNPs and Citrate-Reduced AuNPs. All glassware used in the following procedures was cleaned in a bath of freshly prepared 3:1 (v/v) HNO_3−HCl, rinsed thoroughly in redistilled water, and dried prior to use. The lumAuNPs (14 nm) were prepared via the reduction of $HAuCl_4$ by luminol according to the previous work.[27] In detail, a 100 mL portion of $HAuCl_4$ (0.01% w/w) solution was heated to boiling. While stirring vigorously, 1.80 mL of 0.01 mol/L luminol stock solution was added rapidly. The solution was maintained at the boiling point for 30 min, during which time a color change from yellow to black to purple was observed before a wine-red color was reached. The heating source was removed, and the colloid was kept at room temperature for another 20 min and then stored at 4 °C. Citrate-reduced AuNPs (citAuNPs; 16 nm) were also synthesized according to the previous work.[28] Briefly, 2.00 mL of trisodium citrate (1% w/w) was added to the boiled $HAuCl_4$ solution instead of luminol, while the other experimental conditions remained identical.

To exclude the effects of residual reactants and products such as excess luminol and APA molecules on the FL and ECL measurements, a dialysis procedure was necessary to separate these compounds from the AuNPs. Typically, 100 mL of gold colloid was placed in a homemade semipermeable dialysis bag, which was prepared by the volatilization of collodion. In detail, the inner surface of a conical flask was first soaked by collodion. The residual collodion was then gently heated by using a hair dryer until the solvents ether and ethanol volatilized and a thin membrane was obtained on the inner surface. The dialysis bag was immersed in a beaker with 450 mL of redistilled water in each dialysis operation. The water was renewed at intervals of 12 h. The supernatant during the last dialysis operation was collected as a contrast sample. A typical dialysis procedure required 3 days, and redistilled water was replaced six times in total.

2.3. Instrumentation. FL and photobleaching measurements were performed by using a F-7000 fluorometer (Hitachi, Japan) with a 150 W Xe lamp as the light source. ECL and electrochemical (EC) measurements were performed by using a homemade ECL/EC system, including a model CHI760B electrochemical working station (Chenhua Inc., China), a H-type electrochemical cell (self-designed), a model CR-105 photomultiplier tube (PMT) (Beijing, China), a model RFL-1 luminometer (Xi'an, China), and a computer as described previously.[28,29] A glassy carbon electrode served as the working electrode in the present work. When a potential was scanned on the glassy carbon electrode, an ECL signal was generated and recorded by the RFL-1 luminometer. The curves of ECL intensity versus applied potential (I_{ECL}−E) and the cyclic voltammograms (i−E) were recorded simultaneously.

3. Results and Discussion

Functionalized lumAuNPs were synthesized via the direct reduction of $HAuCl_4$ by luminol in our previous work.[27] According to the surface analysis, luminol and its oxidation product APA were validated to coexist on the surfaces of the AuNPs. Since luminol and APA have ECL and FL activity, respectively, the FL and ECL properties of as-prepared lumAuNPs were subsequently investigated in the present work. Because both the fluorescence of APA and electrochemiluminescence of luminol were dependent on pH, all the FL and ECL experiments were done in a 0.02 mol/L carbonate buffer without special statements. A relatively low concentration of carbonate buffer was selected because AuNPs easily aggregated in the solution with high ionic strength. To avoid the effect of residual luminol and APA molecules on the FL and ECL measurements, dialysis procedures were utilized to separate the residual reactants and products from the lumAuNPs (see Experimental section for details).

3.1. Fluorescence of lumAuNPs. It was observed that the lumAuNPs in a carbonate buffer were fluorescent. The solid,

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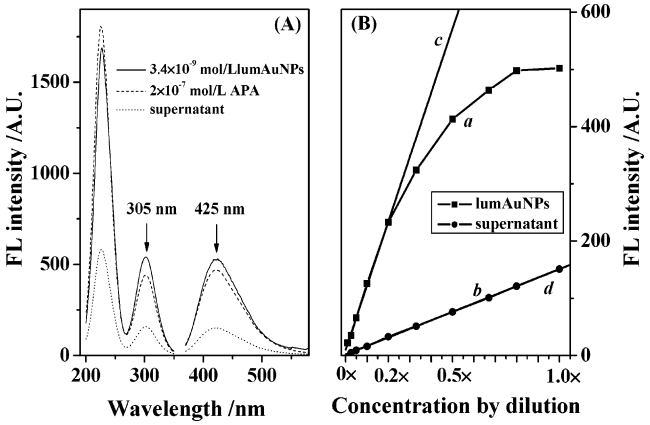

