pH Dependent Change in the Optical Properties of Surface Modified Gold Nanoparticles Using Bovine Serum Albumin

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ABSTRACT

The effect of coatings of Bovine SerumAlbumin (BSA) on the optical properties and reactivity of surface-modified gold nanoparticles in different pH buffer solutions is presented. Aggregation of uncoated gold nanoparticles is seen in all pH buffer solutions. The presence of these aggregations is observed in the absorption spectra by the appearance of a second, red-shifting peak in the spectra and is confirmed in transmission electron micrographs. Coating of individual gold nanoparticles with BSA prevents the aggregation of nanoparticles in solutions with a pH greater than 5. It is concluded, based upon analysis of visible absorption spectra of the coated and uncoated gold nanoparticles, that the BSA coating on the gold nanoparticles also causes a decrease in the scattering of light in lower pH buffer solutions. Analysis of Mie's theory on the optical extinction of light by metal particles supports the interpretation of the spectra.

Keywords: gold, nanoparticle, aggregation, surface plasmon resonance, BSA

1 INTRODUCTION

Gold nanoparticles have been studied with great interest because of its unusual optical properties [1]. According to Mie, who was the first to theoretically explained those properties in the metal colloid solution [2], spherical metal colloids absorb certain wavelengths via a phenomenon known as surface plasmon resonance (SPR). The excitation wavelength of a surface plasmon wave is a function of the size of the particles and the dielectric constants of both the metal and the media surrounding the metallic particles. By monitoring the intensity and position of the SPR peaks in the absorption spectra of gold nanoparticles attached with certain functional molecules, useful information about the interaction between the molecules and other substance in the solution can be acquired. This phenomenon can be used in many advanced bio/chemical sensors [3-5]

Aggregation of gold nanoparticles is a common concern in biosensor applications [6-8] because the SPR signal can not reflect the interaction that happens on the surface of the particles correctly [9]. If used in live cells, large clusters of gold nanoparticles may not be uptaken as easily as individual particles and may selectively collect near the cell membrane. Surface modification of the gold nanoparticles has been used to eliminate aggregation [10-13]. In *in-vivo* biosensing applications, the coating must be biocompatible and robust over the range of intracellular pH, yet sufficiently thin to enable the intracellular fluid to influence the SPR signal of the gold nanoparticles. In this paper, studies are concentrated on optical properties of Bovine Serum Albumin (BSA)-coated gold nanoparticles in different pH environments. Investigation shows that there are significant differences in the absorption spectra of BSA-coated and uncoated gold nanoparticles in solution. Transmission electron microscopy (TEM) micrographs verify the conclusions drawn from these spectra. Because of the biocompatibility of BSA, the surface modification of the gold nanoparticles with BSA may be useful in the design of a live cell biosensor, which can monitor, for example, fluctuations in the intracellular pH in response to biochemical stimuli.

2 EXPERIMENTAL PROCEDURE

Gold nanoparticles with extremely uniform diameter of 20 nm (Figure 1), synthesized according to the method developed by Beesley [14], are purchased from ICN Biomedicals. 0.1 wt % of gold in solution translates to 10^{12} nanoparticles per milliliter. BSA stock is purchased from Sigma and then diluted with nanopure water to a 0.1mmol/L solution. Several different pH buffer solutions are made of phosphate buffer. The solutions are pH 4, 5, 6, 7, and 8, which covers the pH range expected in a live cell biosensor under development by our group. UV-Vis spectra are acquired with Beckman DU640 spectrophotometer. All spectra presented are normalized using Origin (7th edition). No attempt is made to calibrate the intensities of the spectra.

Carbon thin film-coated TEM grids are prepared by drying a small droplet of the solution to be examined in open air. The TEM imaging is performed with Philips EM420T transmission electron microscope. Micro-pipettes are utilized through the experiment to ensure that all of the solutions are accurately measured to within a few µl ranges.

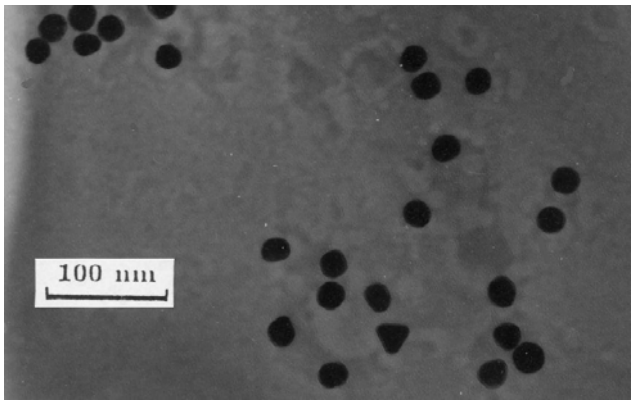

Figure 1: Transmission electron micrograph of uncoated gold nanoparticles on carbon n film coated copper grid.

