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ORIGINAL PAPER

The Fluorescence Enhancement of the Protein Adsorbed on the Surface of Ag Nanoparticle

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Abstract The fluorescence enhancement of the BSA adsorbed on the surface of Ag nanoparticles is reported, where non-fluorescent collagen is used as the separator between the BSA and Ag nanoparticles. The study indicates that Ag nanoparticles can enhance the fluorescence of the BSA, especially the fluorescence of the tyrosine residues with lower quantum. Three types of Ag nanoparticles are evaluated including Ag island film, Ag colloids and fractal Ag electrode. Of them Ag island film is the best. The investigation suggests that the fluorescence enhancement of the BSA is related to the adsorption of the BSA on the surface of Ag island film through the hydrophobic interaction, while the collagen can promote the adsorption of the BSA on the surface of Ag island film and change its conformation, resulting in the interaction between BSA and Ag island film.

Keywords Surface-enhanced fluorescence · BSA · Ag island film · Collagen · Adsorption

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Introduction

Studies on the protein are a very active area for chemist and biologist over the past decades, because of its important role in life science. There are many works focused on the detection of proteins [1–3], the analysis of function and interaction mechanism [4–7], pharmaceutical delivery [8, 9], etc. Fluorescence method stands out since its obvious sensitivity is some orders of magnitude higher than that of other spectral detections. However, the natural fluorescence of the protein is weak, and not all of proteins generate a useful native fluorescent signal. Therefore, the fluorescence probes of protein have been introduced in the detection of proteins.

In recent years, the favorable effects of silver nanoparticles on the fluorophores near the metallic surface have been reported [10–15]. These effects include decreasing the lifetime, increasing photo-stability and quantum yield of fluorophores, particularly those with low quantum yields, which are known as metal-enhanced fluorescence (MEF) or surface-enhanced fluorescence (SEF). The combination of increased brightness, short lifetime and better photo-stability makes MEF become a potential tool for the DNA hybridization [16] and immunoassay [17]. The effect of silver nanoparticles on the fluorescence of the fluorophore has been studied experimentally and theoretically, it is considered that above effects are due to the interaction of the excited state fluorophore with the surface plasmon resonance on the surface of silver nanoparticles. The studies on the surface-enhanced fluorescence through the adsorption of the fluorophores on the surface of metal nanoparticles have not been reported.

In this paper, the fluorescence enhancement of the BSA on the surface of Ag island film is reported, where non-fluorescence collagen is used as the separator between the

BSA and Ag island film. The relationship between the fluorescence enhancement of the BSA and the adsorption properties of the BSA on the surface of Ag island film is studied.

Experimental

Materials

Unless otherwise noted, all reagents and solvents used in this study were analytical grade. Proteins (BSA, HSA and Collagen) were purchased from Sigma Chemical Co. USA without further purification. A 0.05 mol/l Tris–HCl buffer solution was prepared by dissolving 3.03 g of Tris in 500 ml deionized water, and adjusting the pH to 7.0 with HCl measured by a Delta 320-S acidity meter (Mettler Toledo, Shanghai).

Methods

Treatment of the quartz slides: The quartz slides used for silver deposition were first cleaned overnight according to the reported methods [12–16] by soaking in the mixture of H_2SO_4 (95–98%) and H_2O_2 (30%). After washed with ultrapure water, the quartz surface was coated with amino groups by dipping the slides in a 1% aqueous solution of 3-aminopropyltriethoxysilane (APS) for 30 min in room temperature. The slides were washed extensively with water and air-dried.

Preparation of Ag nanoparticles Herein, we prepared three types of Ag nanoparticles:

Ag colloids were prepared by the citrate reduction of $AgNO_3$. 1.0×10^{-3} mol/l $AgNO_3$ solution was boiled with vigorous stirring, followed by the addition of 10 ml (1%) sodium citrate solution. After a further 30 min simmering, the color of the solution changed into brown. Then the solution was rapidly cooled. The method produced a stable, brown dispersion of Ag colloid particles [18]. Then, APS coated glass slides were coated with Ag colloids through immersing in an Ag colloid solution for above 90 h, after which time no further increase in optical density of glass slide was observed.

