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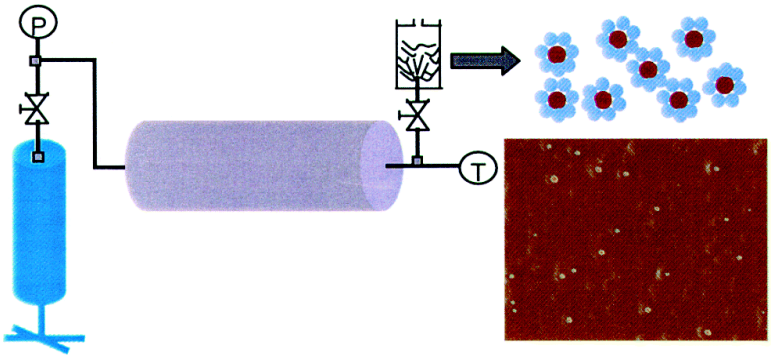

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Protein-Conjugated Nanoparticles from Rapid Expansion of Supercritical Fluid Solution into Aqueous Solution

Mohammed J. Meziani and Ya-Ping Sun*

Contribution from the Department of Chemistry and Center for Advanced Engineering Fibers and Films, Howard L. Hunter Chemistry Laboratory, Clemson University, Clemson, South Carolina 29634-0973

Received February 11, 2003; E-mail: syaping@clemson.edu

Abstract: The method of rapid expansion of a supercritical solution into a liquid solvent (RESOLV) was applied to the preparation of bovine serum albumin protein-conjugated silver sulfide nanoparticles. The conjugate samples were characterized by using a series of instrumental techniques. The results show that the monodispersed nanoparticles in the conjugates are well-coated directly with the protein. Because the protein undergoes solution pH-dependent association and dissociation, the protein-nanoparticle conjugates also assemble and disassemble with changes in solution pH in a reversible fashion.

Introduction

There have been increasing activities in the development of bioactive and biocompatible nanomaterials for a variety of applications. For example, inorganic nanocrystals and nanoparticles are bioconjugated with the attachment of DNA,[1,2] peptides,[3] and proteins.[4] A typical route in the preparation of conjugates involves mixing biomolecules with modified nanoparticles in solution. The modification of nanoparticles prior to the mixing is usually chemical functionalization with a linker that recognizes the biomolecules and also stabilizes the nanoparticles to avoid their uncontrolled growth or agglomeration.[5] The direct conjugation of biological species to inorganic nanoparticles has not been a common practice despite some obvious advantages. This is probably a result of generally incompatible experimental conditions required for biological species and for the formation and stabilization of nanoparticles. In our laboratory, we have developed a method for preparing metal and semiconductor nanoparticles based on the Rapid Expansion of a supercritical solution into a liquid SOLVent (RESOLV), which has generally produced narrowly distributed nanoparticles.[6−8] A unique feature of the method is that it requires no nanoscale templating agents such as surfactants because the templating effect is provided by the supercritical fluid rapid expansion process.[6,9] Thus, it offers a clean way to directly couple inorganic nanoparticles with biological species.[8] Here, we report the preparation of bovine serum albumin (BSA) protein-conjugated silver sulfide (Ag_2S) nanoparticles via RESOLV. The results show that the conjugates are monodispersed nanoparticles well-capped with the protein.

Experimental Section

Materials. Silver nitrate ($AgNO_3$, >99.9%) and sodium sulfide (Na_2S, >99.9%) were purchased from Aldrich. Bovine serum albumin (BSA) protein was obtained from Sigma. Anhydrous ammonia (> 99.9999%) was supplied by Air Products. Absolute methanol was obtained from Fisher Scientific. It was distilled over molecular sieves and then filtered before use. Water was deionized and purified by being passed through a Labconco WaterPros water purification system. The membrane tubing for dialysis was supplied by Spectrum.

