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Metal-enhanced fluorescence using aggregated silver nanoparticles

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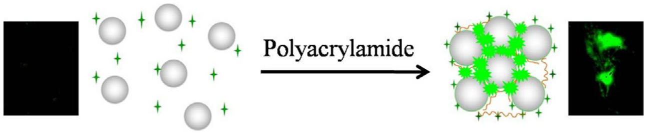
HIGHLIGHTS

- Ag microcomposite was prepared by self-assembly between Ag nanoparticles and cationic polyacrylamide (PAM) in solution.
- The fluorescence intensity of the fluorophore can be increased significantly due to MEF effect.
- This method for preparing this system was simple and novel.

GRAPHICAL ABSTRACT



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ABSTRACT

A simple strategy to prepare aggregated Ag nanoparticle composites in solution by self-assembly is developed. Sodium citrate is used as the reduction agent to produce negatively charged Ag nanoparticles which are then aggregated using cationic polyacrylamide (PAM) to form self-assembled Ag-PAM microcomposites. In these microcomposites, the degree of aggregation of Ag nanoparticles can be controlled by changing the Ag/PAM ratio. This special structural feature can be applied in the study of metal-enhanced fluorescence. Confocal laser scanning microscopy and time-resolved fluorescence measurements are used to examine the fluorescence enhancement phenomenon of the Ag-PAM microcomposites. The microcomposites considerably enhanced the fluorescence intensity of fluorescein isothiocyanate isomer (FITC).

1. Introduction

The unique optical, electronic, and magnetic properties of metal nanoparticles [1,2] have stimulated considerable interest recently for their use in diverse applications in biology and medicine, including biologic and chemical sensing, medical research and diagnosis, microscopy imaging, and optical devices [3–7]. Some noble metal nanoparticles, such as those of Ag and Au, are of particular importance exhibiting an intense absorption band in the visible region caused by surface plasmon resonance (SPR). The fluorescence intensity of fluorophores can be enhanced within a certain distance range by strong electromagnetic fields generated in close proximity to the surface of such metal nanoparticles, commonly referred to as metal-enhanced fluorescence (MEF) [8]. MEF can be used to improve the detection sensitivity of fluorescence analysis and imaging, especially for fluorophores that are typically not effectively detected because of their weak fluorescence [9,10]. For this reason, MEF has received considerable attention in the fields of nanoscience and biology.

MEF can be affected by factors such as elemental species, the distance between metallic surface and fluorophore, and the size and morphology of metallic nanostructures [11–15]. In particular, noble metals (Au, Ag) can promote MEF because of their strong SPR. The interaction distance between a fluorophore and metallic surface plays an important role in MEF [16]. When the interaction distance is appropriate, the intensity of fluorescence from the fluorophore

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can be enhanced. For this reason, researchers have investigated on how to control the interaction distance between a fluorophore and metallic surface [17–19]. Our group has also studied on how to control MEF effects by tuning the distance between a fluorophore and metallic surface [20–22], producing systems that can be applied in cell imaging [23–25].

Although different strategies to prepare MEF systems have been designed, the investigation of MEF based on aggregation of metal nanoparticles and hotspot structures is in its infancy [26]. The very small gap between two aggregated metal nanoparticles can act as a hotspot for MEF [27]. The unique properties of individual metal species selectively excite fluorophores and strongly influence their fluorescence [28–30]. Some hotspot structures for MEF have been fabricated, for example, a gold nanoparticle sandwich film custom-designed with an atomic force microscope cantilever [31,32], fluorophores entrapped in a nanoporous alumina film with an ordered hotspot array [33], and etching technology for producing hotspot structures [34]. Although the above methods have fabricated hotspot structures that induce MEF, planar metal films are mostly used and require complex procedures. To date, only few researchers have fabricated hotspot structures for MEF in solution [35,36].

To acquire a straightforward method to achieve strong MEF in solution, here we attempt to form self-assembled aggregates of Ag nanoparticles which have surfaces with strong electromagnetic fields [37] with a nanogap structure to induce MEF. We use a simple method to fabricate an Ag microcomposite by introducing cationic polyacrylamide (PAM) into as-prepared Ag nanoparticles in aqueous solution. With different Ag/PAM ratio, different size, and compactness Ag microcomposites are obtained. Using this special structured Ag microcomposite, the fluorescence from fluorescein isothiocyanate isomer (FITC) can be improved substantially. This simple method to produce substrates for MEF could be applied in biologic sensing, medical research, and diagnosis.

2. Experimental

2.1. Materials

Silver nitrate ($AgNO_3$) and sodium citrate were analytical grade products and purchased from Aladdin Chemistry Co., Ltd (Shanghai, China). Cationic PAM (*Mw* = 800,000 g/mol, monomodal distribution), was purchased from Sinopharm Chemical Reagent Co., Ltd. FITC was purchased from Sigma–Aldrich. Ultrapure Millipore water (18.3 MΩ) was used throughout the experiments. Reagents were used without further purification unless otherwise stated.