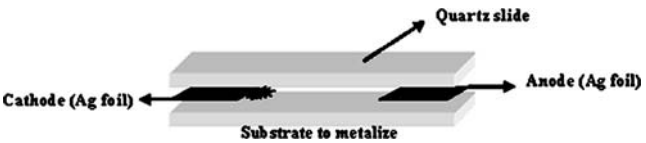
Ag island film deposition was accomplished as described previously [19, 20]. To a fast stirring silver nitrate solution (0.22 g in 26 ml of Millipore water), eight drops of fresh 5% NaOH solution were added. Dark-brownish precipitates formed immediately. Less than 1 ml of ammonium hydroxide was then added drop by drop to redissolve the precipitates. The clear solution was cooled to 5 °C by placing the beaker in an ice bath, followed by soaking the cleaned and dried quartz slides in the solution. At 5 °C, a fresh solution of D-glucose (0.35 g in 4 ml of water) was added. The mixture was stirred for 2 min at that temperature. Subsequently, the beaker was removed from the ice bath. The temperature of the mixture was allowed to warm up to 30 °C. As the color of the mixture turning from yellow green to yellowish brown, the color of the slides became greenish; the slides were removed and washed with water. After being rinsed with water several times, the slides were stored in water for several hours prior to the experiments.

Fractal Ag electrode was generated by using two silver electrodes held between microscope slides (Scheme 1) [12]. The silver electrodes were $10\times35\times0.1$ mm, with about 20 mm between the two electrodes. Deionized water was placed between the slides. A direct current of 100 μA was passed between the electrodes for about 10 min, during which the voltage started near 5 V and decreased to 2 V. After turning on current flow, fractal silver nanostructure grew on the cathode and then on the glass near the cathode.

All of the quartz slides were only half coated with Ag nanoparticles. The UV absorption spectra of three types of Ag nanoparticles are shown in Fig. 1. From this figure, we can see that all of these Ag nanoparticles have the plasmon resonance absorption band at about 417 nm, indicating that the nanometer structures are achieved in three preparations. However, the absorption band of Ag island film is the narrowest, indicating that the size of Ag island nanoparticles is well-distributed compared with those of Ag colloids and fractal Ag electrode.

The adsorption of the collagen on the surfaces of quartz slides. The adsorption of the collagen on the surfaces of quartz slides was accomplished by soaking the Ag nanoparticles coated slides in 6.0×10^{-2} mol/l collagen solution overnight, followed by rinsing with water to remove the unbound material.

Fluorescence measurement Experimental setup used in fluorescence measurements is shown in Scheme 2, where the quartz slides or nano-Ag surfaces were examined in a sandwich configuration in which two coated surfaces faced inward toward an approximate 1 μm thick aqueous sample [18]. The incident angle of excitation light is about 65°. Steady state emission spectra were recorded by a LS 55 spectrofluorometer (PE, USA) with excitation at 290 nm



Scheme 1 Configuration for creation of fractal silver surfaces on silver electrodes and quartz glass

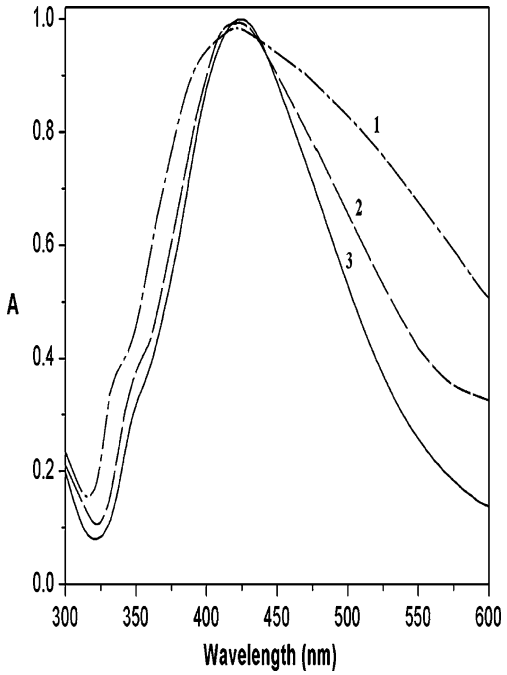


Fig. 1 The absorption of Ag nanoparticles. *1* Fractal silver electrodes; *2* Ag colloids; *3* Ag island film

after the BSA solution was filled in the space between the two quartz slides.