Measurements. UV/vis absorption spectra were recorded on a Shimadzu UV-3100 spectrophotometer. X-ray powder diffraction measurements were carried out on a Scintag XDS-2000 powder diffraction system. Transmission electron microscopy (TEM) images were obtained on a Hitachi 7000 transmission electron microscope. Atomic force microscopy (AFM) analysis was conducted on a Digital Instruments Nanoscope IIIa multimode microscope equipped with an "E"-type piezoscanner (13×13 μm maximum scan range) and a TESP silicon single-crystal cantilever (125 μm length).

(1) (a) Coffer, J. L.; Bigham, S. R.; Li, X.; Pinizzotto, R. F.; Rho, Y. G.; Pirtle, R. M.; Pirtle, I. L. *Appl. Phys. Lett.* **1996**, *69*, 3851.
(2) (a) Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849. (b) Mirkin, C. A. *Inorg. Chem.* **2000**, *39*, 2258.
(3) (a) Dameron, C. T.; Reese, R. N.; Mehra, R. K.; Kortan, A. R.; Carroll, P. J.; Steigerwald, M. L.; Brus, L. E.; Winge, D. R. *Nature* **1989**, *338*, 596. (b) Whaley, S. R.; English, D. S.; Hu, E. L.; Barbara, P. F.; Belcher, A. M. *Nature* **2000**, *405*, 665.
(4) (a) Kreuter, J. In *Microcapsules and Nanoparticules in Medicine and Pharmacy*; Donbrow, M., Ed.; CRC: Boca Raton, 1992. (b) Hermanson, G. T. In *Bioconjugate Techniques*; Academic Press: New York, 1996. (c) Chan, W. C. W.; Nie, S. *Science* **1998**, *281*, 2016. (d) Douglas, T.; Young, M. *Nature* **1998**, *393*, 152. (e) Gestwicki, J. E.; Strong, L. E.; Kiessling, L. L. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 4567. (f) Mamedova, N. N.; Kotov, N. A.; Rogach, A. L.; Studer, J. *Nano Lett.* **2001**, *1*, 281.
(5) Niemeyer, C. M. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 4128.
(6) Sun, Y.-P.; Rollins, H. W.; Jayasundera, B.; Meziani, M. J.; Bunker, C. E. In *Supercritical Fluid Technology in Materials Science and Engineering: Synthesis, Properties, and Applications*; Sun, Y.-P., Ed.; Marcel Dekker: New York, 2002; p 491.
(7) (a) Sun, Y.-P.; Rollins, H. W. *Chem. Phys. Lett.* **1998**, *288*, 585. (b) Sun, Y.-P.; Rollins, H. W.; Guduru, R. *Chem. Mater.* **1999**, *11*, 7. (c) Sun, Y.-P.; Riggs, J. E.; Rollins, H. W.; Guduru, R. *J. Phys. Chem. B* **1999**, *103*, 77. (d) Sun, Y.-P.; Guduru, R.; Lin, F.; Whiteside, T. *Ind. Eng. Chem. Res.* **2000**, *39*, 4663. (e) Sun, Y.-P.; Atorngitjawat, P.; Meziani, M. J. *Langmuir* **2001**, *17*, 5707.
(8) Meziani, M. J.; Rollins, H. W.; Allard, L. F.; Sun, Y.-P. *J. Phys. Chem. B* **2002**, *106*, 11 178.
(9) RESOLV is a modification to the conventional rapid expansion of supercritical solution (RESS) method widely used in the production of micron-size (submicron-size, in some cases) particles. The Asolute droplets@ formed in the rapid expansion probably serve as templates for the particles. For the RESS method, see: (a) Eckert, C. A.; Knutson, B. L.; Debenedetti, P. G. *Nature* **1996**, *383*, 313. (b) Matson, D. W.; Norton, K. A.; Smith, R. D. *Chemtech.* **1989**, *19*, 480.

