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A Highly Sensitive and Selective Surface-Enhanced Nanobiosensor

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ABSTRACT

Nanosphere lithography (NSL) derived triangular Ag nanoparticles were used to create an extremely sensitive and specific optical biological and chemical nanosensor. Using simple UV-vis spectroscopy, biotinylated surface-confined Ag nanoparticles were used to detect streptavidin down to one picomolar concentrations. The system was tested for nonspecific binding interactions with bovine serum albumin and was found to display virtually no adverse results. The extremely sensitive and selective response of the Ag nanoparticle sensor indicates an exciting use for biological and chemical sensing.

INTRODUCTION

Currently, there is great interest in the optical properties of noble metal nanoparticles. Early work with size tunable Ag nanoparticles fabricated by nanosphere lithography (NSL) demonstrated that the localized surface plasmon resonance (LSPR) could be tuned throughout the visible region of the electromagnetic spectrum [1]. The LSPR, the signature optical property of noble metal nanoparticles, arises when electromagnetic radiation excites the conduction electrons of the metal and causes them to oscillate collectively. The primary consequences of this excitation include (a) localized electromagnetic field enhancement and is responsible for the intense signals observed in surface-enhanced spectroscopies and (b) selective photon absorption/scattering (extinction) and can be easily monitored using UV-vis spectroscopy [2].

The formation of alkanethiol self-assembled monolayers (SAMs) on metal surfaces (i.e. nanoparticles) offers a simple and attractive method of surface modification. SAMs are used in many chemical and biological sensor technologies because they can functionalize a metal nanoparticle for specific analyte capture and protect fragile biological molecules from denaturing upon exposure to bare metal surfaces [3-5]. These systems have been implemented in various detection schemes with the goal of creating biosensing systems.

SAMs have been used to link molecules from solution onto nanoparticles and bulk surfaces [6-8]. The extremely high binding affinity of the biotin-streptavidin complex ($\sim 10^{13} - 10^{15}$ M^{-1}) [9] makes SAM modified Ag nanoparticles ideal for model biosensing experiments . The detection of low concentrations of biomolecules with minimal nonspecific binding responses is an elusive goal in many biosensor technologies. Streptavidin (SA), a tetrameric protein, can bind up to four biotinylated molecules (i.e. antibodies, inhibitors, nuclei acids, etc.) with minimal impact on its biological activity [10]. These complexes are extremely stable over a wide range of pH and temperature; accordingly, this system is often used to test potential biosensor systems.

Surface plasmon resonance biosensors have been used to characterize the specific binding of a biomolecule to its immobilized binding partner. These propagating surface plasmon polariton (SPP) sensors, which operate on real-time refractive index changes, offer a label-free method for the detection of biomolecules [11-13].

Recently, we demonstrated that NSL-derived Ag nanoparticles can be used as biological and chemical optical nanosensors by monitoring the peak extinction, λ_{max} [8]. These experiments indicated that Ag nanoparticles could be used in sensor applications much like the widely available SPP biosensors. Similar to the SPP sensor, the LSPR nanosensor operates by detecting refractive index changes near the metal surface. NSL-generated nanoparticles are ideal for optical nanosensor applications for the following reasons: (a) the nanoparticles are confined to a surface at a large enough fixed interparticle spacing so that they function independently rather than as an array [14], (b) the nanoparticles are homogenous in size and shape [15], and (c) the dielectric environment surrounding the particles is easily controlled [8]. In this study, the specific binding of SA to biotinylated Ag triangular nanoparticles was explored. Additionally, detection limits and sensor specificity were determined.

EXPERIMENTAL DETAILS

Materials

11-Mercaptoundecanoic acid (11-MUA), 1-octanethiol (1-OT), hexanes, and methanol were acquired from Aldrich (Milwaukee, WI). 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), bovine serum albumin (BSA), streptavidin (SA), 10 mM and 20 mM phosphate buffered saline (PBS), pH = 7.4 was obtained from Sigma (St. Louis, MO). (+)-Biotinyl-3,6-dioxaoctanediamine (biotin) was purchased from Pierce (Rockford, IL). Absolute ethanol was purchased from Pharmco (Brookfield, CT). Ag wire (99.99%, 0.5 mm diameter) was obtained from D. F. Goldsmith (Evanston, IL). Borosilicate glass substrates, Fisher brand No. 2 18 mm circle cover slips were purchased from Fisher Scientific (Pittsburgh, PA). Tungsten vapor deposition boats were acquired from R. D. Mathis (Long Beach, CA). Polystyrene nanospheres with diameters of 400±7 nm were received as a suspension in water (Interfacial Dynamics Corporation, Portland, OR) and were used without further treatment. Millipore cartridges (Marlborough, MA) were used to purify water to a resistivity of 18 MΩ. All materials were used without further purification.

