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Short communication

Biocompatible, nanogold-particle fluorescence enhancer for fluorophore mediated, optical immunosensor

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Abstract

Fluorophores have been used as effective signal mediators for detecting biomarkers in biosamples. The enhancement of the fluorescence can, therefore, improve the sensitivity of fluorophore-mediated biosensors. A nanogold particle (NGP), when placed at an appropriate distance from a fluorophore, can effectively enhance the fluorescence by transferring the free electrons of the fluorophore, normally used for self-quenching, to the strong surface plasmon polariton field (SPPF) of the NGP. We found that some organic solvents can also enhance the fluorescence significantly. To maximize the fluorescence enhancement, novel, biocompatible nanogold particle reagents (NGPRs) were developed by combining NGPs and biocompatible solvents and tested. The level of enhancement by NGPRs was found to be additively contributed by two enhancers. These NGPRs were able to increase the signal of a fiber-optic biosensor as much as 10 times and accurately quantify some of the important cardiac markers at a tens of picomolar level. These novel enhancers are expected to be effective for fluorophore-mediated bioimaging as well as biosensing.

Keywords: Fluorescence enhancement; Nanogold particle; Biocompatible solvent; Biosensor; Cardiac markers

1. Introduction

Fluorophores with the emission at 600–700 nm have been valuable tools in biosensing/bioimaging for disease screening, diagnosis, and monitoring, because biological systems generate auto-fluorescence noise minimally at these wavelengths (Buschmann et al., 2003; Berlier et al., 2003). However, the low quantum yield (QY) of some of these fluorophores caused by the non-radiative decay, especially self-quenching, limits their effectiveness (Gruber et al., 2000; Anderson and Nerurkar, 2002).

Metallic nanoparticles, e.g. Au or Ag, have been previously studied for coupling the electrons involved in the self-quenching to their strong surface plasmon polariton fields (SPPF), upon photoexcitation (Thomas and Kamat, 2000; Lakowicz et al., 2003; Krenn, 2003). For this electron transfer, it is very important that the fluorophore is placed at a particular distance from a nanoparticle (Fig. 1a). In our study nanogold particles (NGPs) are used for their SPPF. If an NGP is placed too close to a fluorophore, the NGP extracts all electrons in the excited state from the fluorophore, including the ones for radiative emission (Dubertret et al., 2001); if it is too far from a fluorophore, its SPPF may not reach the fluorophore and there will be no effect on the resulting fluorescence intensity; when an appropriate distance is maintained between these two entities by some means, e.g., a self-assembled monolayer (SAM) on the NGP surface (NGP–SAM), the fluorescence may be enhanced due to an effective transfer of the electrons used for self-quenching.

Organic solvents may also affect the fluorescence intensity in biosensing (Jones et al., 1980; Chen et al., 1999), possibly by the shifting excitation/emission spectrums of the fluorophore (Buschmann et al., 2003), by the isomerization of the fluorophore (Rodríguez et al., 1997), by shrinking fluorophore tagged proteins (Bonincontro et al., 1997; Ruckebusch et al., 1999), or by the combination of these three.