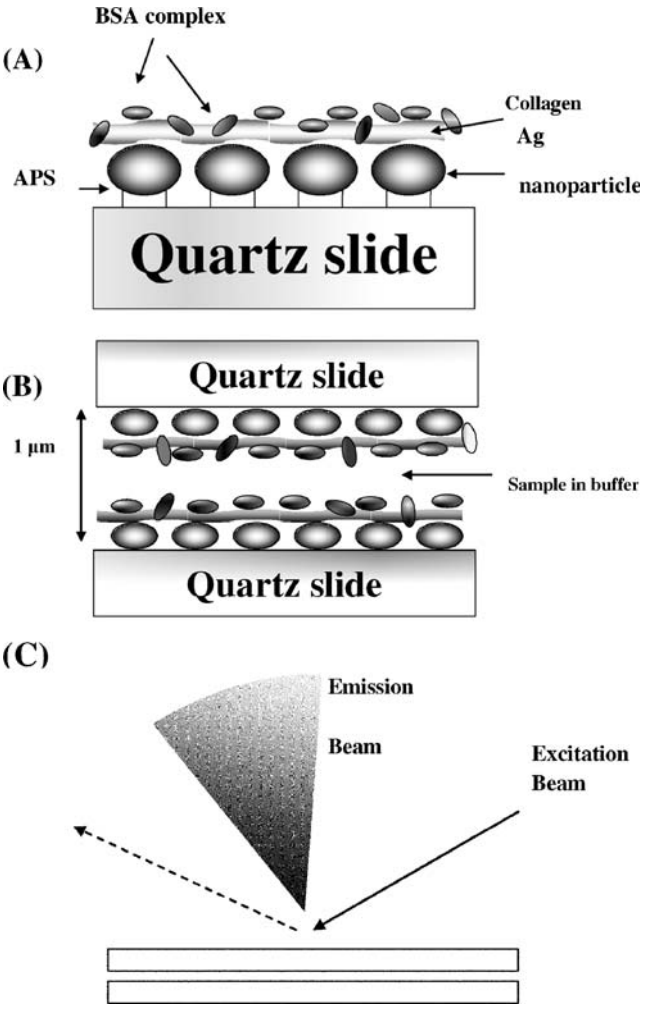
Result and discussion

Choice of the spacer between Ag island film and the fluorophore

The metal-enhanced fluorescence effect requires a suitable space distance between fluorophore and metal nanoparticle. Generally, HSA and BSA were chosen as the spacer in literatures reported [12–15]. However, the aim of our research is to detect the protein. HSA and BSA acting as spacer must interfere with the measurement because of their native fluorescence. Here, we choose collagen as spacer because that there are no tryptophan residues in its molecule to overcome the interference of native fluorescence. In addition, it is considered that there is strong interaction between collagen and other proteins through the hydrophobic force, which can promote the adsorption of BSA on the surface of Ag island film.

The enhancement of BSA fluorescence

The emission of BSA is shown in Fig. 2. It can be seen that the fluorescence intensity of BSA near the surface of Ag island film is about 20-time stronger than that of without Ag island film. In general, the fluorescence of native BSA is mainly attributed to the tryptophan residues due to its higher quantum yield, contrasted by the tyrosine residues with very low quantum yield [15]. Figure 3 is the fluorescence synchronous spectra of BSA with $\Delta\lambda$ of 30 and 60 nm, which can characterize the fluorescence of tyrosine and tryptophan residues, respectively. From Fig. 3, it can be seen that in the presence of Ag island film, two residues emit strong fluorescence, and the contribution of tyrosine residue is the same as that of tryptophan residues, meaning that the fluorescence of the residue with lower quantum yield is increased to a greater extent. This phenomenon is considered that there are probably several contributions to the increased fluorescence intensities of tryptophan and tyrosine residues. These causes include more protein binding, increased rates of excitation due to an enhanced electric field, and larger quantum yields due to increased rates of radioactive decay.



