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Highly sensitive detection of bovine serum albumin based on the aggregation of triangular silver nanoplates

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ABSTRACT

A simple, fast and highly sensitive spectrophotometric method for the determination of bovine serum albumin (BSA) has been developed based on the interactions between triangular silver nanoplates (TAgNPs) and BSA in the presence of Britton–Robison buffer solution (BR). Particularly, the wavelength of absorption maximum (λ_{max}) of TAgNPs is red shifted in the presence of BSA together with Britton-Robinson buffer solution (BR, pH = 2.56), and the color of the solution changed from blue to light blue. This may be due to the interactions between BSA molecules on the surface of TAgNPs through electrostatic forces, hydrogen bonds, hydrophobic effects and van der Waals forces at pH 2.56, which leads to the aggregation of TAgNPs. The determination of BSA was achieved by measuring the change of λ_{max} corresponding to localized surface plasmon resonance (LSPR) from UV–visible spectrophotometry. It was found that the shift value in the wavelength of absorption maximum ($\Delta\lambda$, the difference in absorption maxima of the TAgNPs/BSA/BR mixture and the TAgNPs/BR mixture) was proportionate to the concentration of BSA in the range of 1.0 ng mL^{-1} to 100.0 ng mL^{-1} with the correlation coefficient of r = 0.9969. The detection limit (3 σ/k) for BSA was found to be as low as 0.5 ng mL^{-1}.

1. Introduction

Silver nanoparticles (AgNPs) have recently found a wide range of applications for analytical purposes due to their unique localized surface plasmon resonance (LSPR) optical properties. AgNPs with different sizes and shapes have different colors in the visible spectral range depending on their LSPR absorption [1]. Therefore, many research efforts are focused on preparing different shapes and sizes of AgNPs, such as nanorods, nanowires [2,3], nanoprisms [4], nanodisks/nanoplates [5], nanocubes [6], triangular silver nanoplates (TAgNPs) [7], etc. In particular, TAgNPs, which have a highly active silver atom situated on the three corners of the triangle, are well suited to analytical applications in several chemical, biochemical and biomedical fields [8,9]. For example, TAgNPs have been used for detecting metal ions (such as Hg^{2+}, Mn^{2+}, and Pb^{2+} ions) [10], inorganic anions (Cl^-, Br^-, I^-, $H_2PO_4^-$, and SCN^-) [11–13], and small organic molecules such as cysteine [14] in aqueous solutions. Furthermore, the LSPR absorption of TAgNPs produces unique colors varying from yellow to blue, which makes TAgNPs useful as potential chromogenic probes in colorimetric sensing [14]. To date however, TAgNPs have not been used in protein analysis.

Protein is an important constituent of the cell, which plays a vital role in biological processes. The quantitative analysis of protein has practical significance in the fields of pharmacology, biotechnology, clinical medicine and food nutrition [15–17]. Albumin is the most abundant protein in vertebrate blood. It is used as a transport and depot protein for numerous endogenous and exogenous compounds in the circulation system, to maintain the osmotic pressure of plasma, to scavenge oxygen free radicals, and so on [16,18]. Thus, analytical methods for albumin detection are widely studied. Bovine serum albumin (BSA) is a widely used Albumin analog, and is composed of 582 amino acids residues with 17 cysteine cross-linked residues and 1 free cysteine [19]. Due to its stability and non-interference in biochemical reactions, BSA is commonly used in various biochemical applications as a protein concentration standard, enzyme stabilizer, assay standard, for cell culture protocols, etc.

Due to its wide range of applications, the determination of BSA has received considerable research interest. Various analytical methods have been developed for BSA detection such as resonance light scattering [20–24], voltammetric assays [25], surface enhanced plasmon resonance [26], surface enhanced Raman spectroscopy [27], near infrared reflectance spectroscopy [28], fluorimetric determination [29], circular dichroic methods [19], spectrophotometry methods [18,30–33], and so on. Generally, spectrophotometric methods are the simplest, as they are based on the change in absorption spectrum of probes upon

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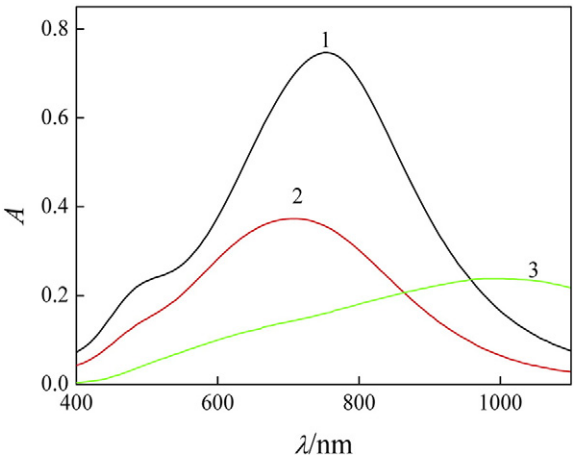

Fig. 1. The absorption spectra of TAgNPs. curve 1, TAgNPs; curve 2, the mixture of TAgNPs/BR after reaction 40 min; curve 3, the mixture of AgNPs/BR/BR. pH 2.56; c_{BSA}, 100 ng mL^{-1}.

binding with BSA. However, until now, the sensitivity of these spectrophotometric methods is not satisfactory. Thus, it is important to develop a new spectrophotometric method for the determination of BSA with high sensitivity for some special application.