Figure 1. (A) FL excitation and emission spectra ($\lambda_{ex}/\lambda_{em}$ = 305/425 nm) of as-prepared lumAuNPs (solid line), 2×10^{-7} mol/L APA (dashed line), and supernatant solution (dotted line) in 0.02 mol/L carbonate buffer. (B) FL intensity ($\lambda_{ex}/\lambda_{em}$ = 305/425 nm) of lumAuNPs (curve *a*) and supernatant (curve *b*) during dilution from 1.0-fold (1.0×, original solution) to 0.01-fold (0.01×, dilution by 100 times) by using 0.02 mol/L carbonate buffer. Curve *c* is the straight line fitted from the data points over the range of 0−0.1-fold in curve *a*, while curve *d* is linearly fitted from all the data points in curve *b*.

dashed, and dotted lines in Figure 1A show the excitation and emission spectra ($\lambda_{ex}/\lambda_{em}$ = 305/425 nm) of the as-prepared lumAuNPs after dialysis, freshly prepared 2×10^{-7} mol/L APA solution, and supernatant during the last dialysis, respectively. It was obvious that the excitation and emission spectra of the lumAuNPs and supernatant were identical to those of the APA solution in shape and wavelength maximum, indicating no changes in the ground-state and excited-state electronic properties of APA when it was bound to the AuNPs. The FL intensity of lumAuNPs was about 3 times stronger than that of the supernatant, indicating that the dialysis was successful and the surface-bound APA molecules retained FL activity. It is worthwhile to mention that it was impossible to achieve a clean supernatant without any APA or luminol molecules, since the desorption of protecting molecules from the AuNP surfaces to the solution was inevitable when the free protecting molecules in solution kept diffusing out. This point was supported by the experimental fact that the FL intensity of the supernatant solution did not proportionally decrease any more after two to three dialysis procedures. Moreover, long-time dialysis would lead to the formation of precipitation due to the dissociation of stabilizers. It was found that a 3 day dialysis was sufficient to exclude the influence caused by residual protecting molecules while lumAuNPs still retained their dispersity. The FL intensity of lumAuNPs was calculated to be equal to that of the free APA solution of 2.3×10^{-7} mol/L under identical conditions. The concentration of the 14 nm lumAuNPs was estimated to be 3.4×10^{-9} mol/L by assuming a spherical particle according to the previous report.[30] In other words, the FL intensity of a single nanoparticle was ∼70 times brighter than that of one APA molecule.

The quenching effect of gold cores on the vicinal organic fluorophores was often reported, which was usually caused by the electron/energy transfer from the excited-state FL molecules to the metal nanoparticles.[4,13,14] A similar quenching effect was also observed in the present work. The FL intensity of the supernatant solution was found to linearly decrease with the dilution from 1.0-fold (1.0×, original solution) to 0.01-fold (0.01×, dilution by 100 times) by using 0.02 mol/L carbonate buffer solution, as shown in Figure 1B (curve *b*). The dilution of lumAuNPs, however, led to a nonlinear decrease in FL intensity according to curve *a*. This phenomenon indicated the quenching effect of the lumAuNPs on the neighboring nanoparticles and free APA molecules by excited-state interactions and by adsorbing the incoming light.[31] The decrease in FL intensity caused by the surface plasmon absorption of AuNPs at the excitation wavelength (305 nm) was considered in the interparticle quenching effect here. This kind of interparticle quenching became weaker with increasing dilution due to the increase of particle distance and the decrease of solution absorbance at the excitation wavelength. Consequently, a linear relationship was finally obtained in the diluted solution with the concentration below 0.1-fold (curve *a*). From the linearly fitted equation (curve *c*) between FL intensity and the lumAuNP concentration below 0.1-fold, the FL intensity of lumAuNPs at 1.0-fold was calculated to be 1100. The interparticle quenching efficiency $\Delta I/I$ was 52% (calculated from $\Delta I/I = (1100 - 530)/1100$), since the experimental FL intensity was only 530. Therefore, the FL intensity of a single gold nanoparticle was actually 145 times brighter (calculated from $70/(1 - 0.52)$) than that of one free APA molecule without interparticle quenching.