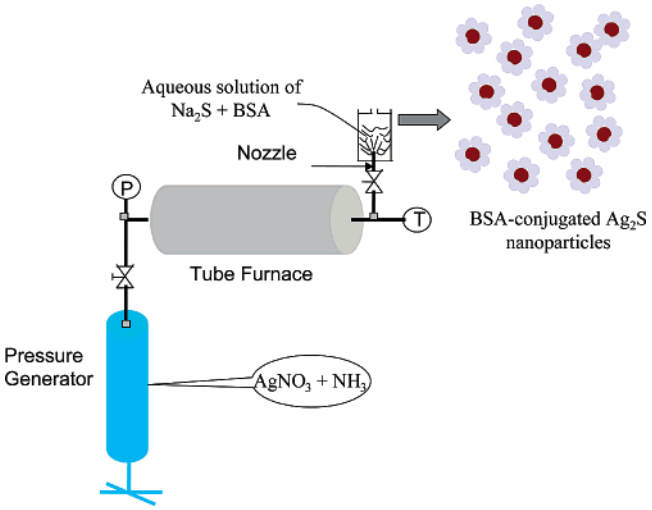


Figure 1. Experimental setup for preparing BSA-conjugated Ag_2S nanoparticles via RESOLV.

The RESOLV apparatus for the preparation of protein-conjugated Ag_2S nanoparticles is illustrated in Figure 1. It consists of a syringe pump (60 mL capacity) for pressure generation and pressure maintenance during the rapid expansion process and a gauge for monitoring the system pressure. The heating unit consists of a cylindrical solid copper block of high heat capacity in a tube furnace. The copper block is wrapped with stainless steel tubing to ensure a close contact between the tubing coil and the copper block for efficient heat transfer. The copper block/tubing coil assembly is preheated to a set temperature before each rapid expansion experiment. The expansion nozzle is a fused silica capillary hosted in a stainless steel tubing, which is inserted into a chamber containing the room-temperature receiving solution.

In a typical experiment for BSA protein-conjugated Ag_2S nanoparticles, a solution of $AgNO_3$ in methanol (0.15 M, 0.5 mL) was added to the syringe pump, followed by the evaporation of the solvent methanol. The syringe pump was then filled with liquid ammonia. When pumped through the heating unit, the ammonia solution of $AgNO_3$ was heated and equilibrated at 160 °C before reaching the expansion nozzle. The supercritical solution was rapidly expanded via a 50-microns fused silica capillary nozzle into an ambient aqueous Na_2S solution (1.3 mg/mL, 20 mL). The system pressure was maintained at 4000 psia during the rapid expansion. The aqueous receiving solution also contains BSA protein (5 mg/mL) for conjugating with the Ag_2S nanoparticles produced in the rapid expansion process. After the rapid expansion, the ambient suspension of nanoparticles was transferred to a membrane tubing (cutoff molecular weight 100 000) and dialyzed against water to remove excess BSA and other salts and reagents.

The modified Lowry procedure[10] was used to determine the total protein content in the BSA-nanoparticle conjugate sample. In a typical experiment, a dilute sample solution in sodium chloride buffer was prepared. To a small aliquot of the solution (0.2 mL) was added Biuret reagent (2.2 mL). After the solution was allowed to stand at room temperature for 10 min, Folin−Ciocalteu's phenol reagent (0.1 mL) was added. The resulting solution was kept at room temperature for another 30 min before the visible absorption spectrum was measured. The same experimental procedure was applied to pristine BSA to produce a solution for reference.

Results and Discussion

The RESOLV process resulted in the formation of Ag_2S nanoparticles in an aqueous suspension. The yellowish suspension containing BSA was stable, without any precipitation under ambient condition (at least within the 4-month observation period). The UV/vis absorption spectrum of the nanoparticle suspension is a structureless curve with gradually increasing absorbance toward shorter wavelengths (Figure 2). Since the band gap for bulk Ag_2S is ~1 eV (1,240 nm),[11] the observed absorption spectrum indicates that these nanoparticles are quantum confined.

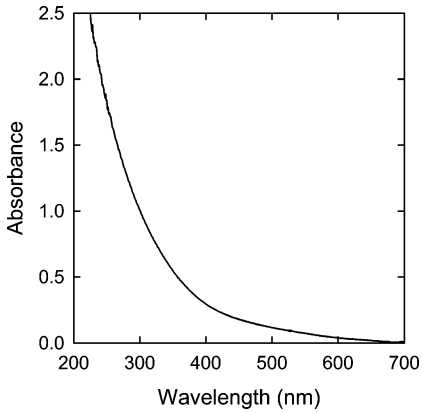


Figure 2. Absorption spectrum of the BSA-conjugated Ag_2S nanoparticles in an aqueous suspension (pH ≈ 7).