Scheme 2 **a** Glass (quartz) surface geometry. APS is used to functionalize the surface of glass with amine groups that readily bind silver nanoparticle; **b** the sample geometry; **c** experimental geometry

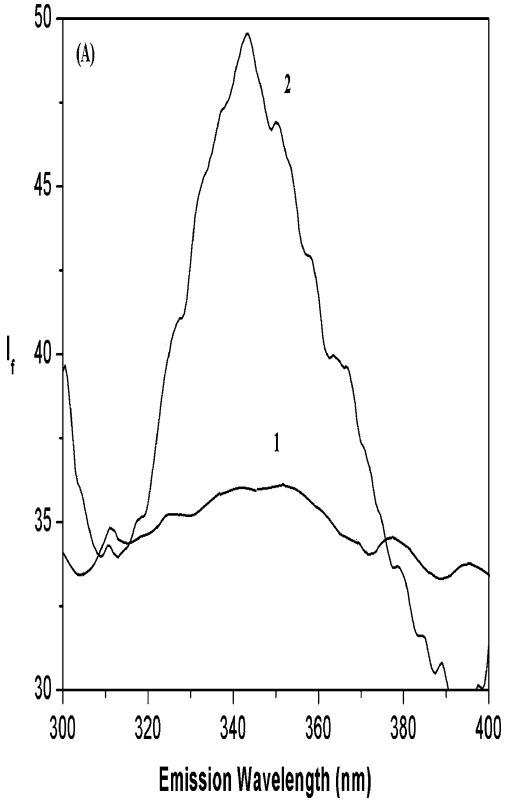


Fig. 2 The emission spectra of BSA at the excitation wavelength of 290 nm. *1* BSA without Ag island film; *2* BSA with Ag island film

The choice of Ag nanoparticle form

We evaluated the effect of three types of Ag nanoparticles on the enhancement of BSA (1.0×10^{-5} g/ml) fluorescence. From the results shown in Table 1, it can be seen that Ag island film is the best, consistent with the previous reports [19, 20] that Ag island film is more stable for eliminating the coagulation of Ag nanoparticle. In addition, the size of nanoparticles in Ag island film is well-distributed, which is another reason to enhance the fluorescence. So Ag island film is chosen in the future research.

Adsorption of BSA on Ag island film coated quartz slides

The interaction of the protein (BSA as example) with Ag island film coated quartz slides is studied by monitoring the intrinsic fluorescence of tryptophan residues in protein. This can avoid the labeling or the addition of the probe those may exert some effect on the adsorption pattern.

Excited by 290 nm with about 65° incident angle, the fluorescence of BSA is greatly enhanced by near Ag island film, which is shown in Fig. 2. According to the basic principle of metal-enhanced fluorescence, the enhancement is only attributed to BSA molecules near the surface of Ag island film, whereas the fluorescence intensities of other free BSA molecules in the solution are not enhanced. So we can quantify the concentration of adsorbed BSA by aid of the enhancement of its fluorescence intensity.

First, the characteristic penetration depth of incident light, d, is a function of the angle of incidence θ, wavelength λ, and refractive indices (n_1 and n_2) of the solid and liquid materials [21]:

$$d = \lambda \Big/ 2\pi\left(n_1^2 \sin^2\theta - n_2^2\right)^{1/2} \quad (1)$$

herein, n_1=1.515, n_2=1.333, θ=65°.

