Korean J. Chem. Eng., **26**(1), 258-260 (2009)

SHORT COMMUNICATION

Applications of silver nanoplates as colorimetric indicators of pH-induced conformational changes in cytochrome c

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(*Received 21 April 2008 • accepted 5 July 2008*)

Abstract−Silver nanoplates (AgNPs) were used as a colorimetric indicator of pH-induced conformational changes in the protein, cytochorme c (cyt c). Cyt c was covalently bound to the surface of AgNPs, and UV-vis spectroscopy was used to identify changes in the optical properties of the resulting cyt c-AgNP conjugates in colloidal solution over pH, with values ranging from 3.1 to 10.6. Transmission electron microscopy (TEM) results indicated that the morphological changes in cyt c-AgNP conjugates were dependent on pH. Moreover, pH-induced conformational changes in cyt c could be detected by visual inspection of the color changes in cyt c-AgNPs, which occurred as a result of the coupling effect of localized surface plasmon resonance (LSPR) by aggregated AgNPs.

Key words: Silver Nanoparticle, Colorimetric, Cytochrome, pH

INTODUCTION

The use of noble metal nanoparticles as nanoscale optical biosensors has received significant attention since the Mirkin group reported assembling DNA-linked gold nanoparticles (AuNPs) [1]. This application arises from the unique nanoparticle optical properties of certain materials such as copper, silver and gold [2]. This optical excitation of the LSPR of copper, silver and gold results in a strong enhancement of the local electromagnetic fields at or near the nanoparticle surface [3]. These LSPR spectra are strongly dependent on their size, shape, interparticle spacing, and local dielectric environment [4]. AuNP-aggregates with interparticle distances substantially greater than the average particle diameter appear red, but as the interparticle distances in these aggregates decrease to less than the average particle diameter, the color becomes blue [5]. The Zare group reported a colorimetric sensor for protein conformational changes using the optical properties of AuNP aggregates. The cyt c was bound on the surface of AuNPs to detect the conformational change of protein *in situ* [6]. A conformational change of proteins can be achieved in various conditions, such as high temperature, high concentration of denaturants, high pH, or the addition of surfactants [7]. The ability to detect and track conformational change in real time is essential to understanding the dynamic relationship between structure and function in biological molecules [8]. Cyt c is one of the excellent models for investigating conformational change of pH-dependent protein [9]. In addition, Cyt c has a thiol group from a single cysteine amino acid, and this feature can be used to form a self-assembled monolayer (SAM) of cyt c at Ag surface without another treatment [10]. In this work, we suggest a simple colorimetric system to gauge the pH-induced conformational change of cyt c using silver nanoplates (AgNPs) in real time. AgNPs with a nanodisc shape were fabricated to use as efficient colorimetric sensors because the LSPR of AgNPs is stronger than that of AuNPs. It is also reported that alkanethiols on Ag nanoparticles produce a ca. 3.5 times larger LSPR shift than do AuNPs [3].

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EXPERIMENTAL SECTION

1. Synthesis of AgNPs

AgNPs were prepared by a method proposed by Jiang et al. [11]. A 0.02 M aqueous $AgNO_3$ and sodium bis(2-ethylhexyl) sulfosuccinate (NaAOT) solution was added to DI water, followed by stirring for 10 min. A 0.1 M citrate acid and L-ascorbic acid aqueous solution were then added to the solution. Finally, a 0.002 M $NaBH_4$ aqueous solution was added to the above mixed solution. At this point, the color of the solution changed from light yellow to blue. The as-prepared solution was aged overnight for formation of platelet shape and stability of the colloidal solution under a fluorescent lamp. After aging, the color of the colloidal solution turned from blue to purple.