3 RESULTS AND DISUSSION

2mL of each pH (4, 5, 6, 7, and 8) buffer solution is mixed with 0.25mL of the uncoated gold nanoparticles solution in a disposable cuvette at room temperature. The buffer-nanoparticle solution is then immediately placed in the UV-Vis spectrophotometer and spectra are collected between 450 nm and 750 nm every 10 minutes (each scan takes approximately one minute to collect). The first maximum (around 528 nm) in absorption spectra (Figure 2) is the SPR signal, characteristic of 20 nm gold nanoparticles [15]. The second red-shifting peak is indicative of aggregation of uncoated gold nanoparticles [13, 16]. It is clear that the aggregation of the gold particles happens more slowly with increasing solution pH.

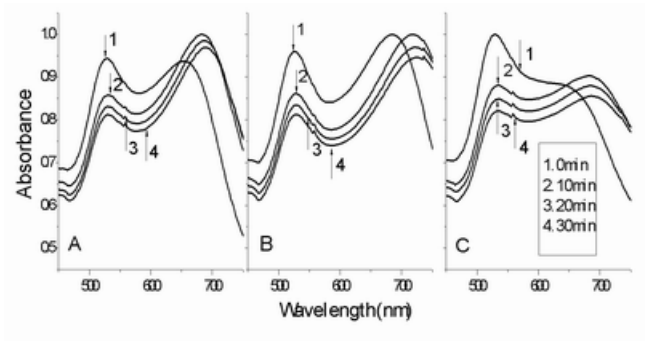

Figure 2: Optical absorption spectra as a function of time of 0.25 mL of gold nanoparticles solution following the addition of 2 mL of pH buffer. A. pH4 B. pH6 C. pH8

In original solution, gold particles are electrostatic stabilized with an electrical double layer on individual particles [17]. Upon mixing the nanoparticles solution with the different pH buffer solutions, the double layer is destroyed as the buffer alters the charge balance in the solution and, thus, aggregation forms.

Figure 3 presents the optical absorption spectra of both uncoated and BSA-coated gold nanoparticles as a function of time. The coating process happens immediately upon mixing the BSA stock and gold colloid solution (1:1 volume ratio). There is a minor change in the spectra of the BSA-coated gold nanoparticles in the first five minutes, after which the absorption spectrum of the mixture is quite stable. The significant increase and the slight red shift of the SPR peak of the BSA-coated nanoparticles as compared to the uncoated particles can be interpreted by Mie's theory.

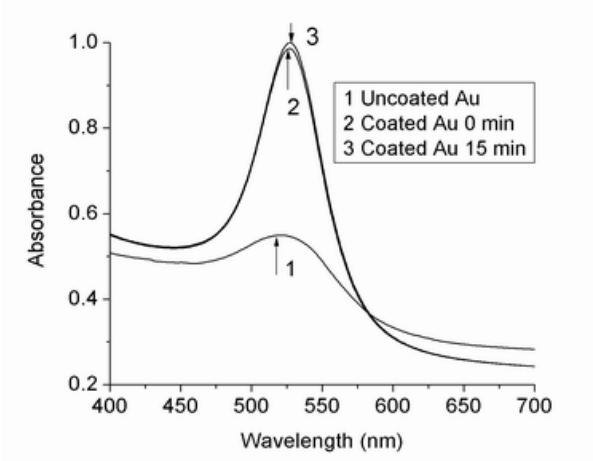

Figure 3: (1) Absorption spectra of uncoated gold particles (2) Same solution upon mixed with BSA stock (3) 15 minutes afterwards

The measured attenuation of light of intensity, I_0, passing through a colloid solution with N particles per unit volume over a light path, d cm, is defined as:

$$A = \log_{10} I_0 / I_d = NC_{ext}\, d/2.303 \qquad (1)$$

where C_{ext} is the extinction cross section of a single particle. For spherical particles embedded in a media of dielectric function ε_m, C_{ext} is equal to

$$C_{ext} = 2\pi/k^2 \sum (2n+1)\,\mathrm{Re}(a_n + b_n) \qquad (2)$$

where $k = 2\pi\sqrt{\varepsilon_m}/\lambda$ and coefficients a_n and b_n are spherical Bessel functions of complex dielectric constant $(\varepsilon = \varepsilon' + i\varepsilon'')$ of colloid metal, which describe the contribution of n^{th} electric and magnetic partial oscillation, respectively.