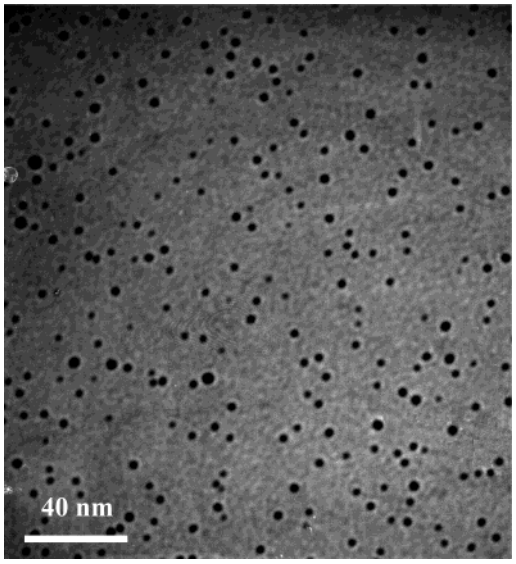


Figure 3. Typical TEM image of the BSA-conjugated Ag_2S nanoparticles prepared via RESOLV and then dialysis.

The X-ray powder diffraction analysis was used to identify the Ag_2S nanoparticles in the solid state. The observed diffraction pattern matches well with that of the bulk (monoclinic) Ag_2S in the JCPDS library. The diffraction peaks are broad, corresponding to an estimated average Ag_2S particle size of ~10 nm in terms of the Debye−Scherrer equation.[12] Transmission electron microscopy (TEM) imaging allowed a more direct observation of the nanoparticles. The TEM sample was prepared by depositing a few drops of the diluted nanoparticle suspension onto a carbon-coated copper grid. Shown in Figure 3 is a typical TEM image of the BSA-conjugated Ag_2S nanoparticles. These

(10) (a) Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. *J. Biol. Chem.* **1951**, *193*, 265. (b) Ohnishi, S. T.; Barr, J. K. *Anal. Biochem.* **1978**, *86*, 193. (b) Peterson, G. L. *Anal. Biochem.* **1979**, *100*, 201.

(11) (a) Junod, P.; Hediger, H.; Kilchör, B.; Wullschleger, J. *Philos. Mag.* **1977**, *36*, 941. (b) Motte, L.; Billoudet, F.; Pileni, M. P. *J. Phys. Chem. B* **1995**, *99*, 16 425.

(12) Klug, H. P.; Alexander, L. E. *X-ray Diffraction Procedures*; John Wiley & Sons: New York, 1959.

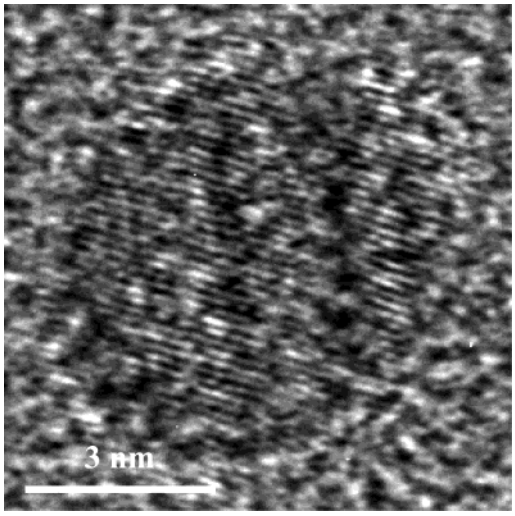


Figure 4. High-resolution TEM image of a typical BSA-conjugated Ag_2S nanoparticle (6 nm) prepared via RESOLV and then dialysis.