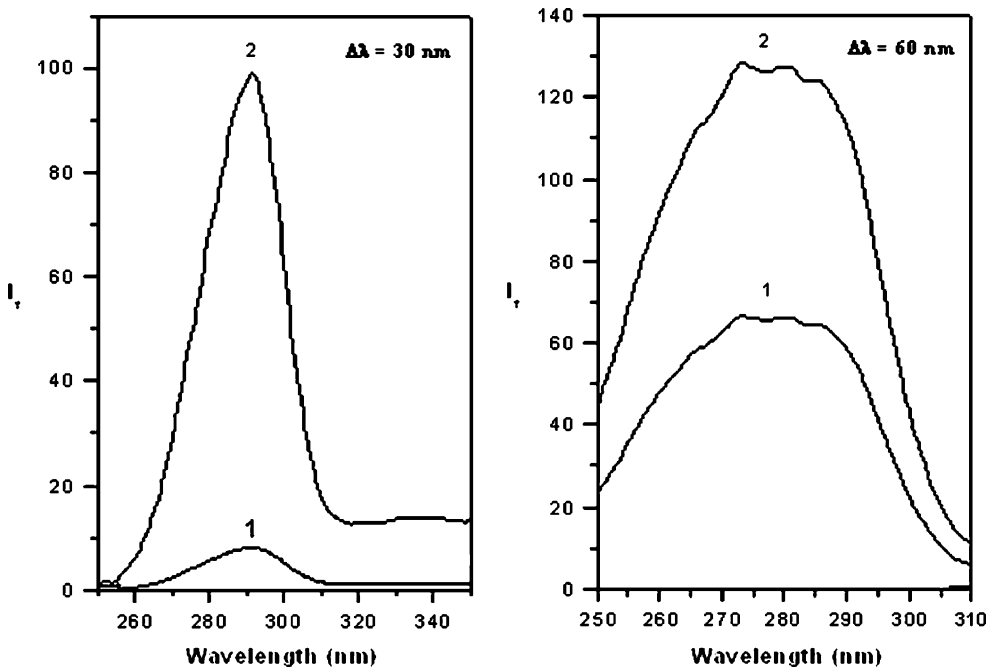


Fig. 3 Fluorescence synchronous spectra. *1* BSA in solution; *2* BSA on the surface of Ag island film

Table 1 Comparison of fluorescence enhancing factor induced by Ag nanoparticles (1.0×10^{-5} g/ml BSA)

	Ag island film	Ag colloids	Fractal Ag electrode
Enhancement factor	12.2	5.3	3.8

At given excitation and emission wavelengths, the fluorescence intensity emitted by the fluorophore molecules in the evanescent wave region is related to its extinction coefficient ε, the quantum yield φ, the concentration of molecules c, and the intensity of the evanescent wave I, whereas the concentration and intensity are a function of the distance z from the interface to the solution. Therefore, the observed fluorescence signal S can be written as:

$$S = f\varepsilon\varphi\int_0^\infty c(z)I(z)\mathrm{d}(z) \tag{2}$$

where f is an instrumental factor.

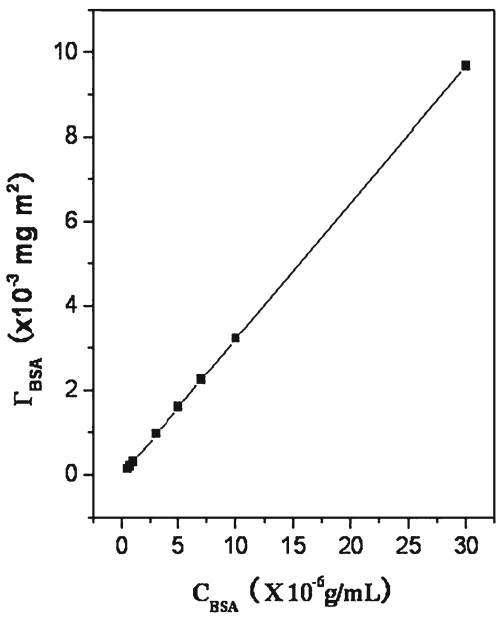
To relate the measured fluorescence intensity to the surface concentration of the fluorophore, a calibration procedure is required. The fluorescence intensity depends on properties of both the fluorophore and the instrumental setup. According to the described in the reported paper [22], a series of tryptophan solution with different concentrations were used to calibrate the instrumental property. On the other hand, to convert the fluorescence signal of the adsorbed proteins into surface concentrations, the fluorescence of the protein is normalized to the increment of fluorescence signal per increment of the concentration (in units of absorption) of tryptophan. After rewriting the normalized Eq. 2, we can get Eq. 3 [22]:

$$\Gamma_\mathrm{p} = \frac{S_\mathrm{p}d}{2\varepsilon_\mathrm{p}\,\Delta S_\mathrm{t}/\,\Delta(c_\mathrm{t}\varepsilon_\mathrm{t})}\,\frac{\varphi_\mathrm{t}}{\varphi_\mathrm{p}} \tag{3}$$

where Γ_p is the surface adsorption amount; the subscripts p and t refer to the solutions of the protein and tryptophan standard, respectively. The only unknown parameter is the quantum yield. We used the L-tryptophan (quantum yield= 0.14) as the criterion to measure the quantum yields of the tryptophan and BSA near surface of Ag island film, which are 0.19 and 1.52, respectively. So in the bulk solutions with different concentration of BSA, the surface adsorption amount of BSA on surface of Ag island film (Γ_BSA) were obtained and listed in Table 2. It can be seen that when the concentrations of BSA solution are ranged from 5.0×10^{-7} to 3.0×10^{-5} g/ml, there is a linear relationship between the Γ_BSA and the concentration of BSA in the bulk solution, the adsorption ratio is about 32.3%, which is much greater than that on the surface without collagen. Compared to the detection limit of BSA near quartz slides (8.0×10^{-6} g/ml BSA), the detection limit of BSA near Ag island film is greatly improved, which can be deciphered as the result of the enhanced fluorescence of BSA molecules near Ag island film (shown in Fig. 2)

Table 2 The relationship between concentration (C_BSA) of BSA in the bulk solution and its surface adsorption amount (Γ_BSA) on the surface of Ag island film

C_BSA (g/ml)	Γ_BSA (mg m^2)	C_BSA (g/ml)	Γ_BSA (mg m^2)	Linear relationship
1.0×10^{-8}	1.5×10^{-6}	5.0×10^{-6}	1.6×10^{-3}	
3.0×10^{-8}	2.4×10^{-6}	7.0×10^{-6}	2.3×10^{-3}	
5.0×10^{-8}	3.2×10^{-5}	1.0×10^{-5}	3.2×10^{-3}	
7.0×10^{-8}	5.2×10^{-5}	3.0×10^{-5}	9.7×10^{-3}	
1.0×10^{-7}	7.3×10^{-5}	5.0×10^{-5}	1.0×10^{-2}	
3.0×10^{-7}	9.6×10^{-5}	7.0×10^{-5}	1.5×10^{-2}	
5.0×10^{-7}	1.6×10^{-4}	1.0×10^{-4}	2.0×10^{-2}	
7.0×10^{-7}	2.4×10^{-4}	3.0×10^{-4}	2.2×10^{-2}	
1.0×10^{-6}	3.3×10^{-4}	5.0×10^{-4}	2.7×10^{-2}	
3.0×10^{-6}	9.5×10^{-4}			



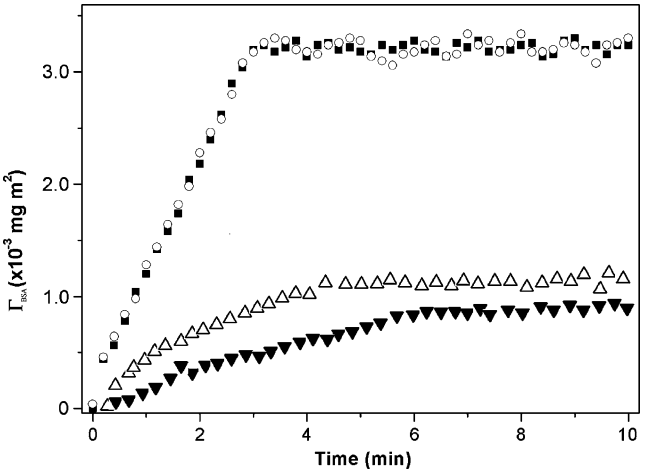


Fig. 4 Adsorption kinetics. *Filled squares* BSA and *open circles* BSA at ionic strength (0.20 mol/l NaCl) on the surface of collagen–Ag–APS–quartz; *filled inverted triangles* BSA on the surface of APS–quartz; *open triangles* BSA on the surface Ag–APS–quartz

Based on the research on the adsorbed amount of BSA, the adsorption kinetics of BSA onto the three different surfaces is studied here, which is shown in Fig. 4. It can be seen that the adsorption equilibrium of BSA on the surface of collagen–Ag–APS–quartz is reached after about 3 min adsorption time, which is shorter than those of APS–quartz (6 min) and Ag–APS–quartz (5 min). In addition, the adsorbed amount of BSA on the surface of collagen–Ag–APS–quartz is the biggest among them.