2. Conjugation of AgNPs and Cyt c

We mixed the AgNP solution and a 10-ppm cyt c (from horse heart) solution in PBS buffer (pH 7.0) at various ratios, from 1 : 5,000 to 1 : 1, to determine the optimum. The optimum ratio of cyt c and AgNPs was determined to be 1 : 1,000 cyt c to AgNP by measuring an absorption maximum peak change of cyt c-AgNP conjugates. After cyt c was mixed with the AgNP solution at this ratio, the pH of the solution was adjusted to 11.0, and the solution was aged overnight to allow full conjugation. After aging, the pH of the solution was adjusted from 3.1 to 10.6 by adding 1 M HCl solution. The absorption spectra of each sample were measured by UV-vis absorption spectroscopy (K-mac, Korea) and the morphological changes of cyt c-AgNP conjugates were identified by TEM (JEM 1010, Japan). The size distribution of cyt c-AgNP conjugates at different pH was measured by electrophoretic light scattering spectrophotometer (ELS) (Otsuka Electronics, Japan).

RESULTS AND DISCUSSION

The ratio of 1 : 1,000 cyt c to AgNP, respectively, was selected as the optimum ratio, because the absorbance intensity of the band

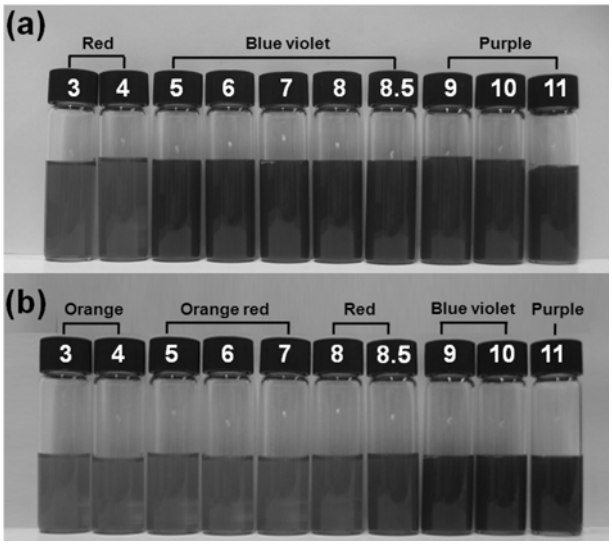

Fig. 1. Color change of (a) bare AgNPs colloidal solution, and (b) cyt c coated AgNPs solution with pH ranging from 3 to 11.

centering around 570±5 nm, attributed to the surface plasmon resonance of AgNP, was maintained at its maximum at this ratio. Before mixing the AgNP colloidal solution and the cyt c, the pH of the AgNP solution was adjusted to 11.0 to prevent acidic denaturation of the cyt c, due to the resulting thiol-silver bond release of protons; Cyt c-SH+Ag→Cyt c-SAg+H^+ [2]. The cyt c-AgNP conjugates were aged overnight and then adjusted to various pH values, from 3.1 to 10.6, by adding the HCl solution. The color of cyt c-AgNP conjugates changed at low pH as soon as the HCl solution was added and the solution began to violently vortex.

The pH effect on the cyt c-AgNP conjugates can be identified by the naked eye, as shown in Fig. 1. The color of the bare AgNP solution was changed at approximately pH 4 from purple to red (Fig. 1a), and the color of the cyt c-AgNP conjugates was changed at approximately pH 8 from purple to red. At approximately pH 4, the color changed to orange (Fig. 1b). The spectroscopic measurement of the colors of the solutions was conducted by UV/VIS absorption spectroscopy and the results are shown in Fig. 2. Absorbance of bare AgNP solution had a maximum peak at 569 nm at pH 10.5. As pH decreased, the absorption maximum of the bare AgNP solution shifted gradually to 458 nm. However, the absorption maximum of cyt c-AgNP conjugates was dramatically changed from 575 to 450 nm, as shown in Fig. 2. This sharp optical change was due to the pH-induced conformational change of the cyt c absorbed on the AgNPs. The redox properties of cyt c are pH-sensitive. When cyt c solution is exposed to high pH, cyt c undergoes conformational transitions accompanying the substitution of an axial methionine ligand (Met 80) of heme iron [12] in the range of pH 7-12. Santucci et al. have ascribed the weakness of a Met 80-Fe(III) bond caused by denatured acid to the conditions employed. In neutral pH, the Met 80-Fe(III) bond is strengthened because the hydrogen bonds between loops decreases their mobility. In addition, in an acidic environment, this condition cannot be satisfied because the high proton concentration will leave the protein with a high positive charge [13].