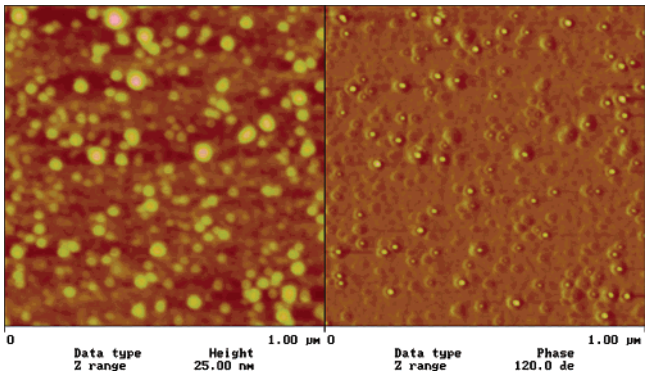


Figure 5. Height (left) and phase (right) images from the AFM analysis (on a mica substrate) of the BSA-conjugated Ag_2S nanoparticles prepared via RESOLV and then dialysis.

particles are obviously well dispersed. A statistical analysis of ~200 particles (by hand) yields an average particle size of 6.3 nm and a size distribution standard deviation of 1.6 nm. The high-resolution TEM image of a typical BSA-conjugated Ag_2S nanoparticle in Figure 4 suggests a single crystal. The interreticular distance is 0.26 nm, corresponding to (−121) lattice planes.

Atomic force microscopy (AFM) was used to look more closely at the BSA-nanoparticle conjugation. The analysis was conducted with the sample on a mica substrate. The height and phase images were recorded at a scan rate of 4 Hz and over a scan area of 1 μm^2. The images in Figure 5 show well-dispersed Ag_2S nanoparticles surrounded by soft materials, with the overall circular features of 20−30 nm in size. Apparently, the Ag_2S nanoparticles are uniformly coated with BSA in a core−shell like arrangement, with each nanoparticle seemingly immersed in a small pot of BSA protein (Figure 5). The particle sizes observed in the AFM analysis (~10 nm on average) are somewhat larger than those determined by TEM. The larger apparent particle sizes may be attributed to the tip convolution effect, which is a well-established scanning probe microscopy phenomenon.[13] Nevertheless, the AFM results provide strong evidence for the conclusion that well-dispersed Ag_2S nanoparticles directly conjugated (no need for stabilization and mediation by organic ligands) with protein species can be obtained via the RESOLV method. The preparation of the conjugates is insensitive to the BSA concentration over a relatively wide range.

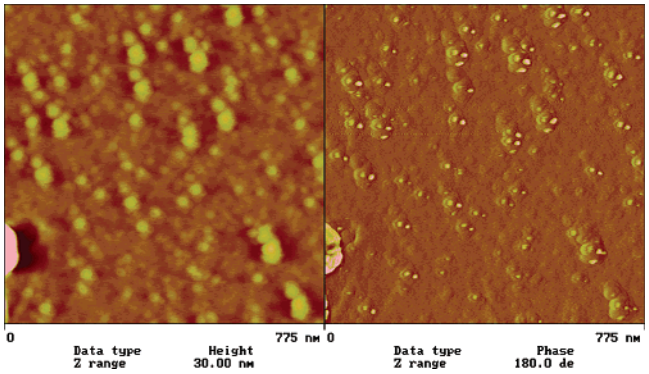


Figure 6. Height (left) and phase (right) images from the AFM analysis (on a mica substrate) of the BSA-conjugated Ag_2S nanoparticles from the as-prepared suspension (pH ≈ 12).

The BSA protein contains 60 amino moieties in lysine residues and 26 arginine moieties in guanidino side chains.[14] It also contains 17 disulfide bonds with one free thiol in cysteine residues,[14] which may be attracted to the Ag_2S nanoparticles for surface binding via thiolate linkages.[15] Results in the literature have also shown that alkylamines form weak covalent bonds with colloidal surfaces.[16] These binding interactions are probably responsible for the direct BSA-Ag_2S nanoparticle conjugation.

The BSA protein remained bioactive after the conjugation with the Ag_2S nanoparticles. This was based on results from the modified Lowry procedure, a commonly used total protein analysis method that measures the tryptophan and tyrosine contents in the analyte.[10] Thus, the BSA protein essentially plays dual roles of protecting the Ag_2S nanoparticles from agglomeration in a stable aqueous suspension and providing bioactive functionalities throughout the nanoparticle surface for further biological interactions or couplings (with antibodies, for example).