It is well known that the adsorption rate and adsorbed amount are limited by the protein flux toward the surface and is further affected by interaction between the protein and adsorbent surface which determine whether the protein adsorbs or not. In general, the major driving forces for protein adsorption are considered as electrostatic and hydrophobic interactions. It is observed in Fig. 4 that BSA adsorption curves without NaCl resembles that with 0.20 mol/l NaCl, which indicates that electrostatic interactions have a minor influence on the adsorption behavior, so the major driving force for BSA adsorption on the surface of collagen–Ag–APS–quartz is hydrophobic interaction.

The conformational change of the BSA on the Ag island film coated quartz

It is well known that fluorescence of tryptophan residues in proteins is very sensitive to the polarity of its local environment. In Fig. 2, there is a blue-shift and narrowing for the emission spectrum of BSA near the surface of collagen–Ag–APS–quartz. The blue-shift indicates that the local polarity of tryptophan residue is decreased, which is considered as the result of tryptophan residue buried in the structure from the water molecules. The narrowing of the spectrum indicates the homogeneous distribution of protein structure, that is, most of BSA molecules adsorbed on the surface of collagen–Ag–APS–quartz have the same unfolding. UV absorption spectra are also used to study the conformational change of BSA. The absorption peak at about 200 nm is sensitively affected by the conformational change of protein. From Fig. 5, it can be seen that this peak of BSA reduces remarkably when it is adsorbed on the surface of collagen–Ag–APS–quartz, which indicates that BSA is the unfolding [21].

All of the factors mentioned above are considered to be the result of the strong hydrophobic interaction between BSA and the collagen, where the conformation of BSA is changed into more expanding and BSA molecules keep close to the surface of the collagen. This adsorption shortens the distance between BSA and Ag island film, and promotes the interaction between BSA and metal nanoparticles.

Conclusion

In conclusion, the study on the surface-enhanced fluorescence through the adsorption properties of the BSA on the surface of Ag island film is reported, where non-fluorescence collagen is used as the separator between the BSA and Ag nanoparticles. It is considered that the fluorescence enhancement of the BSA is related to the adsorption of the BSA on the surface of Ag island film through the hydrophobic interaction, while the collagen can promote the adsorption of the BSA on the surface of Ag island film and change its conformation, resulting in the interaction between BSA and Ag island film. The investigation is helpful for us to understand the fluorescence enhancement of the fluorophores on the surface of metal nanoparticles.

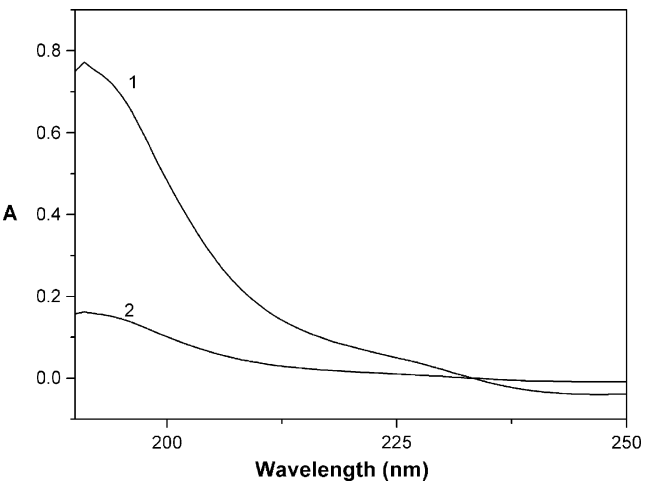


Fig. 5 BSA conformational changes reflected by UV absorption. *1* BSA without Ag island film; *2* BSA with Ag island film

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