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Studies on the interaction of colloidal gold and serum albumins by spectral methods

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Abstract

The interactions of colloidal gold and serum albumins, including bovine serum albumin (BSA) and human serum albumin (HSA), were studied by fluorescence and absorption spectrometry. Fluorescence quenching spectrometry was applied to study the interactions between colloidal gold and serum albumins. At pH 7.4 phosphate-buffered saline (PBS), the intensity of fluorescence emission spectrum of serum albumins decreased in the presence of colloidal gold, which indicated that colloidal gold quenched the fluorescence of serum albumins. Experimental results indicated that the combination reactions of colloidal gold and serum albumins were static quenching processes. Based on the effect of colloidal gold on fluorescence intensity, the binding constants, the numbers of binding sites and the acting forces between colloidal gold and serum albumins were found.

Keywords: Fluorescence quenching; Colloidal gold; Serum albumins

1. Introduction

Colloidal gold has unique physical and chemical characteristics, which has been studied extensively in many areas [1–7]. Different size of colloidal gold can be prepared by changing the rate of $HAuCl_4$ to reducing agent. Based on the spectral data and the molar extinction coefficients, the concentration of different size of colloidal gold particles can be obtained [6,8]. Gold nanoparticles are red in color because of the Mie absorption by their surface-plasmon oscillation. Based on the surface plasmon resonance (SPR), colloidal gold was used to determine the antibody or antigen and to study molecular recognition process using the interaction between antigen and antibody [2]. In SPR investigation, colloidal gold can be used to enhance the surface plasmon resonance signal by preparation of protein–Au conjugates [2,3]. Colloidal gold was also applied extensively in self-assembled monolayers [4], optical absorption study [5], and resonance light scatting spectroscopy [6], immunoassy [7]. In these assay, most of them are based on the interactions of colloidal gold and proteins or thiol-containing substances. Both bovine serum albumin (BSA) and human serum albumin (HSA) are proteins that have thiol groups. BSA was often used to stabilize the colloidal gold against coagulation and inhibit the nonspecial adsorption [3]. Therefore, studies of the interactions of colloidal gold and serum albumins are very important.

Serum albumins have intrinsic fluorescence, mainly because there exist tryptophan residues, which can emit light at about 340 nm when excited by ultraviolet wavelength light around 280 nm. The information of interactions of serum albumins and other substances can be obtained by measuring fluorescence of serum albumins, including synchronous fluorescence [9] and quenching fluorescence. In recent years, the fluorescence quenching technique was applied extensively to study the interactions of biomedical molecules and serum albumins [10–13], and the effect of metal ion on the bindings of serum albumins and other substances [14]. But so far the studies of interactions of colloidal gold and serum albumins have not been reported by fluorescence technique in our knowledge. In this paper, the fluorescence quenching

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technique was applied to study the interactions of colloidal gold and serum albumins. Based on colloidal gold quenching fluorescence and Stern–Volmer equation, the dynamic quenching constants were calculated. According to the plots of $\log((F_0 - F)/F)$ versus $\log([Au_t] - n[P_t](F_0 - F)/F_0)$ and thermodynamic equations, the binding constants, the numbers of binding sites and the acting forces between colloidal gold and serum albumins were discussed.

2. Experimental

2.1. Apparatus

Fluorescence spectrum recordings and intensity measurements were performed with a Shimadzu RF-5301PC Spectrofluorometer equipped with a xenon lamp and 1.0 cm × 1.0 cm × 4.0 cm quartz cells. Absorption spectra were measured with an Australian GBC Cintra 10e UV–vis Spectrometer equipped with 1.0 cm × 1.0 cm × 4.0 cm quartz cells. Agitating heater was used to heat and stir the solution, which purchased from Guohua Instrument Company. The temperature was controlled by an electronic thermostat waterbath.