There are several isomeric conformations of BSA, corresponding to different solution pH values.[14] The BSA protein coating the well-dispersed Ag_2S nanoparticles (Figure 5) at neutral pH is predominantly in the native form. However, in the original suspension from RESOLV before dialysis, the pH value is close to 12 due to the expansion of ammonia. Under the high pH condition, BSA is expected to be in the aged form, in which the protein drastically expands and forms soluble aggregates through intermolecular disulfide exchanges.[14,17] The aggregation is reflected by the clustering of Ag_2S nanoparticles

(13) Waner, M. J.; Gilchrist, M.; Schindler, M.; Dantus, M. *J. Phys. Chem. B* **1998**, *102*, 1649, and references therein.

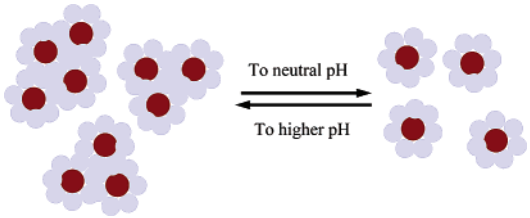
(14) (a) Foster, J. F. In *Albumin Structure, Function and Uses*; Rosenoer, V. M., Oratz, M., Rothschild, M. A., Ed.; Pergamon: Oxford, U.K., 1977; p 53. (b) Reed, R. G.; Putnam, F. W.; Peters, T., Jr. *Biochem. J.* **1980**, *191*, 867. (c) Hirayama, K.; Akashi, S.; Furuya, M.; Fukuhara, K. I. *Biochem. Biophys. Res. Commun.* **1990**, *173*, 639. (d) Adamczyk, M.; Gelber, J. C.; Mattingly, P. G. *Bioconjugate Chem.* **1996**, *7*, 475.

(15) (a) Sasaki, Y. C.; Yasuda, K.; Suzuki, Y.; Ishibashi, T.; Satoh, I.; Fujiki, Y.; Ishiwata, S. *Biophys. J.* **1997**, *72*, 1842. (b) Brelle, M. C.; Zhang, J. Z.; Nguyen, L.; Mehra, R. K. *J. Phys. Chem. A* **1999**, *103*, 10 194.

(16) (a) Leff, D. V.; Brandt, L.; Heath, J. R. *Langmuir* **1996**, *12*, 4723. (b) Keating, C. D.; Kovaleski, K. M.; Natan, M. J. *J. Phys. Chem. B* **1998**, *102*, 9404. (c) Keating, C. D.; Kovaleski, K. M.; Natan, M. J. *J. Phys. Chem. B* **1998**, *102*, 9414.

(17) Peters, T., Jr. In *All About Albumin. Biochemistry, Genetics, and Medical Applictions*; Academic Press: San Diego, 1995; p 22.

Scheme 1



in the as-prepared suspension. Such clustering is clearly demonstrated in the AFM images of the sample before dialysis (Figure 6). In these clusters, individual Ag_2S nanoparticles are still separated by protein species and protected from agglomeration into larger Ag_2S nanoparticles. Upon a reduction in pH via dialysis, the conversion of BSA into the more compact native form without aggregation is accompanied by the disassociation of the nanoparticle clusters (Scheme 1).

This pH dependent assembly and disassembly of the BSA-conjugated Ag_2S nanoparticles, driven by the isomeric conversion of the protein, is qualitatively reversible (Scheme 1). For example, when the neutral suspension of well-dispersed BSA-Ag_2S conjugates, from which results in Figures 3 and 5 were obtained, was basified to a high pH $\approx$ 12, there were significant aggregation of BSA protein and clustering of Ag_2S nanoparticles. The AFM images of the sample became similar to those of the as-prepared suspension before dialysis. In the isomeric conversion, BSA protein is relatively unharmed at high pH and can mostly return to its native configuration, thus making it possible to maintain the reversibility.

The same RESOLV method should be applicable to the preparation of other well-dispersed BSA-nanoparticle conjugates. For example, preliminary results show that BSA-conjugated cadmium sulfide nanoparticles can be similarly produced via RESOLV.

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