2.2. Measurements

The maximum surface plasmon absorption of Ag nanoparticles was measured using a Hitachi U3900 spectrophotometer with quartz cells (path length = 1 cm) at room temperature. For transmission electron microscopy (TEM) observation, the aqueous Ag nanoparticle suspension and Ag-PAM microcomposite colloids were added dropwise onto carbon-coated Cu grids (200 mesh) and allowed to dry overnight in air. TEM images were recorded on a JEOL JEM 2100 TEM with an accelerating voltage of 200 kV. The hydrodynamic diameters and zeta (ζ) potentials of the Ag nanoparticles and Ag-PAM microcomposites were measured by dynamic light scattering (DLS) using a Nano ZS90 zetasizer (Malvern Instruments Ltd.) at room temperature.

2.3. Preparation of Ag nanoparticles

Citrate-coated Ag nanoparticles were synthesized according to a reported procedure [38]. Briefly, silver nitrate (10 mg) was dissolved in distilled water (50 mL) and heated to 95 °C under rapid stirring. After 1 min, 1% (w/w) sodium citrate solution was added in four aliquots of 1 mL, and then the solution was maintained at 95 °C for 1 h with continuous stirring. The final concentration of $AgNO_3$ was 1.09 mM, and that of sodium citrate was 6.30×10^{-2} M. The reduction reaction was performed in the dark. The resulting Ag nanoparticle colloidal sol was separated by centrifugation for 20 min at a rotation speed of 20,000 rpm, the corresponding centrifugal force is 24,596 × *g*. The isolated nanoparticles were washed twice with deionized water.

2.4. Preparation of Ag-PAM microcomposites

Ag-PAM microcomposites were prepared through the electrostatic attraction of negatively charged Ag nanoparticles with positively charged PAM. To investigate the effect of different amounts of PAM on the aggregation of Ag nanoparticles, we prepared aqueous solutions of PAM of various concentrations. Ag-PAM microcomposites were synthesized as follows: an aqueous solution of PAM (100 μL, 7.80×10^{-4}, 4.68×10^{-3}, and 6.24×10^{-3} M) was added dropwise to Ag nanoparticle colloid (1.90 mL, 0.34 mM). Each mixture was stirred vigorously for 10 min and then left at room temperature for 2 h. The final concentration of Ag nanoparticles in each mixture was 0.32 mM and the final concentrations of PAM were 3.90×10^{-5}, 2.34×10^{-4}, and 3.12×10^{-4} M, respectively. These Ag-PAM microcomposite systems are designated Ag-PAM-1, Ag-PAM-2, and Ag-PAM-3, respectively. The molar ratios of Ag to PAM in these microcomposite systems are 8.2:1, 1.4:1, and 1:1, respectively.

2.5. Measurement of fluorescence

To measure MEF, aqueous solution of FITC (4 μL, 1 mM) was added to each Ag-PAM microcomposite solution (2 mL, pH = 7.0), and then each mixture was stirred vigorously in the dark. The final concentration of FITC was 2 μM. Fluorescence measurements were performed in a standard quartz cuvette with a path length of 1 cm and the excitation wave via a 488 nm (xenon lamp). Fluorescence spectra were measured on a Hitachi F-7000 fluorescence spectrophotometer 1 h after adding fluorescent dye. To measure MEF, the same amount of FITC was also added to deionized water (2 mL) and Ag nanoparticles colloids (2 mL, 0.32 mM); the fluorescence of these solutions was measured after stirring for 1 h.

2.6. Fluorescence imaging

Fluorescence imaging studies were performed with a confocal laser scanning microscope (CLSM, Olympus, F V1000-IX81). FITC-linked Ag-PAM microcomposite solution (5 μL) was added dropwise onto a clean glass slide (18 mm × 18 mm), covered with a coverslip (15 mm × 15 mm), and placed on the objective table. An excitation laser (488 nm) was irradiated on the sample directly through a 10 nm bandpass excitation filter and reflected by a dichroic mirror. The sample was observed through an oil immersion objective (Olympus 100X). CLSM images were recorded by raster scanning the sample over the focused spot of the incident laser.

2.7. Time-resolved fluorescence

Time-resolved fluorescence spectra were recorded with an Edinburgh Instruments F900 spectrometer using vertically polarized excitation pulses of ~100 fs, excitation wavelength of 488 nm and laser repetition rate of 250 kHz. The detector polarizer was set at the magic angle orientation and a time range of 0–999 ns was used. To detect the change of decay rate, lifetimes curves of an