Fig. 3 shows the absorption maximum peak changes of bare AgNPs

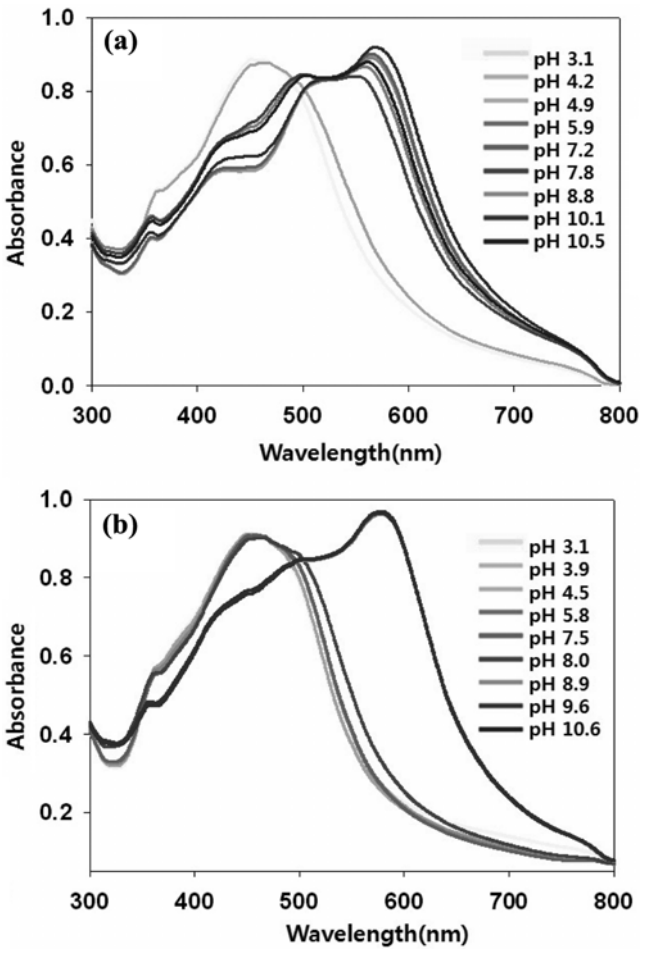

Fig. 2. UV-vis absorbance spectra of (a) bare AgNPs solution and (b) cyt c-AgNPs conjugates in the range of pH 3.1-10.6

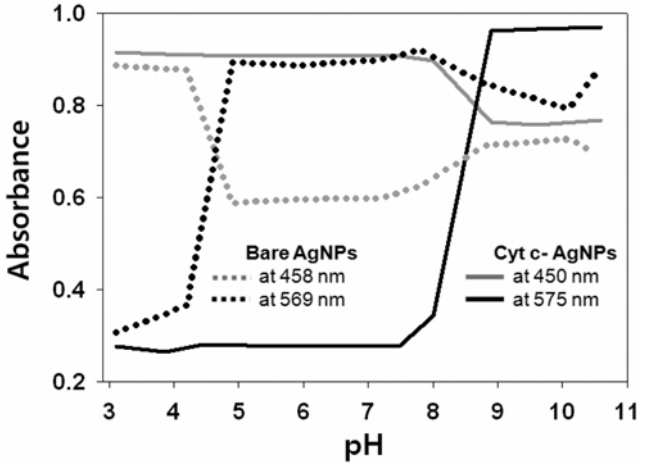

Fig. 3. Comparison of the pH-induced absorption change for bare AgNPs (dotted line) and cyt c-AgNP conjugates (bold line) at each maximum peak.

and cyt c-AgNP conjugates over a pH range from 3 to 11. The maximum peak for absorption of bare AgNPs changed at around pH 4. However, cyt c-AgNP conjugates changed in the range of pH 8-9. Increasing the pH from 8.5 to 9.0 induces a progressive loss of redox