Besides interparticle quenching, the intraparticle quenching effect which reflected the quenching of gold cores to the surface-bound APA molecules could not be negligible. The content of surface-bound APA molecules was obtained by both ligand-exchange experiments and theoretical estimation. From the experimental aspect, 3×10^{-3} mol/L cysteine was added to the dialyzed lumAuNP solution with 0.02 mol/L carbonate buffer for 24 h to achieve complete substitution of surface-bound APA. The as-processed and blank colloids were subsequently centrifuged. Because 3×10^{-3} mol/L cysteine did not show any observable quenching effect on the FL intensity ($\lambda_{ex}/\lambda_{em}$ = 305/425 nm) of 1×10^{-6} mol/L APA in 0.02 mol/L carbonate buffer, a difference in the FL intensity of the supernatant could be used to estimate the content of the surface-bound APA molecules. According to this method, the content of surface-bound APA was determined to be 2.6×10^{-6} mol/L. From the theoretical aspect, the average area/molecule of tryptophan on the AuNPs was reported to be 20 Å^2.[32] Since the molecular sizes of luminol and APA were similar to that of tryptophan, and the interactions between ligands and gold cores were both validated to be Au−N interactions,[27,32] it was therefore reasonable to assume that the average ligand molecular area was 20 Å^2 for the lumAuNPs. Consequently, the number of ligand molecules on a 14 nm lumAuNP was calculated to be ∼3000. Because it was difficult to quantitatively distinguish the amount of luminol and APA on the surfaces of AuNPs, it was assumed that the number of APA molecules was 1500. Since the AuNP concentration was estimated to be 3.4×10^{-9} mol/L, this result meant that the content of surface-bound APA was 5.1×10^{-6} mol/L, which was about 2 times that of the experimental results. Considering the inevitable desorption of ligands during the 3 day dialysis and centrifugation, it was understandable that the experimental result was lower than the theoretical estimation, in which 100% surface-coverage was assumed. According to the experimental results, the number of surface-bound APA molecules on one single lumAuNP was 765 (calculated from $2.6 \times 10^{-6}/3.4 \times 10^{-9}$), and only 145 of them retained FL activity in diluted solution (without interparticle quenching). Therefore, the intraparticle quenching efficiency was 81% (calculated from (765 − 145)/765), which was close to that in a previous report on fluorophore functionalized AuNPs.[10]

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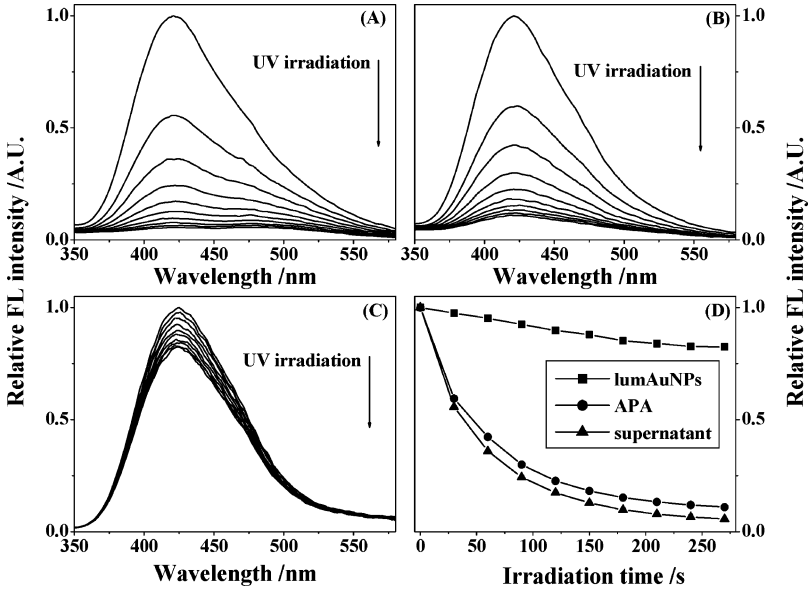

Figure 2. Relative FL spectra of supernatant (A), 1×10^{-6} mol/L APA (B), and lumAuNPs (C) solutions with 0.02 mol/L carbonate buffer under continuous irradiation ($\lambda_{ex} = 230$ nm). (D) Relative FL intensity at 425 nm for the above cases during 10 measurements. Conditions: sample volume 0.5 mL, excitation slit width 10 nm, and interval 30 s.

Although 765 APA molecules were bound to one gold nanoparticle, only 70 of them were observed in the as-prepared lumAuNPs because of both inter- and intraparticle quenching. The total quenching efficiency was thus 91% (calculated from (765 − 70)/765).

3.2. Photostability of lumAuNPs. It was found that the FL intensity of APA and lumAuNPs decreased with irradiating time, that is, photobleaching. The photostability of the APA solution and lumAuNPs was investigated by measuring 10 emission spectra ($\lambda_{ex} = 230$ nm) at equivalent intervals, while the excitation light kept irradiating the sample solution even during the interval time. The relative FL intensity of the *i*th measurement I_i/I_1 at 425 nm and the corresponding photobleaching efficiency $(I_{10} - I_1)/I_1$ were utilized to quantitatively describe the photobleaching phenomenon. Figure 2 shows 10 emission spectra of the supernatant (A), 1×10^{-6} mol/L APA (B), and lumAu (C) solutions with 0.02 mol/L carbonate buffer under continuous excitation by the light source (150 W Xe lamp) with equivalent measurement intervals. Figure 2D displays the relative FL intensity at 425 nm in each measurement. A dramatic decrease in the FL intensity of both the APA and supernatant solutions was observed during light irradiation in less than 10 scans (about 5 min). However, 80% FL intensity of lumAuNP colloids remained under the same conditions. Because the photobleaching efficiency was concentration-dependent, the concentration of the contrast APA solution should be selected properly. The criterion was that the contrast APA solution should give a comparable FL intensity to that of lumAuNPs. Since interparticle quenching could be weak if the lumAuNPs are used as FL probes, the concentration of the APA solution should be at least 4.8×10^{-7} mol/L (calculated from $2.3 \times 10^{-7}/(1 - 0.52)$) to compare the photostability of a single lumAuNP with that of one free APA molecule. Finally, 1×10^{-6} mol/L APA solution was selected in the experiments below.

