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Quenched Emission of Fluorescence by Ligand Functionalized Gold Nanoparticles

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A fluorescence-based detection scheme that uses ligand functionalized gold nanoparticles is proposed. The transduction scheme is based on the strong quenching of the fluorescence emission exerted by metallic surfaces on fluorophores positioned in their immediate vicinity (<5 nm). Binding of fluorophore-labeled anti-biotin to biotinylated gold nanoparticles resulted in decreased fluorescence emission intensity. Subsequent competitive dissociation of labeled anti-biotin with D-biotin resulted in increased fluorescence emission intensity. These interactions occurred by means of specific molecular recognition because when the binding sites of anti-biotin were saturated with D-biotin prior to interaction with the gold nanoparticles; changes in the fluorescence emission intensity were not observed.

KEY WORDS: Gold nanoparticles; self-assembled monolayers; surface modification; fluorescence; energy transfer.

INTRODUCTION

During the last decade there has been a large amount of interest in metallic nanoparticles for a variety of nanotechnology applications. Examples of this can be found in the use of gold nanoparticles (or other noble metals) as contrast agents for electron microscopy [1], colorimetric assays for DNA detection [2,3], biosensors [4,5], catalysis [6] and the assembly of quantum dots into ordered arrays or nanocrystal superlattices [7]. One area where the use of these systems has had an enormous growth is in biotechnology, where nanoparticles and biological molecules are combined to take advantage of the optical properties of the former and the specific molecular recognition capabilities of the latter.

The optical properties of metallic nanoparticles are readily susceptible to changes in the dielectric constant of the medium and/or their degree of aggregation and this has been exploited to achieve optical detection of biomolecules [2,3]. However, metallic surfaces also exert a strong influence in the emission characteristics of fluorophores positioned in their vicinity and two opposite phenomena can be observed depending on their separation distance. When the separation distance between fluorophores and metallic surfaces is smaller than 5 nm, strong quenching of fluorescence emission and a dramatic reduction on the lifetimes of the excited states is observed [8–11]. When this distance is in the range of 10–20 nm, enhanced fluorescence emission can result because of local concentration of the incident excitation field by the metallic nanoparticles [12–14]. Although these phenomena are well known, their application in biotechnology has not been extensively explored.

This work shows how surface-modified gold nanoparticles can be used as platforms for displaying ligands in fluorescence-based biosensors that exploit the quenching phenomena exerted by metallic surfaces on fluorophores. Although this scheme has been explored before on planar surfaces [15], using metallic nanoparticles may offer additional advantages because of their larger specific area and the fact that their size is on the same order

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of magnitude as biomolecules. Thus, the proposed system could be implemented in combination with biomolecules in a variety of assays and devices.

The implementation of a detection scheme based on quenching by metals and the use of gold nanoparticles whose surface was modified with alkane thiols follows from the fact that the thickness of self-assembled monolayers (SAMs) of alkanethiolates is on the order of 1–3 nm [16]. Thus, a SAM offers the means to position fluorophores at a distance from the metal where the fluorescence emission and lifetime of the excited states is more sensitive to changes in the separation distance [8–15].

EXPERIMENTAL SECTION

The experimental system consisted of biotinylated gold nanoparticles (core diameter of 20 nm) and Alexa^{TM} 488 labeled monoclonal 2F5 anti biotin. Biotinylated gold nanoparticles were prepared by reacting 2-(2-aminoethoxy) ethanol and (+)-biotinyl-3-6,9,-trioxaundecanediamine to carboxyl-modified gold nanoparticles using *N*-(3-dimethylaminopropyl)-*N′*-ethyl-carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) as coupling reagents (Fig. 1). The preparation of stable carboxyl-modified gold nanoparticles was accomplished by chemisorbing 16-mercaptohexadecanoic acid in the presence of a non-ionic surfactant as described before [17].

Materials

Gold nanoparticle dispersions (monodisperse, 20 nm average particle size), hydrogen peroxide, sulfuric acid, *N*-Hydroxysuccinimide (NHS) and sodium phosphate monobasic were obtained from Sigma. Polyoxyethylenesorbitan monolaurate (Tween® 20), absolute ethanol, 2-(2-aminoethoxy)ethanol, and 16-mercaptohexadecanoic acid (16-MHDA) were purchased from Aldrich. *N*-3-(dimethylaminopropyl)-*N′*-ethyl-carbodiimide (EDC) was from Fluka, (+)-biotinyl-3,6,9,-trioxaundecanediamine (BA) from Pierce, and Alexa^{TM} 488 labeled monoclonal 2F5 anti biotin from Molecular Probes. All chemicals were used as received.