Substrate preparation

Glass substrates were cleaned in a piranha solution (1:3 30 % H_2O_2:H_2SO_4) at 80°C for 30 minutes. Once cooled, the glass substrates were rinsed with copious amounts of water and then sonicated for 60 minutes in 5:1:1 H_2O:NH_4OH:30% H_2O_2. Next, the glass was rinsed repeatedly with water and was stored in water until used.

Nanoparticle preparation

NSL was used to fabricate monodisperse, surface-confined Ag nanoparticles [16]. For these experiments, single layer colloidal crystal nanosphere masks were prepared by drop coating ~2 μL of nanosphere solution onto glass substrates. Once the nanosphere masks were dry, the substrates were mounted into a Consolidated Vacuum Corporation vapor deposition system. A Leybold Inficon XTM/2 quartz crystal microbalance (East Syracuse, NY) was used to measure the thickness of the Ag film deposited over the nanosphere mask. Ag films were

deposited to a 50.0 nm thickness for all samples in this study. Following Ag deposition, the nanosphere mask was removed by sonicating the sample in ethanol for 3 minutes.

Ultraviolet-visible extinction spectroscopy

Macroscale UV-vis extinction measurements were collected using an Ocean Optics (Dunedin, FL) SD2000 fiber optically coupled spectrometer with a CCD detector with a resolution of ~0.6 nm. All spectra were collected in standard transmission geometry with unpolarized light. The probe beam area was approximately 12 mm^2.

Nanoparticle modification

A home built flow cell [8] was used to control the external environment of the Ag nanoparticle substrates. Prior to modification, the Ag nanoparticles were solvent annealed [8]. Dry N_2 gas and solvent were cycled through the flow cell until the λ_{max} of the sample stabilized. The UV-vis extinction maxima, λ_{max}, of identically-prepared bare Ag nanotriangles, varies slightly due to small differences in nanoparticle size and roughness [8]. This difference, however, does not affect the overall sensitivity of the nanoparticle sensor. As a result, the λ_{max} must be recorded before and after chemical modification. Once solvent annealed, samples were then incubated in 1 mM 3:1 1-OT:11-MUA ethanolic solutions for 24 hours. After incubation, the nanoparticle samples were rinsed with ethanol and dried by flowing N_2 gas through the sample cell. Next, 1 mM biotin in 10 mM PBS was covalently linked to the surface carboxyl groups using EDC coupling over a 3 hour period. Following thorough rinsing and N_2 drying, the samples were incubated in SA solutions in 10 mM PBS for 3 hours. Samples were rinsed thoroughly with 10 mM PBS, 20 mM PBS, and water to remove electrostatically bound molecules.

Atomic force microscopy (AFM)

AFM images were collected using a Digital Instruments Nanoscope III microscope operating in tapping mode. Etched Si nanoprobe tips (TESP, Digital Instruments, Santa Barbara, CA) were used. These tips had resonance frequencies between 280 and 320 kHz and are conical in shape with a cone angle of 20• and an effective radius of curvature at the tip of 10 nm. All images shown here are unfiltered data that were collected in ambient conditions.

DISCUSSION

NSL-derived Ag nanoparticles with an in-plane dimension, a=93 nm and an out-of-plane height, b=50.0 nm were synthesized and solvent annealed (Figure 1). The Ag nanoparticles were then functionalized with a mixed monolayer of 11-MUA/1-OT resulting in a surface coverage of = 0.1 [17] of carboxylate binding sites. Next, biotin was covalently attached via amide bond formation to carboxylated surface sites. Upon exposure to 10 pM SA, the LSPR extinction maximum shifted +15.3 nm (Figure 2). It should be noted that the signal transduction mechanism in this sensor is unique in that it is a reliably measured wavelength shift rather than an intensity change.

Figure 1. Tapping mode AFM image of the Ag nanoparticle LSPR nanosensor. After solvent annealing, the resulting nanoparticles have in plane widths of ~100 nm and out-of-plane heights of 51 nm. Defects are present in sensing probe volume but do not contribute to the optical sensing events.

The sensitivity limits of the biotinylated Ag nanoparticle biosensor were also examined. By varying the concentration of SA, the detection limit of LSPR shift versus SA concentration was determined (Table 1). In these studies, the SA concentration was varied from $1x10^{-15}$ M to $1x10^{-6}$ M. The LSPR shift response remained constant for concentrations between $1x10^{-10}$ M and $1x10^{-6}$ M. The LSPR response drops off drastically between $1x10^{-13}$ M and $1x10^{-10}$ M. Overall, it was found that the maximum SA response of +26.5 nm decreased rapidly to a detectable +4 nm shift for 1 pM SA. Fitting the SA response to the function yields a value for the saturation response, R_{TOT}=26.5 nm, and the surface-confined thermodynamic affinity binding constant, $K_{a,surf}=10^{11}$ M^{-1} [18]. This value is smaller than the widely accepted $K_{a,surf}$ value [9]. By adjusting the length of the biotin tether, the $K_{a,surf}$ value will approach the widely accepted value of 10^{13}-10^{15} M^{-1} [9].