To further clarify the photobleaching phenomenon, the effects of several factors on the FL photobleaching of 1×10^{-6} mol/L APA solution with 0.02 mol/L carbonate buffer were tested. Figure 3A displays various photobleaching curves when different intervals were selected. A longer interval meant longer irradiation time. It was clearly observed that the decrease in FL intensity was greater if the interval was longer. Furthermore, a larger irradiation flux could also lead to greater photobleaching as well as longer irradiation time. The irradiation flux could be controlled by adjusting the excitation slit width. The rate of FL intensity decrease was much faster at larger excitation slit widths as shown in Figure 3B. On the contrary, it was thus natural to imagine that the higher concentration and larger volume of the APA solution might promote its resistance to photobleaching according to the irreversible photo-oxidation mechanism, since the size of the excitation light spot was fixed. Figure 3C and D shows the effects of sample concentration and volume, respectively, on photobleaching efficiency. Samples with higher concentration and larger volume indeed displayed better photostability. These results supported that APA molecules in carbonate buffer suffered from an irreversible photobleaching process that led to a decrease in FL intensity.

Several experiments were designed to explore the photobleaching mechanism of APA molecules. Figure 4A shows the effect of solution pH on photobleaching by adjusting the pH with NaOH. The photobleaching efficiency was found to initially increase with the increase of pH and subsequently reached a stable level when pH ≥ 10.7. Considering that the concentration of both the hydroxide anion and carbonate anion changed with pH, the effect of carbonate was individually tested by simply adjusting the concentration of the carbonate buffer from 0.01 to 0.1 mol/L with an identical pH at 10.0. It was found that the photobleaching efficiency monotonically increased with the increase of carbonate anion concentration as shown in Figure 4B. These results demonstrated that the carbonate anion instead of hydroxide anion was involved in photobleaching. This point was also supported by the results that no obvious photobleaching of APA molecules was observed for 0.02 mol/L phosphate buffer under identical conditions (data not shown).

The photobleaching curve was found to be dependent on the excitation wavelength, as shown in Figure 5A. Figure 5B further displays the relationship between photobleaching efficiency $(I_{10} - I_1)/I_1$ and the excitation wavelength, which shows two obvious maxima at 230 and 305 nm, respectively. The similarity between the curve in Figure 5B and the excitation spectrum of APA in Figure 1A strongly indicates that the photobleaching was related to the excited-state APA molecules by absorbing the incoming light. It was reported that the carbonate anion could be oxidized