For particles whose size is far less than the wavelength of light, only the first term, the electric dipole, plays a significant role. Then equation (2) can be simplified to

$$C_{ext} = \frac{24\pi^2 R^3 \varepsilon_m^{3/2}}{\lambda} \frac{\varepsilon''}{(\varepsilon' + 2\varepsilon_m)^2 + \varepsilon''^2} \quad (3)$$

where R is the radius of the particle. As the dielectric constant of water (1.33) is little less than that of BSA (1.37), the red-shifted and more intense peak of absorption spectra of BSA-coated gold particles is expected compared to uncoated gold particles.

0.5mL (0.25mL of colloid gold and 0.25mL of BSA) of the coated gold nanoparticles solution is added to 2mL of each of the pH buffer solution. The optical absorption spectra are presented in Figure 4. A red shift of the surface plasmon resonance along with a reduction in the intensity of the resonance is observed in lower pH (pH 4 and 5) buffers. The larger the red shift and the larger the magnitude of the decrease in the maximum of the spectra indicate a higher degree of aggregation of gold particles occurs in the solution [13, 16] (as seen in Figure 5A and 5B). The color of these mixture solutions gradually changes from pink to clear in the first few minutes after mixing, again indicative of less absorption in the visible spectrum. At higher pH value (pH 6, 7 and 8) solutions, the peak of the resonance remains constant with respect to time, up to several days, as does the color of the solution.

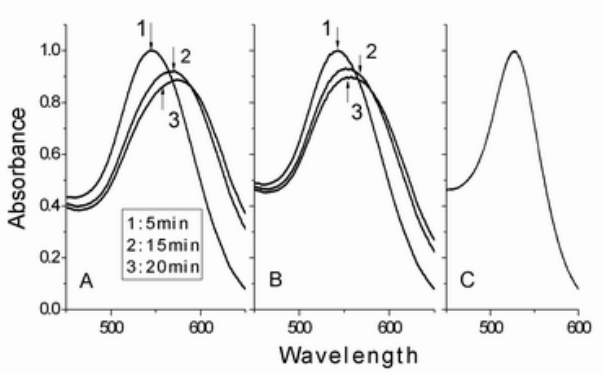

Figure 4: Optical absorption spectra of 0.5 mL of BSA-coated gold nanoparticles solution mixed with 2 mL of pH buffer as a function of time. Spectra of pH 6, 7 and 8 are identical A. pH4 B. pH5 C. pH6, 7 and 8

The isoelectric point of BSA is between 4.5-4.9 [18-20]. Therefore, in the buffer solution with pH value greater than 5, the BSA molecule has a significant number of negative ions attached on the surface, which forms the attractive columbic interaction between the protein and the gold nanoparticles; yet repels similarly BSA-coated gold nanoparticles. Thus, the BSA molecules prevent the gold nanoparticles from interacting with one another to form aggregates.

It should be noted that, although the aggregation of gold still occurs in pH 4 and 5 buffer solutions, the spectra of the BSA-coated gold nanoparticles is not similar to that of the uncoated gold nanoparticles. The second maximum in absorption spectra of uncoated gold particles solution (Figure 2) does not appear in the spectra of the coated particles. We believe this is, in part, due to surface plasmon enhancement of the hyper-Rayleigh scattering (HRS). HRS has been observed to increase by at least a factor of 10 when the physical dimensions of the scatterer is altered from a symmetric to an asymmetric scattering center while there is minimal change in the Rayleigh scattering [21, 22]. As the BSA proteins are likely to attach randomly on the gold nanoparticles and as aggregation of the gold nanoparticles occurs, the symmetry of isolated gold nanoparticles is lost and HRS at the surface plasmon resonance frequency increases. Given the normalization technique used in this study, this will also lead to a decrease in the magnitude of the optical absorption calculated at other wavelengths. An additional effect may be due to a "lens effect" introduced by the BSA coating. Since it has slightly higher index of refraction than water, the thin layer of BSA on the gold nanoparticles will bend the incoming light towards the particles. This increases the optical cross-section of an individual gold nanoparticle, which would increase the number of photons interacting with the individual Au nanoparticle, resulting in a larger surface plasmon wave being excited at 528nm.