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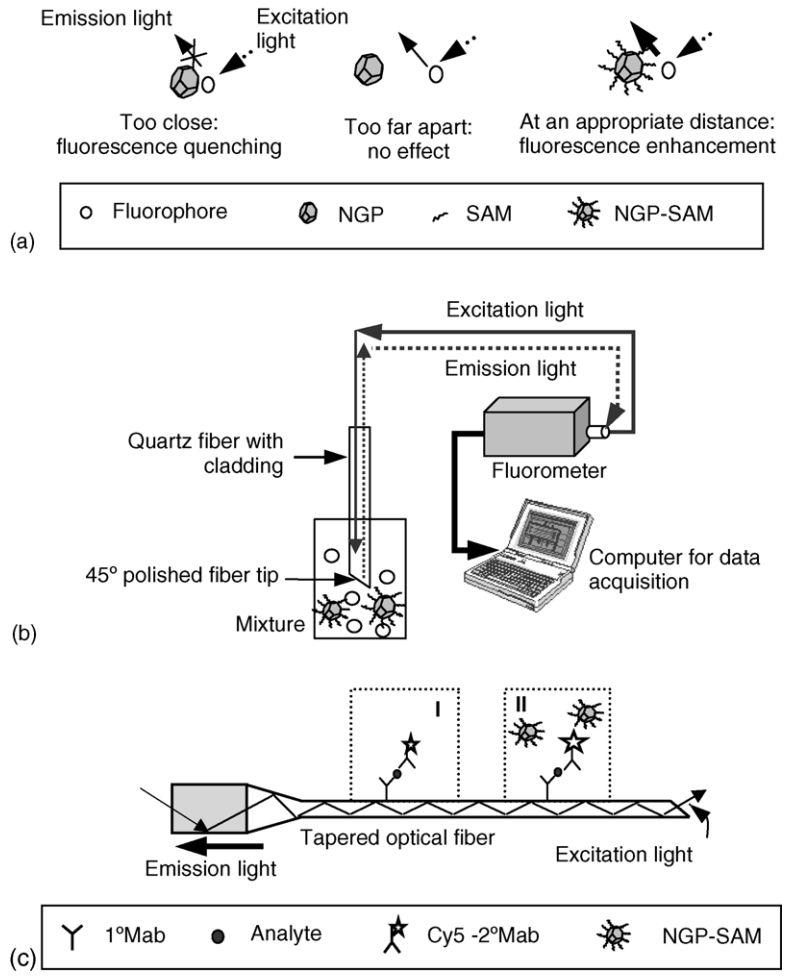

Fig. 1. Schematic diagrams of: (a) the effect of distance between an NGP and a fluorophore on the fluorescence emission. (b) Fluorescence measurement for free fluorophores in solution. The tip of a quartz fiber polished at 45° was used. (c) Fluorescence measurement in biosensor: (I) regular immuno-sensing; (II) immuno-sensing using NGP–SAMs.

In this paper, the effects of the NGP size, the distance between an NGP and a fluorophore, the quantum yield (QY) of a fluorophore, and the solvent on fluorescence intensity are reported. The fluorescence intensity affected by the mixture of the NGP and the solvent (nanogold particle reagent; NGPR) is also presented. The effect of these NGPRs on the sensitivity of cardiac marker sensors is also demonstrated.

2. Materials and methods

2.1. NGP–SAM and NGPR preparation

Nanogold colloids, which are nanogold particles (2, 5 and 10 nm) coated with tannic acid (surfactant; approximately, 3 nm), were from Ted Pella (Redding, CA). The thickness of a surfactant layer or a self-assembled monolayer (SAM) was estimated using the software, HyperChem 7.0 (Hypercube, Inc.; Gainesville, FL). L-Glutathione (approximately, 1 nm) and 16-mercaptohexadecanoic acid (approximately, 2 nm) used as SAMs, ethanol, methanol, and tetrahydrofuran (THF) were purchased from Sigma/Aldrich (St. Louis, MO). The SAM of L-glutathione or 16-mercaptohexadecanoic acid was linked on the surface of NGPs by dissolving these molecules in DI water or dimethyl sulfoxide; transferring them to the nanogold colloid; heating the mixture up to 70 °C; stirring them for 5 h; and purifying the products using Slide-A-Lyzer® dialysis cassette (Pierce; Rockford, IL) or DispoDialyzer® dialysis tube (Spectrum Laboratories, Inc.; Rancho Dominguez, CA) to eliminate the unreacted SAM molecules and replaced surfactant molecules. The NGPRs were produced by mixing the NGP–SAMs and the solvent to be tested.

2.2. Biosensor preparation

Fluorophores, Fluorolink™ Cyanine 5 (Cy5) and Alexa Fluor® 647 (AF647), were obtained from Amersham Pharmacia Biotech (Uppsala, Sweden) and Molecular Probes