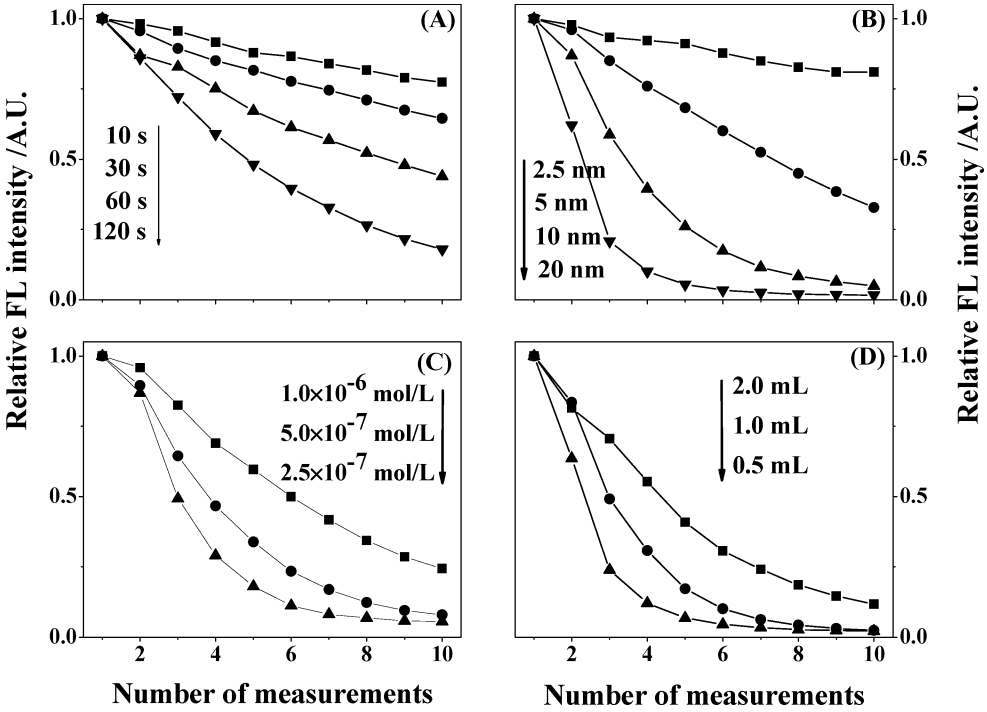

Figure 3. Effects of scan interval (A), excitation slit width (B), sample concentration (C), and volume (D) on the photobleaching of APA solution in 0.02 mol/L carbonate buffer. Conditions: (A) slit width 5 nm, sample volume 1.0 mL; (B) sample volume 0.5 mL; (C) slit width 20 nm, sample volume 2.0 mL; and (D) slit width 20 nm. Scan intervals of 30 s and a sample concentration of 1×10^{-6} mol/L were used without special statements.

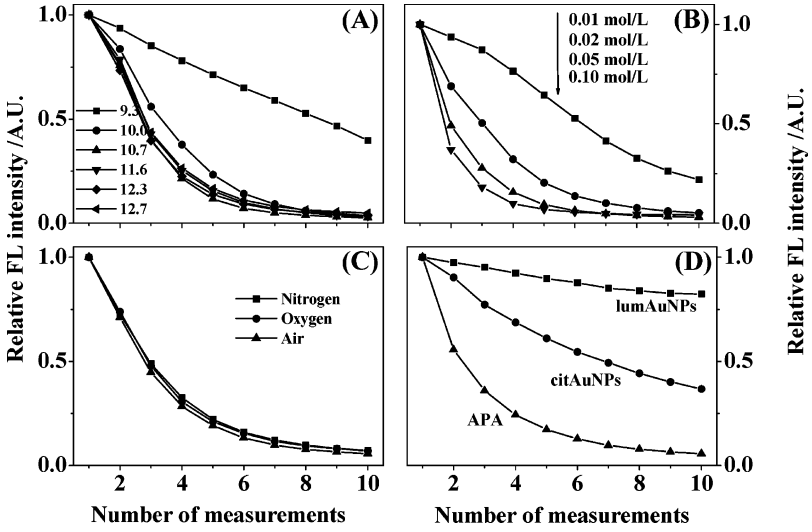

Figure 4. Effects of solution pH (A), carbonate buffer concentration (B), atmospheres (C), and AuNPs (D) on the photobleaching of 1×10^{-6} mol/L APA solution. Conditions: scan interval 30 s, slit width 10 nm, and sample volume 0.5 mL.

by the triplet dye sensitizer to generate an oxidative carbonate radical, which could further react with aromatic compounds.[33] Therefore, it was deduced that the carbonate anion might react with excited triplet state APA molecules formed via rapid and efficient intersystem crossing from the excited singlet state to generate carbonate radicals.[33] The reactive carbonate radicals further oxidized the aromatic APA molecules, leading to a decrease in FL intensity, that is, photobleaching. Moreover, the relationship in Figure 5B also excluded another possibility of carbonate radical generation by direct photolysis of the carbonate anion,[34] because a monotonic increase of the photobleaching efficiency with the decrease of excitation wavelength was supposed in that case due to the higher photon energy at lower wavelength. Oxygen is another important species generally involved in the photobleaching process.[35] Photobleaching was also examined in nitrogen/oxygen/air atmospheres to explore the role of dissolved oxygen. The nitrogen or oxygen saturated solution was achieved by bubbling the corresponding gas into the solution for 15 min, and maintained under the corresponding atmosphere above the solution surface during FL measurements. However, no obvious difference in the photobleaching efficiency was found under various atmospheres as shown in Figure 4C, indicating that the dissolved oxygen was not involved in photobleaching. The effect of dialyzed 16 nm citAuNPs was also tested to explore role of AuNPs in the improvement of

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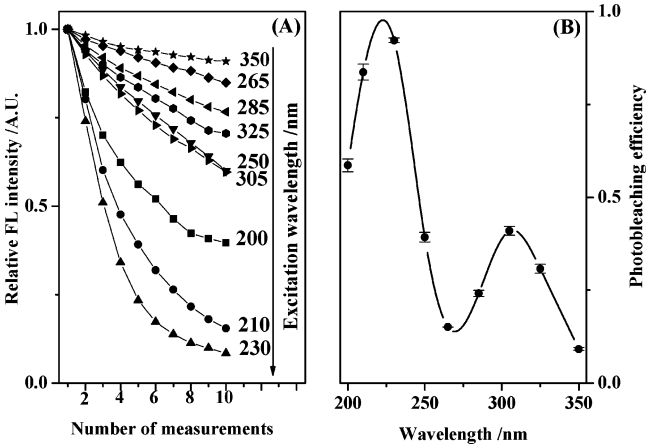

Figure 5. (A) FL photobleaching curves of 1×10^{-6} mol/L APA solution in 0.02 mol/L carbonate buffer at various excitation wavelengths. (B) Relationship between photobleaching efficiency $(I_{10} - I_1)/I_1$ and the excitation wavelength, which was calculated from the curves shown in (A). Experimental conditions were the same as those in the Figure 4 caption.