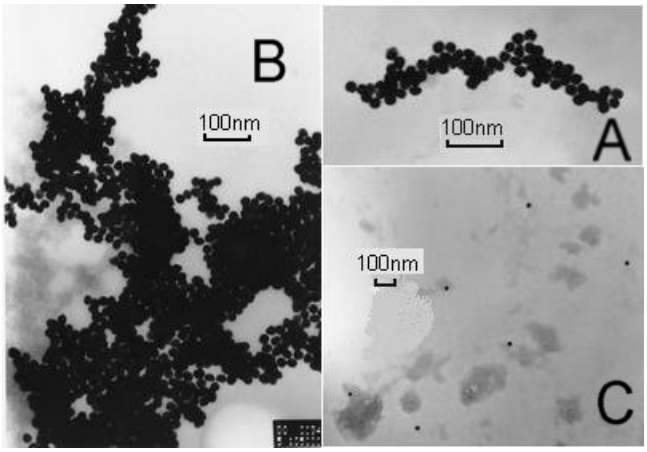

Figure 5: A. BSA-coated gold particle aggregation is observed immediately after mixing with pH 5 buffer; B. Much larger clusters appear in the same pH 5 solution 20 minutes later; C. No aggregation of BSA-coated gold nanoparticles is observed in pH 8 buffer

In order to determine the effect of the concentration of protein on the aggregation of gold particles, two additional mixtures are made. 0.025mL and 0.125mL of 0.1mmol/L BSA stock are mixed with 0.25mL of the gold colloid solution, respectively, prior to the addition of 2mL of pH 4 buffer to the solutions. The peaks of optical absorption spectra (Figure 6) of both solutions are compared to that observed for a solution containing 0.25mL of the gold colloid solution mixed with 0.25mL of the 0.1mmol/L BSA stock and 2mL of pH 4 buffer (Figure 4A). Since the

aggregation happens as soon as the coated gold particles solution is introduced to pH 4 buffers, the peak wavelength of each curve is different from each other even at time equals to zero. Based upon the magnitude of the shift in the wavelength peak of the spectra as a function of time, it is observed that the few molecules of BSA introduced into the solution, the faster the aggregation of the gold nanoparticles takes place.

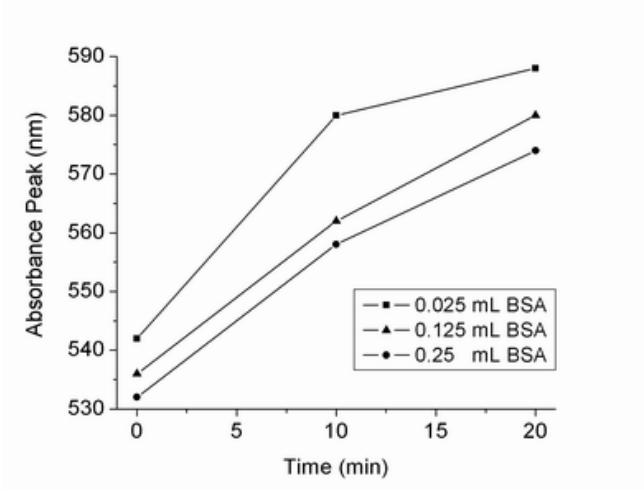

Figure 6: The peak of optical absorption spectra of 0.25mL of the gold particles coated with different amount BSA in 2mL of pH 4 buffers

In conclusion, the pH dependent optical properties of gold nanoparticles coated with BSA are quite different from the uncoated particles. The BSA coating prevents the gold nanoparticles from aggregating in solutions of pH greater than 5 and, thus, eliminating the time-varying red-shifting peak observed in the spectra of the uncoated gold nanoparticles. The absorption peak of the aggregated BSA-coated gold nanoparticles associated with the excitation of the surface plasmons is enhanced and the optical scattering at longer wavelengths by the aggregates is not observed at lower pH value solutions. The present study demonstrates that the BSA-coated gold nanoparticles may be used in biosensors to reduce the aggregation of the gold nanoparticles, which can prevent the uptake of these nanoparticles in live cells and reduce their applicability as bioprobes. More research is needed to identify the role of BSA coating at lower pH level in the aggregation of the nanoparticles. Additional studies are needed to determine an optimal thickness for the BSA coating and to identify alternatives to BSA to prevent gold nanoparticle aggregation at pH lower than 5.

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