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The interaction between Ag^+ and bovine serum albumin: A spectroscopic investigation

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ABSTRACT

By using spectroscopic methods, we probed the interaction of Ag^+ with bovine serum albumin (BSA) in an aqueous environment. Fluorescence of BSA quenched by Ag^+ is a dynamic quenching process. Two binding modes–a strong one at low concentration of Ag^+ and a weak one at high concentration were found. The association constant (K_A) and the number of binding sites (n) were $4.88\times10^3\ M^{-1}$ and 1.17 for strong binding, and $17.6\ M^{-1}$ and 0.547 for weak binding at 293 K. The results of thermodynamic parameters ΔH^θ, ΔG^θ and ΔS^θ for instinct binding modes at different temperatures indicated that the hydrogen bonding and van der Waals interaction play a major role for low Ag^+/BSA ratio while electrostatic association for high Ag^+/BSA ratio. Data of UV–Vis and Circular dichroism (CD) suggested that with the increasing amount of Ag^+, the secondary structure undergoes a decrease in α-helix and an increase in β content and the backbone of BSA experiences a micro-environmental alteration. Furthermore, the distance r between donor (Trp-212) and acceptor (Ag^+) was evaluated to be 10 nm according to nonradiative energy transfer theory.

1. Introduction

As a major soluble protein constituent of circulatory system (~60% of the total), serum albumin plays an essential role in binding and transport a wide range of endogenous and exogenous compounds such as fatty acids, heme, bilirubin, metal ions and drugs, in the bloodstream to their target organs (Hu et al., 2009; Tian et al., 2005). Its functional and physiological properties have been extensively studied in recent years (Dubois et al., 2006; Throop et al., 2004). Moreover, these proteins have long been used as model proteins in both industrial and academic research areas (Charbonneau and Tajmir-Riahi, 2010; Dubeau et al., 2010; Hu et al., 2009; Liu et al., 2009; Mandeville and Tajmir-Riahi, 2010; Tayeh et al., 2009; Zhao et al., 2009).

The crystallographic analyses of human serum albumin revealed that the principal regions of ligand binding sites in albumin are located in hydrophobic cavities in subdomains IIA and IIIA (Sudlow et al., 1976). In the current work, bovine serum albumin (BSA) was selected as our model protein because of its structures homology with human serum albumin and lone-standing interest in the protein community. Like other serum albumin, BSA has a wide range of physiological functions involving the binding and delivery of organic macromolecules and metal ions.

Recent years heavy metal toxicity has drawn wide and deep attention (Demirel et al., 2009; Fini et al., 2009; Venkatachalam et al., 2009), and silver is one of them. Silver has long been valued as a precious metal, and it is used to make ornaments, jewelry, high-value tableware, utensils, and currency coins. Today, silver metal is also used in electrical contacts and conductors, in mirrors, in thermos bottles and in catalysis of chemical reactions. Its compounds are used in photographic film and dilute silver nitrate solutions and other silver compounds are used as disinfectants and microbiocides. Although silver is not an essential element for life beings, it has been found to be widely distributed in nature, including tissues and organisms (Shen et al., 2003). Previous studies have demonstrated toxicity to human cells (Cortese-Krott et al., 2009), plant (Mikelova et al., 2007) and microbial growth (Choi et al., 2008). However there is a severe lack of study on toxicity and binding mode targeted to biomacromolecules. In this research, spectroscopic technologies including fluorescence spectroscopy, synchronous fluorescence spectroscopy, ultraviolet-visible absorption spectroscopy and circular dichroism spectroscopy were used to investigate the binding sites, modes, and forces for Ag^+ binding to BSA, and the secondary and tertiary structure transitions of BSA. Besides, the micro-environment changes of some typical amino acid residues were also studied.

2. Experimental section

2.1. Reagents

Bovine serum albumin (BSA) were bought from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China) and was dissolved in ultrapure water to form a 0.5 g/L solution, then preserved at 4 °C for

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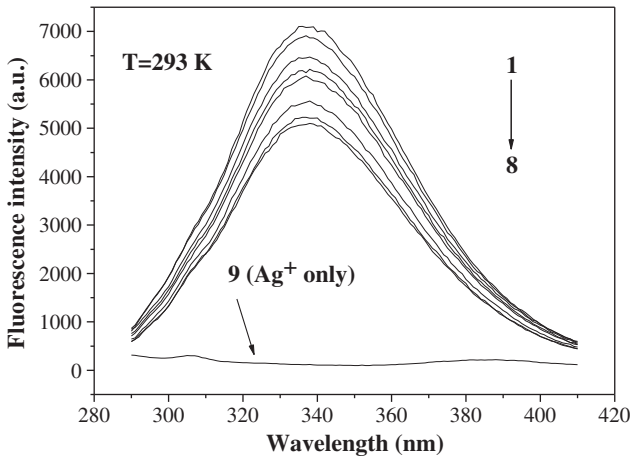

Fig. 1. Fluorescence spectra of BSA in the presence of various concentrations of Ag^+. Concentration of BSA was fixed at 7.5×10^{-7} M; c (Ag^+) from 1 to 8 were 1×10^{-5}, 5×10^{-5}, 1×10^{-4}, 2×10^{-4}, 5×10^{-4}, 8×10^{-4} and 1×10^{-3} M. Curve 9 (dashed line) shows the fluorescence spectrum of Ag^+ only.

later use. $AgNO_3$ was purchased from Sinopharm Chemical Reagent Co., Ltd. HAc and NaAc were all of analytical reagent grade, and were prepared as 0.10 M buffer to stabilize pH at 5.5. Solutions were prepared with ultrapure water throughout.