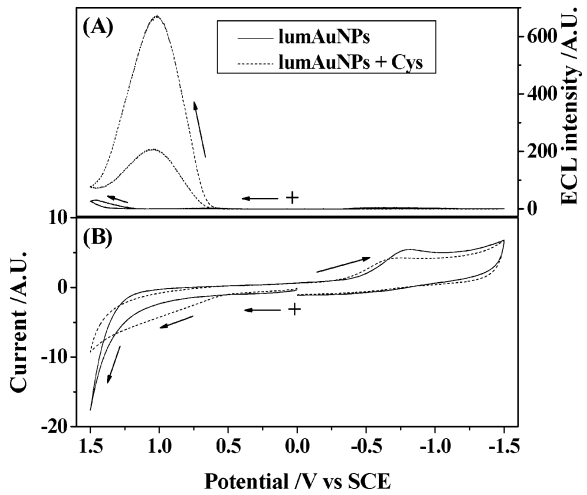

Figure 6. Typical $I_{ECL}-E$ curves (A) and cyclic voltammograms (B) of lumAuNPs in 0.02 mol/L carbonate buffer at a glassy carbon electrode with (dashed line) and without (solid line) 1 mmol/L cysteine.

photostability. The addition of 0.9-fold citAuNPs led to an obvious inhibition of the photobleaching efficiency of APA molecules as shown in Figure 4D. This result can probably be attributed to the reactivity of the AuNPs, which were reported to act as radical scavengers[36−38] to decrease the local radical concentration around the AuNP surface and protect the APA molecules from oxidation by the carbonate radicals. The protection was not sufficient enough by simply mixing the APA solution and citAuNPs because APA molecules were not bound to the citAuNPs and were relatively far from the AuNP surface in that case. As for the lumAuNPs, almost all of the APA molecules were bound to the surfaces of the AuNPs and the best photostability was therefore obtained due to the efficient protection by the AuNPs to decrease the local concentration of carbonate radicals as shown in Figure 4D.

3.3. Electrochemiluminescence of lumAuNPs and Cysteine. The solid lines in Figure 6A shows the $I_{ECL}-E$ curves of the lumAuNPs with 0.02 mol/L carbonate buffer, and Figure 7 shows

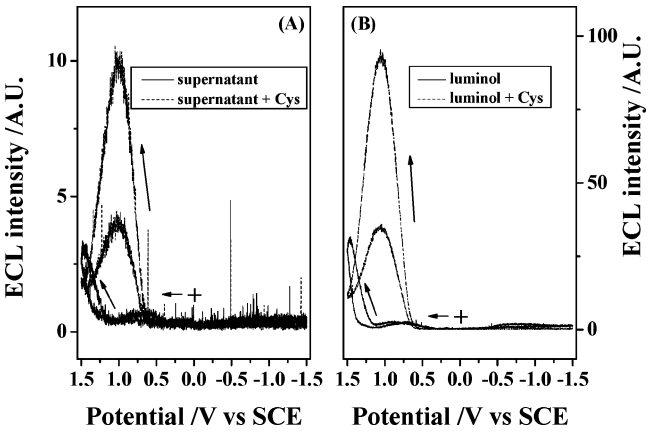

Figure 7. Typical $I_{ECL}-E$ curves of supernatant (A) and 2×10^{-7} mol/L luminol (B) in 0.02 mol/L carbonate buffer at a glassy carbon electrode with (dashed line) and without (solid line) 1 mmol/L cysteine.

the $I_{ECL}-E$ curves of the supernatant solution and 2×10^{-7} mol/L luminol solution with 0.02 mol/L carbonate buffer. It was found that the ECL profiles and peak potentials were identical in all the cases. The ECL intensity of the lumAuNPs after dialysis (solid line in Figure 6A) was about 10 times larger than that of the supernatant solution during the last dialysis (solid line in Figure 7A), indicating again that the dialysis procedure was effective and the surface-bound luminol molecules retained ECL activity. The ECL intensity of the as-prepared lumAuNPs was comparable with that of the 2×10^{-7} mol/L luminol solution. Therefore, 2×10^{-7} mol/L luminol was selected in all the contrast experiments below.