It is important to realize that the maximum number of SA molecules detected at saturation coverage is approximately 600 per nanoparticle. At 1 pM SA concentration, a maximum of 60 SA molecules per nanoparticle is being detected. Previously, it has been demonstrated that the macroscopic measurements (12 mm^2 spot size) are equivalent to

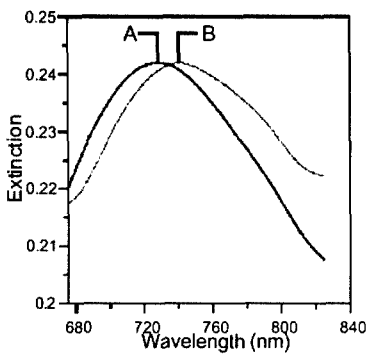

Figure 2. UV-vis extinction spectra of Ag nanoparticles before and after modification with 10 pM SA in a N_2 environment. (A) Ag nanoparticles after modification with 1 mM biotin, λ_{max} = 727.5 nm. (B) Ag nanoparticles after modification with 10 pM SA, λ_{max} = 742.8 nm.

Table 1. SA concentration vs LSPR shift (SA-biotin) obtained for Ag nanoparticles in a N_2 environment.

[SA] (M)	LSPR Shift (nm)
$1x10^{-6}$	23.9
$1x10^{-7}$	26.5
$1x10^{-9}$	26.4
$1x10^{-10}$	26.5
$1x10^{-11}$	15.3
$1x10^{-12}$	3.8
$1x10^{-13}$	0.6
$1x10^{-14}$	0.1
$1x10^{-15}$	0.3

microscopic measurements (12 μm^2 spot size) [19]. Based on the previous result and the principle that the individual nanoparticles act independently, the extinction spectrum of an array of nanoparticles is equivalent to the extinction spectrum of one nanoparticle. As the experiments near the 1 nanoparticle limit, a reasonable extrapolation of the data indicates yoctomole sensitivity.

Additionally, control experiments were performed to verify that the induced LSPR shifts are a result of SA binding to a biotinylated surface [18]. To test nonspecific protein interactions with a biotinylated Ag nanoparticle surface, BSA interactions with the Ag nanoparticle surface were tested (Figure 3). Ag nanoparticles were functionalized with a mixed monolayer of 11-MUA/1-OT. Next, biotin was covalently attached using EDC coupling. The biotinylated LSPR nanosensor is now ready for nonspecific binding tests. For this study, $1.5x10^{-5}$ M BSA was exposed to the biotinylated surface. A 0.6 nm LSPR shift was detected from this exposure. Because this shift is insignificant (i.e. less than the detection limit of the system) [18], this suggests that there is no non-specific binding occurring on the nanoparticle biosensor.

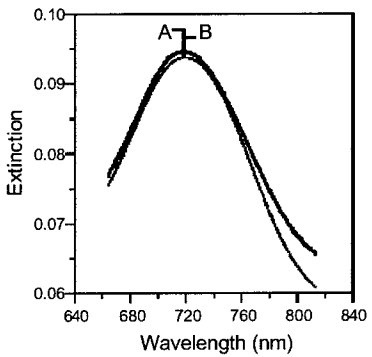

Figure 3. UV-vis extinction nonspecific binding measurements for Ag nanoparticles before and after modification with BSA. Ag nanoparticles (A) after modification with 1 mM biotin, λ_{max} = 718.3 nm and (B) after modification with $1.5x10^{-5}$ M BSA, λ_{max} = 718.9 nm.

CONCLUSION

In conclusion, Ag nanoparticles were fabricated using the technique of NSL. Using the model system of biotin-SA, several exciting features of this novel nanoparticle-based biosensor was demonstrated: 1) the extinction maximum, λ_{max}, of the LSPR shifts +26.5 nm for a complete monolayer coverage of streptavidin and shift +15.3 nm for 10 pM SA; 2) the limit of detection of this ultrasensitive nanoparticle based system is 1 pM streptavidin; and 3) a nonspecific binding tests using BSA yielded desirable results. Current work is aimed at studying systems with weaker binding constants than the biotin-SA system. All in all, a new class of nanobiosensors is being developed using triangular Ag nanoparticles modified with biomolecules. These nanosensors work by detecting shifts in the LSPR induced by changes in the local refractive index caused by analyte binding events. We anticipate the findings reported here will have a significant impact on biological and chemical sensing technologies.

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