Buffers and Solutions

Sodium phosphate monobasic buffer solutions were prepared to a 10 mM concentration at pH 7. Exact pH values for buffer solutions were measured using an Accumet AB15 pH meter. Deionized water (electric resistivity >18 MΩ-cm) was obtained from a Barnstead Nanopure Infinity UV/UF water purification system. All glassware

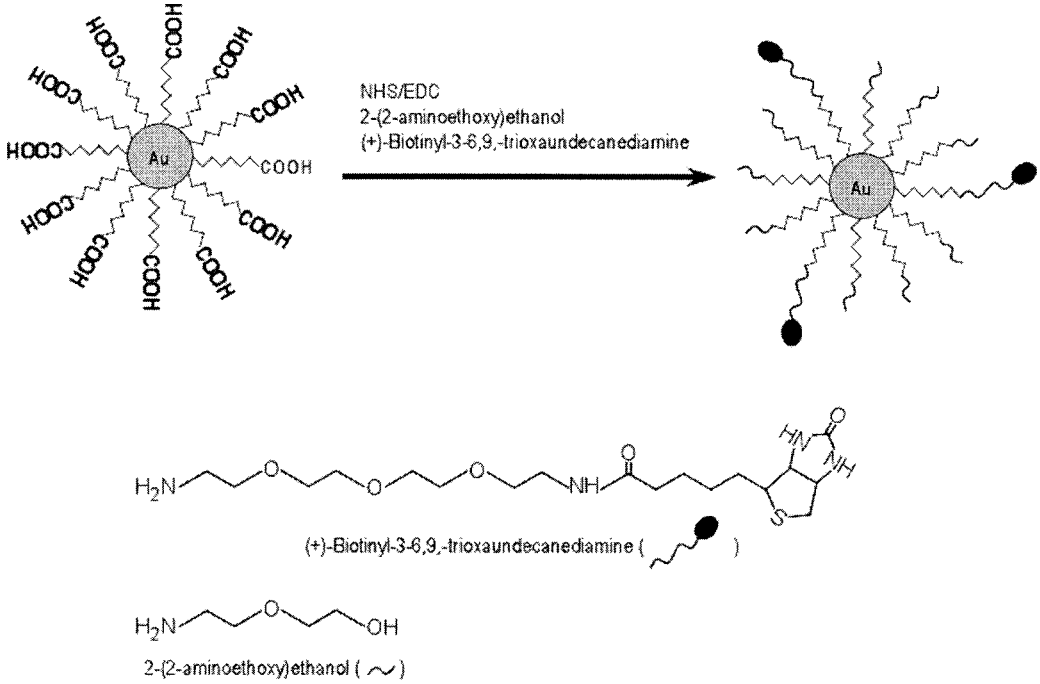

Fig. 1. Schematic procedure of ligand immobilization on gold.

used was treated with "piranha solution" (3:7 30% hydrogen peroxide/concentrated sulfuric acid: CAUTION! *piranha solution reacts violently with most organic materials and should be handled with extreme care*).

Solutions of 0.5 mM 16-MHDA were prepared in degassed ethanol. Tween 20 solutions were prepared in sodium phosphate buffer at pH 7.

Surface Modification of Gold Nanoparticles

Gold nanoparticle dispersions with a concentration of 0.8 nM as purchased were degassed before use. Equal volumes (400 μL) of gold nanoparticle dispersions and Tween® 20 (1.82 mg/mL) in pH = 7 buffer were gently mixed and allowed to stand for 30 min to allow for the physisorption of Tween® 20 to the gold nanoparticles to occur. Then, 400 μL of 0.5 mM 16-MHDA were added and the final mixture (final concentrations: [gold nanoparticles] = 0.27 nM, [Tween® 20] = 0.61 mg/mL, [16-MHDA] = 0.17 mM) was allowed to stand for 3 hr. The final solution (gold nanoparticles, Tween® 20, and 16-MHDA at pH 7) was centrifuged (15 min, 15,700*g*) and resuspended three times in phosphate buffer and the supernatant was discarded. The centrifugate was resuspended in sodium phosphate buffer containing 1.82 mg/mL Tween® 20 at pH 7. The 16-MHDA modified-gold nanoparticles were reacted with a mixture of freshly prepared 50 mM NHS and 200 mM EDC solution for 5 min. The resulting colloidal solution was centrifuged (5 min, 15,700*g*) and the centrifugate was then resuspended in sodium phosphate buffer containing 1.82 mg/mL Tween® 20 at pH 7, and centrifuged again (5 min, 15,700*g*). After discarding the supernatant, the remaining NHS ester-alkane thiol modified-gold nanoparticles were reacted with a freshly prepared mixture consisting of 2-(2-aminoethoxy)ethanol (AEE) and (+)-biotinyl-3-6,9,-trioxaundecanediamine (BA) in a molar ratio of 10:1 for 10 min. The unreacted BA and AEE were separated from the resulting colloidal solution by repeated ultrafiltration (a minimum of three times) using Microcon30 (MW cut off 30,000) centrifugal filters for 5 min at 6,000*g*. The centrifugate (biotinylated gold nanoparticles) was then stored at 4°C for future use. All solutions having gold nanoparticles were stored in polypropylene centrifuge tubes in the dark to prevent light-induced flocculation of the nanoparticles.