It was previously reported that luminol could react with cysteine (Cys) to generate electrochemiluminescence by applying a positive potential to the electrode.[39] The strong affinity between the cysteine molecules and the AuNPs offered us an opportunity to simultaneously attach luminol and the co-reactant cysteine molecules to the AuNP surfaces. A 20-fold ECL intensity was recorded after adding 1×10^{-3} mol/L cysteine to the lumAuNPs as shown in Figure 6A. The corresponding cyclic voltammograms with and without cysteine are shown in Figure 6B. The novel ECL peak around 1.0 V was found to be related to the electrooxidation of cysteine according to the cyclic voltammograms in Figure 6B. Therefore, the ECL peak was associated to the electrooxidation of cysteine. The ECL reactions of cysteine with the supernatant and 2×10^{-7} mol/L luminol solution were also studied as shown in Figure 7 (dashed lines). Similar changes in the ECL shapes were observed for both the supernatant and luminol solutions, implying that the ECL reaction followed the same mechanism in all the cases. However, the greatest enhancement of ECL intensity was observed for the lumAuNP solution. The ECL intensities with and without cysteine in each case are shown in Table 1. The listed values are the maximal ECL intensity in the corresponding $I_{ECL}-E$ curves. An approximately 3-fold ECL intensity was observed for the luminol and supernatant solutions after the addition of 1×10^{-3} mol/L cysteine, while a 19.3-fold ECL intensity was observed for the lumAuNP system. Since luminol was the only ECL species in the supernatant solution, it was reasonable to observe similar ECL behaviors in the supernatant as that in the luminol solution. The role of AuNPs in lumAuNP electrochemiluminescence was also studied by the addition of 16 nm citAuNPs to the solution of luminol with and without cysteine. In the luminol solution without cysteine, 50% of the ECL intensity was inhibited by

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Table 1. ECL Intensity with and without Sulfur-Containing Compound (S-Compound)

no.	solution	without S-compound	with 1 mmol/L S-compound	enhancement factor	average enhancement factor
a[a]	lumAuNPs	35.4	687.5	19.4	19.3
		35.9	647.4	18.0	
		33.1	678.2	20.5	
b[a]	supernatant	4.3	10.6	2.5	2.8
		3.5	11.1	3.2	
		3.8	10.7	2.8	
c[a]	2×10^{-7} luminol	34.0	90.8	2.7	2.7
		36.4	101.2	2.8	
		36.2	92.8	2.6	
d[a]	2×10^{-7} luminol + citAuNPs	16.8	48.2	2.9	2.3
		20.6	39.2	1.9	
		19.5	42.1	2.2	
e[b]	lumAuNPs	34.5	35.5	1.0	1.0
		33.5	32.0	1.0	
		32.0	34.3	1.1	
f[b]	2×10^{-7} luminol	37.7	36.5	1.0	1.0
		34.5	33.8	1.0	
		34.1	34.1	1.0	

[a] S-compound: cysteine. [b] S-compound: methionine.

citAuNPs as shown in Table 1 (lines c and d), which was consistent with the previous study on the electrochemiluminescence of luminol bound to AuNPs.[11] Further addition of cysteine to the mixture of luminol and citAuNPs led to 2.3-fold enhancement according to Table 1 (line d), indicating that the 19.3-fold ECL enhancement was not due to the catalytic effect of the AuNPs. These results demonstrated that the lumAuNPs−cysteine system displayed different ECL properties from those of both the luminol solution and the mixture of luminol and citAuNPs, in which luminol was not attached to the surfaces of AuNPs.

Since cysteine molecules could be attached to the AuNP surfaces via Au−S bonds, there were two possible ECL pathways in the lumAuNPs-cysteine system. One part of the ECL intensity was the contribution of dissociated luminol molecules from the lumAuNP surfaces. And another part came from the luminol molecules still bound to the AuNPs. Further dialysis or centrifugation separation of the reacted mixture of lumAuNPs and cysteine was able to distinguish these two contributions. However, either the dialysis or centrifugation procedure took about 1 h to achieve effective separation, while the enhancement of lumAuNP electrochemiluminescence was observed immediately after the addition of cysteine in several seconds. Since the ligand-exchange reaction on the surfaces of AuNPs was a dynamic process, such a long-time operation would inevitably change the surface status of lumAuNPs and could not offer real information on the initial status when cysteine was added to the lumAuNP solution. Consequently, another experiment was used to indirectly distinguish the main ECL sources by recording the ECL intensities before the addition of cysteine and at 0, 0.5, 1.0, and 2.0 h after the addition of cysteine. Based on the ligand-exchange mechanism, it was inevitable that some of the surface-bound luminol molecules would dissociate from the surfaces of AuNPs in several hours. If the electrochemiluminescence of dissociated luminol was the main contribution to the enhanced electrochemiluminescence, that is, the first pathway, the ECL intensity should increase gradually due to the continuous ligand-exchange. However, the ECL intensity was found to decrease with time after the additon of cysteine as shown in Figure 8 (curve *a*). The decrease in curve *a* could be well explained if the reaction of surface-bound luminol with cysteine was the main source in the ECL signal, that is, the second pathway, since the