In this report, we present a facile BSA assay method utilizing distinct changes in the morphology and absorption spectrum of TAgNPs to detect trace BSA amounts based on the interaction between TAgNPs and BSA in pH 2.56. This interaction of TAgNPs with BSA in the presence of Britton Robison buffer solution (BR) causes the aggregation of TAgNPs, leading to corresponding changes in the optical properties. Thus, BSA concentrations could be determined even at low levels up to 1.0 ng mL^{-1}, with a linear range of 1.0 ng mL^{-1} to 100.0 ng mL^{-1} by UV–visible spectroscopy. Moreover, this method could be used to detect the synthetic samples under optimized conditions.

2. Experiment

2.1. Apparatus

The UV–visible absorption spectra were obtained using a Lambda-35 UV–visible spectrophotometer (Perkin Elmer Instruments Inc., City, USA) and a quartz cell (1 × 1 cm^2). TEM images were obtained with a Tecnai G20 transmission electron microscopy instrument (FEI Company, Hillsboro, USA). Zeta potential of the mixtures measurements were conducted on a Zetasizer Nano ZS dynamic light scattering instrument (Malvern Instru- ment Inc., Westborough, MA) in solutions containing TAgNPs, BSA, and BR. The pH value of the solution was measured by a pH meter (Mettler-Toledo International Inc., Shanghai, China). A DF-101B magnetic stirrer (Yu Hua Instrument Inc., Gongyi, China) was used to blend the solutions in an Erlenmeyer flask to prepare the TAgNPs.

2.2. Reagents

All commercially available reagents were of analytical grade and redistilled water was used for preparing solutions. BSA stock solution (100 mg/L) was prepared by dissolving 5.0 mg pure BSA with water in a 50 mL flask. The other different concentrations were prepared by directly diluting the stock solution with water. Silver nitrate solution with a concentration of 0.01 M was prepared by dissolving 0.4247 g pure Silver nitrate with water in a 250 mL flask. The pH of the reaction system was controlled by the Britton–Robison buffer solution (BR), which was prepared from a mixture of three acids (H_3BO_3, CH_3COOH, and H_3PO_4 (0.04 M each)) with the suitable volumes of 0.2 M NaOH solution.

2.3. Preparation of TAgNPs

The glassware used for the preparation of TAgNPs was washed thoroughly with nitric acid before using. TAgNPs were synthesized according to a previously reported procedure [13]. In a typical process, 49.5 mL of redistilled water was added to a 100 mL Erlenmeyer flask and magnetically stirred at room temperature in air. Subsequently, silver nitrate (0.01 M, 0.50 mL), trisodium citrate (30.0 mM, 3 mL), and H_2O_2 (15 wt%, 240 μL) were added to the above flask under vigorous stirring at room temperature. Then, 250 μL of 100 mM freshly prepared $NaBH_4$ in ice water was rapidly injected into the mixture. The color of the originally colorless solution immediately turned to light-yellow suggesting the reduction of silver. After about 2 min, the colloidal solution turned to deep yellow due to the formation of silver nanoparticles. Within the next several seconds, the morphology started to change from nanoparticles to nanoplates accompanied by color change of the solution from deep yellow to red, green, and finally blue. The entire transition from nanoparticles to nanoplates typically took 2 to 3 min. The TAgNPs synthesis process gave reproducible results with the same conditions, with a yield about 80–90%. The formation of TAgNPs was confirmed by TEM characterization. As showed in Fig. S1(a) in the supporting information, the absorption maximum of TAgNPs is about 755 nm. The morphology of the TAgNPs also showed clearly triangular corners as expected (Fig. S1(b) in the supporting information).

2.4. Spectral detection procedures

A suitable concentration of BSA was thoroughly mixed with 1.0 mL of a specific concentration of TAgNPs solution for 20 min. Then, 200 μL BR (pH 2.56) was added to the above mixture and the solution was diluted to 2.0 mL with water. After standing for 50 min, the mixture was directly used for UV–visible spectral analysis and TEM detection.

2.5. Preparation of samples

Sample 1: 1000 ng mL^{-1} BSA was mixed with 5000 ng mL^{-1} L-Histidine, 5000 ng mL^{-1} DNA, 5000 ng mL^{-1} Zn^{2+}, and 5000 ng mL^{-1} Cd^{2+}.

Sample 2: 1000 ng mL^{-1} BSA was mixed with 5000 ng mL^{-1} L-Tyrosine, 5000 ng mL^{-1} Methionine, 5000 ng mL^{-1} Mg^{2+}, and 5000 ng mL^{-1} Ni^{2+}.

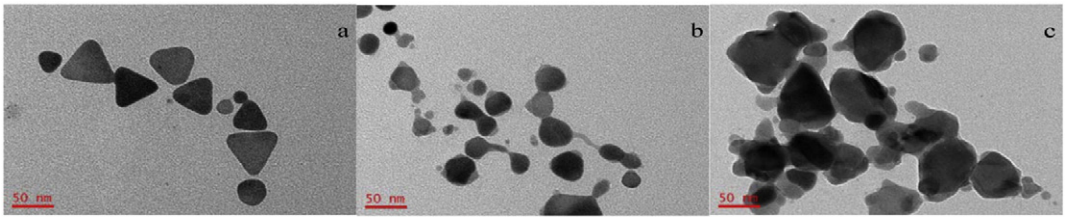

Fig. 2. (a) TEM image of original TAgNPs; (b) TEM image of TAgNPs/BR mixture; (c) TEM image of TAgNPs/BSA/BR mixture (pH 2.56). c_{BSA}, 100 ng mL^{-1}.