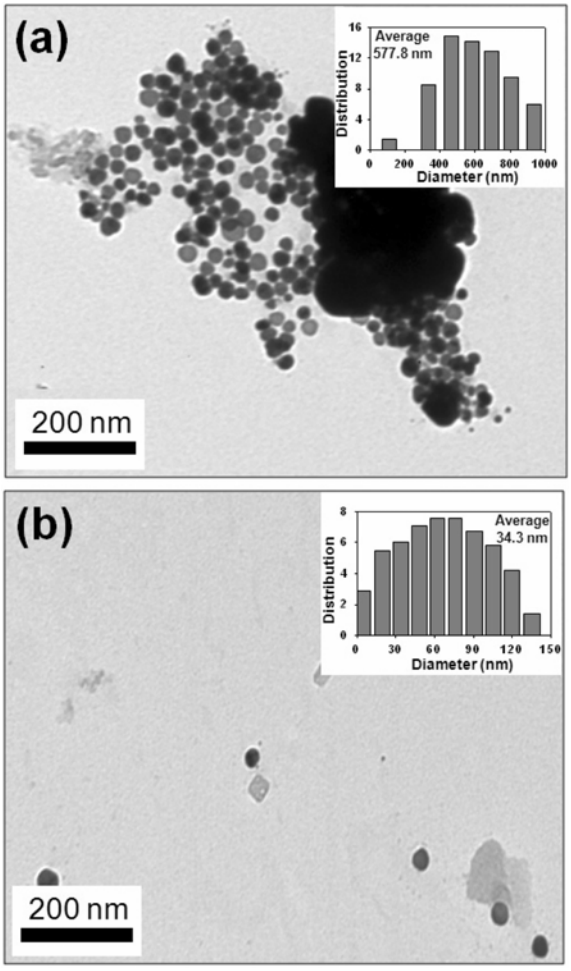

Fig. 4. TEM images of cyt c-AgNPs conjugates (a) at pH 4 (b) at pH 10.6. The inset is size distribution.

activity of cyt c, as reported by Desideri and co-workers [14]. This sharp change of optical absorbance can make cyt c-AgNP conjugates suitable for use as a sensitive pH colorimetric sensor.

Further evidence of the conformational change came from the TEM images of conjugates at pH 4 and pH 10.6. The images of cyt c-AgNP conjugates at pH 4 show large assembled networks of Ag colloids (Fig. 4a). On the other hand, the cyt c-AgNP conjugates at pH 10.6 did not aggregate and maintained a monodispersed state (Fig. 4b). ELS data represented the morphological change of cyt c-AgNPs at different pH. As shown in inset of Fig. 4, average size distribution at pH 4 and 10.6 was 577.8 and 34.3 nm, respectively. As a function of pH-induced conformational change of cyt c to the short-distance coupling of surface plasmons, color changes of cyt c-AgNP conjugation are induced. The interparticle distance of the colloids in the aggregated state approached the colloidal radius that results in a blue shift in the absorbance spectrum, and the cyt c-AgNP conjugation solution consequently appears orange [15]. Deng et al. reported a simple process to achieve pH-switched reversible aggregation and dispersion of gold nanoparticles by controlling the pH of a solution involving ethylenediamine [16]. Hydrogen bonding and electrostatic interactions are common driving forces that account for the reversible aggregation of nanoparticles in this report. Bare AuNP maintain stability regardless of the pH, whereas the AgNP show instability by increasing the amount of H^+ at low pH [17]. The difference of stability of bare nanoparticles on pH would makes AuNP-protein and AgNP-protein conjugates different, such as color change in the different pH range.

CONCLUSION

We report here on a color change of cyt c-AgNP conjugates by the pH-induced conformational change of cyt c. The color of cyt c-AgNP conjugates was changed from purple to red rapidly over a pH range from 8 to 9. The pH-induced denaturant of absorbed cyt c on the Ag surface resulted in decreased interparticle distances that were concurrent with a change in color, and the aggregates of cyt c-AgNP conjugates that induce a coupling of the localized surface plasmon resonance of AgNPs, may influence the color change. These results suggest a possibility for the use of cytochrome c coated silver nanoplates as colorimetric pH sensors.

ACKNOWLEDGMENT

This work was supported by a research grant of the Seoul R&BD Program of Seoul City and research grant of Kwangwoon University in 2008.

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