2.2. Chemicals

All stock solutions were prepared with doubly deionized water. Gold chloride ($HAuCl_4 \cdot 4H_2O$ $M_r = 411.85$) was obtained from Shanghai Reagent Company (China). HSA and BSA purchased from Shanghai Biological Products Institute (Shanghai, China) were directly dissolved to prepare stock solutions of 1×10^{-5} mol l^{-1}, respectively. Phosphate-buffered saline (PBS) pH 7.4 was prepared by dissolving 0.2 g KCl, 8.0 g NaCl, 0.24 g KH_2PO_4, 1.44 g Na_2HPO_4 in 1000 ml water. Other reagents used in this study were of analytical grade or best grade commercially available.

2.3. Procedure

Preparation of 2.6 nm colloidal gold [6,15]: H_2O of 90 ml was added into 1 ml of 1% $HAuCl_4$ at 20 °C. After 1 min of stirring, 2.00 ml of 38.8 mmol l^{-1} sodium citrate was added. One minute later, 1.00 ml of fresh 0.075% $NaBH_4$ in 38.8 mmol l^{-1} sodium citrate was added. The solution was stirred for 5 min to make the reaction react completely. At last, the solution was stored in dark bottle at 4 °C.

In a series of 10 ml colorimetric tubes, 0.2 ml of 1×10^{-5} mol l^{-1} BSA (or HSA), PBS buffer solution and appropriate colloidal gold were added, respectively. So there were a series of solutions containing different concentration of colloidal gold and the same concentration of BSA (or HSA). Then the mixture was vibrated in order to make colloidal gold and BSA (or HSA) combine completely and placed into electronic thermostat waterbath in suitable temperature for 10 min. The fluorescence spectrum was recorded with the Shimadzu RF-5301PC Spectrofluorometer within the wavelength region from 300 to 500 nm. The exciting wavelength was 280 nm, the slit width was 5 nm/5 nm for BSA and 5 nm/10 nm for HSA, respectively. The absorption spectra were recorded with the Australian GBC Cintra 10e UV–vis Spectrometer within wavelength from 300 to 800 nm, the slit width was 1.5 nm.

3. Results and discussion

3.1. The absorption spectrum of colloidal gold

The absorption spectrum of colloidal gold solution prepared according to the above method was measured with UV–vis Spectrometer. The intensity and position of surface plasmon oscillation peak of colloidal gold are related to the size, shape and dispersity of nanoparticle. As shown in Fig. 1, the shape of the peak is very symmetry and the peak width is very narrow, which indicated that the prepared colloidal gold had good monodispersity and spherical shape [16]. Colloidal gold has a maximum absorbance at about 512 nm, which is surface plasmon oscillation peak of colloidal gold. This almost accorded with the reported assay. Based on the same process of preparation of colloidal gold, the concentration of 2.6 nm diameter colloidal gold was determined to be 1×10^{-6} mol l^{-1} [6,8].

3.2. The fluorescence spectrum analysis

Most of proteins have intrinsic fluorescence and absorbance at around 280 nm, because there exist tryptophan, tyrosine and phenylalanine residues in serum albumins, which can absorb and emit ultraviolet wavelength light. Because of the different chromophores of tryptophan, tyrosine and phenylalanine residues, their spectra were different. The fluorescence intensity increased in the order:

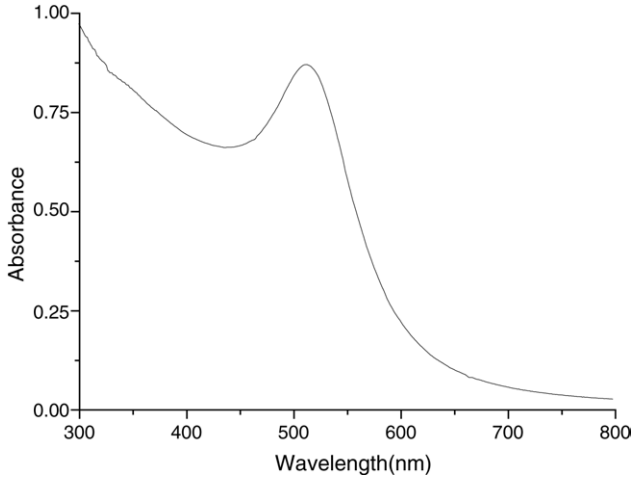

Fig. 1. The absorption spectrum of 2.6 nm colloidal gold at room temperature.