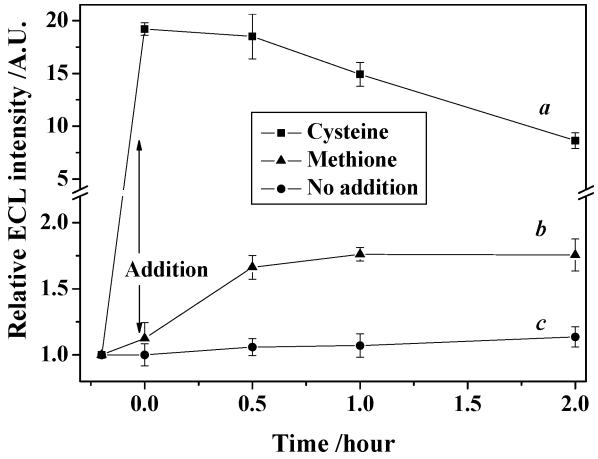

Figure 8. ECL kinetic curves of lumAuNPs without (curve *c*) and with 1 mmol/L cysteine (curve *a*) or methionine (curve *b*).

amount of surface-bound luminol molecules gradually decreased during the aging process. Other independent experiments without the addition of cysteine (curve *c*) were also tested. No obvious change in ECL intensity of lumAuNPs was observed in 2 h without cysteine, indicating weak influences of environmental factors and experimental operations.

Furthermore, the addition of methionine (Met) was also tested as a contrast to support the discussion above. Methionine is another sulfur-containing amino acid with a structure similar to that of cysteine. Although the affinity of methionine to AuNPs was weaker than that of cysteine, it was also reported that methionine could be attached to AuNPs via similar Au−S bonds.[40,41] The ECL behaviors of luminol and lumAuNPs with and without methionine were subsequently studied as shown in Table 1 (lines e and f). No obvious changes in ECL intensity and peak shape were observed when adding 1×10^{-3} mol/L methionine to the lumAuNPs or luminol solution, indicating no ECL reactions between luminol and methionine. In other words, methionine only acted as the substitution reagent while cysteine was not only the substitution reagent but also the co-reactant. When methionine was mixed with lumAuNPs for 2 h, some of the luminol molecules gradually dissociated from the AuNPs. It was reported that the ECL intensity of surface-bound luminol was about 1/3 of that of the luminol in solution due to the quenching effect of AuNPs.[11] And it was also observed that the addition of citAuNPs would lead to a decrease in the ECL intensity of luminol, according to lines c and d in Table 1. Therefore, the dissociation of luminol molecules from the AuNPs would increase the concentration of free luminol molecules in the solution and lead to an enhancement the of ECL intensity, as shown in Figure 8 (curve *b*). From curves *a* and *b*, it was concluded that the ECL intensity was mainly caused by the reaction of surface-bound luminol with cysteine molecules. The platform effect of AuNPs is proposed to be the reason for the electrochemiluminescence enhancement. Since both luminol and cysteine were negatively charged in 0.02 mol/L carbonate buffer, the electrostatic repulsion obstructed their collisions and reactions. When they were both bound to the surfaces of AuNPs, however, the molecular distance between the reactants was much shorter. The larger collision opportunity accelerated the reaction rate, resulting in the electrochemiluminescence enhancement.

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4. Conclusions

The lumAuNPs exhibited several specific FL and ECL properties. (1) The as-prepared lumAuNPs with a concentration of 3.4×10^{-9} mol/L displayed comparable FL emission to that of the 2.3×10^{-7} mol/L APA solution. As the FL intensity of a gold nanoparticle was ~70 times brighter than that of a single APA molecule, the detection limit was supposed to be much lower if lumAuNPs were used as FL probes. (2) APA molecules were found to suffer from serious photobleaching in carbonate buffer, which was associated with the oxidative carbonate radicals generated from the reaction of excited triplet state APA molecules with carbonate anions. Photobleaching was greatly inhibited for the lumAuNPs compared with free APA molecules in solution. The mechanism for the improvement of photostability was attributed to the gold cores which acted as carbonate radical scavengers to protect the surface-bound APA molecules from oxidation. The proposed radical scavenger mechanism was different from the previously reported radical blocking mechanism for the dye doped SiO_2 nanoparticles,[19] and it offered new insight into the photostability improvement of surface-bound organic chromophores. (3) The ECL intensity of as-prepared lumAuNPs was greatly enhanced in the presence of cysteine. It is proposed that the platform effect of AuNPs was responsible for the electrochemiluminescence enhancement. Luminol and cysteine molecules were simultaneously attached to the surfaces of AuNPs, which acted as a platform to overcome the electrostatic repulsion of the reactants and increased the reaction rate, resulting in enhanced ECL intensity.

The present work on the interactions between gold cores and functional ligands was of significance to improve the ligand properties and promote the applications of functionalized nanoparticles. Combined with the excellent biocompatibility of AuNPs, such advantages may make them promising biomarkers for applications in biosensors and bioimaging.

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