The interaction of biotinylated gold nanoparticles and Alexa™ 488 labeled antibody was studied by first placing a known amount of the labeled antibody in a quartz cuvette and measuring its emission intensity. After this, a known amount of biotinylated gold nanoparticles was quickly mixed into the cuvette and allowed to bind to the antibodies. After the intensity emission reached a plateau, the antibody-nanoparticles were competitively displaced by introducing an aliquot of free biotin in phosphate buffer to a final concentration in the cuvette of 1 mM biotin. A SPEX Fluoromax-3 spectrofluorometer was used for the measurements and the emission fluorescence was recorded as a function of time using an Argon ion laser as the excitation source (using the laser line with wavelength of 488 nm) and measuring the emission at 518 nm.

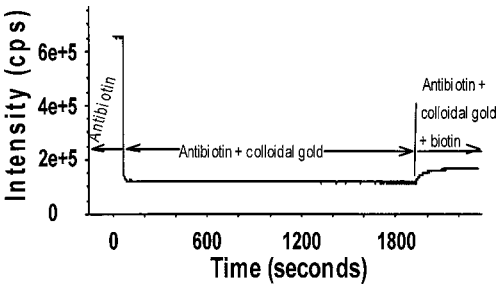

Fig. 2. Emission intensity of Alexa™ 488 labeled anti-biotin before and after binding to biotinylated gold nanoparticles and after competitive dissociation with d-biotin.

Correction for Dilution and Absorption/ Scattering Effects

The emission intensities in Figure 2 are affected by the scattering/absorption of the gold nanoparticles (for these nanoparticles absorption the absorption effect is more significant than scattering) and also by the dilution resulting from introducing aliquots of gold nanoparticles and free biotin solution during the experiment. Thus, the data were corrected for dilution effects (by multiplying the measured intensities by the appropriate dilution factors) and the strong absorption by the gold colloids. The dilution corrected intensities were further divided by the normalized intensity (Fig. 3) corresponding to the concentration

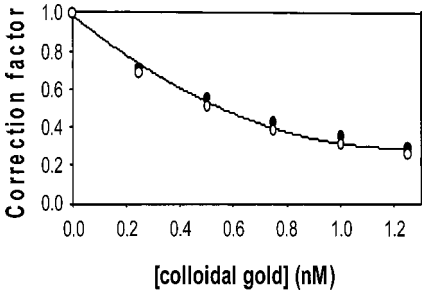

Fig. 3. Emission intensity of Alexa™ 488 in the presence of varying concentrations of biotinylated gold nanoparticles (•) 5 nM Alexa™ 488 and (○) 20 nM Alexa™ 488.

of gold nanoparticles present in the cuvette at the different stages of the experiment (*vide infra*).

RESULTS AND DISCUSSION

Figure 2 shows the time progression of the emission intensity from Alexa^{TM} 488 labeled anti-biotin (4.30 nM) and its interaction with biotinylated gold nanoparticles (0.86 nM). Immediately after addition of biotinylated gold nanoparticles, there is a strong decrease of the emission intensity, which is due to three combined effects: 1) a dilution effect after adding an aliquot of biotinylated gold nanoparticles to the cuvette; 2) strong absorption/scattering caused by the presence of biotinylated gold nanoparticles; and 3) actual quenching of the emission intensity by the gold surfaces as the antibody binds to biotin groups on the surface of the nanoparticles. The latter occurs according to the intrinsic kinetics of the protein-hapten interaction. Evidence that quenching of emission occurs due to specific biomolecular recognition between nanoparticles and anti-biotin is observed upon competitive dissociation of the bound antibody. As Figure 2 shows, introducing free biotin in solution (1 mM) results in increased fluorescence emission. This happens because the antibody molecules separate from the gold surface as they are displaced into the bulk of the solution, thus resulting in concomitant separation distance of the fluorophores from the metallic colloids. In order to separate the effects of quenching and dilution from those due to absorption/scattering by the gold nanoparticles, the emission intensity of standard solutions of Alexa^{TM} 488 in the presence of known concentrations of biotinylated gold nanoparticles were measured. Biotinylated gold nanoparticles were used to avoid possible interactions between the fluorophores and the gold surfaces [18] that may introduce quenching to the measurements.

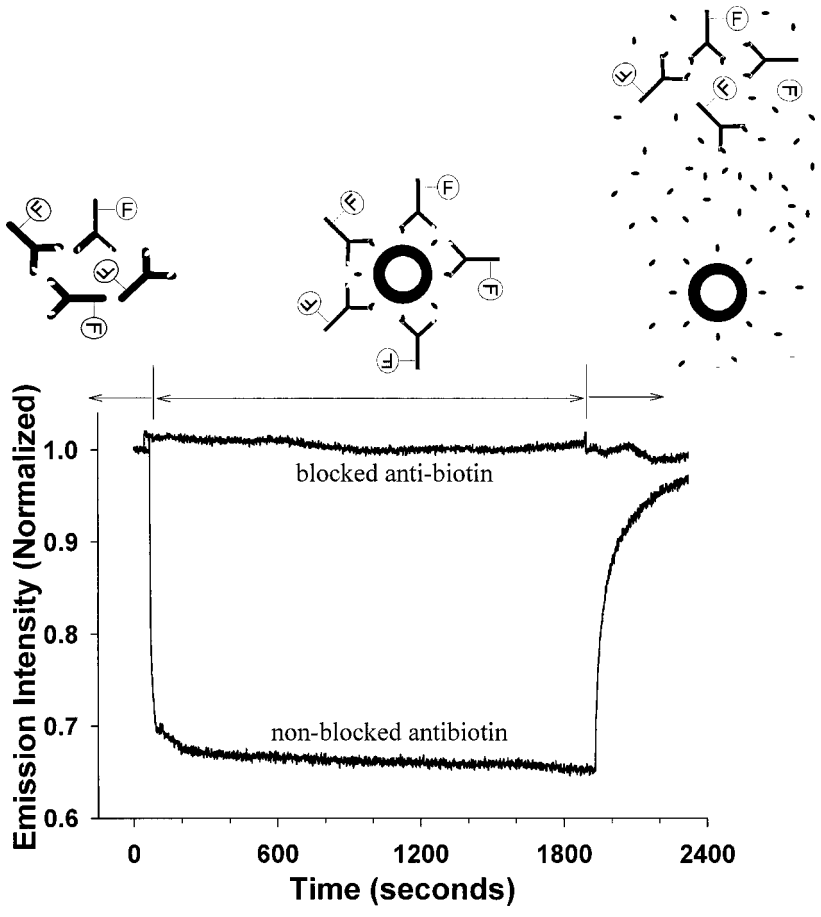

Fig. 4. Fluorescence emission intensity (normalized) of Alexa^{TM} 488 labeled anti-biotin interacting with biotinylated gold nanoparticles.

Figure 3 shows the emission intensity of Alexa^{TM} 488 at varying concentrations of gold nanoparticles normalized to the intensity observed in the absence of gold nanoparticles. The fact that the experimental points fall within the same curve regardless of the fluorophore concentration in the experiment suggests that the normalized intensity can be used as a correction factor to compensate for the effects of absorption and scattering and thus, to determine the actual contribution from the quenching effect [19].

Figure 4 shows the interaction of Alexa^{TM} 488 labeled anti-biotin with biotinylated gold nanoparticles, which is corrected for dilution and absorption/scattering effects and normalized to the intensity of labeled antibody in the absence of gold particles. Thus, the corrected data shows the pure quenching effects resulting from the specific molecular recognition between antibodies and biotinylated gold nanoparticles. Figure 4 also shows the interaction of antibodies whose biotin binding sites were blocked by incubation in 1 mM biotin. The corrected data of Figure 4 for blocked antibodies does not show changes in the emission intensity. This demonstrates that the interaction between biotinylated gold nanoparticles and Alexa^{TM} 488 labeled anti-biotin occurs exclusively by means of specific biomolecular recognition; and that the quenching exerted by metallic nanoparticles on the emission properties of fluorophores can be implemented as a detection scheme for specific biomolecular interactions between proteins/antibodies and ligand/hapten functionalized metallic nanoparticles provided that the appropriate molecular composition is achieved on the surface of the nanoparticles.

In summary, this work demonstrates that metallic nanoparticles can be used as platforms for the display of ligands in fluorescence-based applications. The use of alkane thiol based SAMs for the surface modification of gold nanoparticles offers the advantage of facile monolayer formation with a thickness that positions the fluorophores in the region where the quenching exerted by a metal is the strongest. This scheme could be easily generalized to any other ligand-protein pair and/or system where a change in the molecular architecture at the surface results in separation between metallic nanoparticle and a fluorophore attached to a biomolecule. The proposed technique also offers the potential for rapid, sensitive, and selective detection of small molecules through measurements of the emission intensity and/or lifetimes of the excited states of fluorescent molecules. The results of the detailed investigation of this work will be reported in due course.

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