2.2. Apparatus

In all the work, absorption spectra were recorded on a double beam UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were measured using a FL-4500 fluorescence spectrophotometer (Hitachi Co. Ltd., Tokyo, Japan). Circular dichroism (CD) spectra were gauged by a J-810 Spectropolarimeter (Jasco, Tokyo, Japan) under constant nitrogen flush. Transmission electron microscopy (TEM) images were taken on a JEM-100X transmission electron microscope (JEM Ltd. Tokyo, Japan). KQ-100E ultrasonic cleaner (Jiangsu Kunshan Ultrasonic Instrument Co., Ltd.) and electric blender (Jintan Xiaoyang Electronic Instrument Factory) were used for sample preparation.

3. Methods

Fluorescence measurements were equipped with a 1 cm × 1 cm quartz cell, and using excitation and emission slit widths of 5 nm. Fluorescence spectra were recorded at two different temperatures (283 K and 293 K). The systems were excited at 278 nm, and emission wavelength was adjusted from 290 nm to 410 nm with the scanning speed of 1200 nm/min and the PMT (Photo Multiplier Tube) voltage of 750 V.

CD measurements were operated over the range of 200–250 nm by 0.5 nm intervals. The scanning rate was adjusted to 200 nm/min. The spectra were measured in a temperature-controlled 1 cm path length cell.

As to UV–Vis absorption spectra, a series of Ag^+ solutions with diverse concentrations and a fixed amount of BSA were added to eight 10 ml volumetric flasks in sequence, and then diluted with ultrapure water to mark line. The equilibrated solution was poured into the quartz cells and scanned in the ultraviolet range of 190–350 nm using Ag^+ solutions mixed with buffer as references.

We use HAc-NaAc solution system as buffer throughout. All measurements were performed at 293 K if they are not particularly indicated.

Table 1
Stern–Volmer quenching constants of the system of Ag^+–BSA at different temperatures.

T(K)	c (Ag^+) ≤ 1×10^{-4} M			c (Ag^+) ≥ 2×10^{-4} M		
	K_q (10^{11} M^{-1} s^{-1})	K_{SV} (10^3 M^{-1})	R	K_q (10^{10} M^{-1} s^{-1})	K_{SV} (10^2 M^{-1})	R
293	1.51	1.51	0.9889	2.87	2.87	0.9902
283	0.958	0.958	0.9992	3.25	3.25	0.9994

4. Results and discussion

4.1. Fluorescence intensity changes of BSA caused by Ag^+

Fluorescence spectroscopy has been widely used to investigate the interaction between small molecules and proteins. This technology can be used to expound the interaction of proteins and quenchers such as accessibility and quenching mechanism.

In case of inner filter effect (IFE) which was caused by the absorption of excitation and emission radiation, primary experiment was done. The sum of the absorbance at 278 nm (excitation wavelength) and 337 nm (fluorescence peak) could cause no more than 5% percentage error for each sample, so IFE was ignored here.

The fluorescence spectra of BSA obtained in the absence and presence of Ag^+ is shown in Fig. 1. If the small molecule can quench the tryptophan residues, the residues must be located in or near the binding position. The gradual addition of Ag^+ led to a decrease in the fluorescence intensity of BSA, which indicates the strong interaction

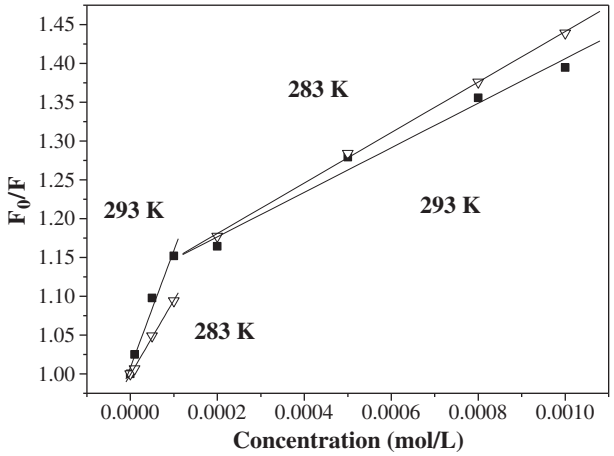

Fig. 2. Stern–Volmer plots of fluorescence quenching of BSA by the addition of Ag^+ at diverse temperatures. Concentration of BSA was fixed at 7.5×10^{-7} M.

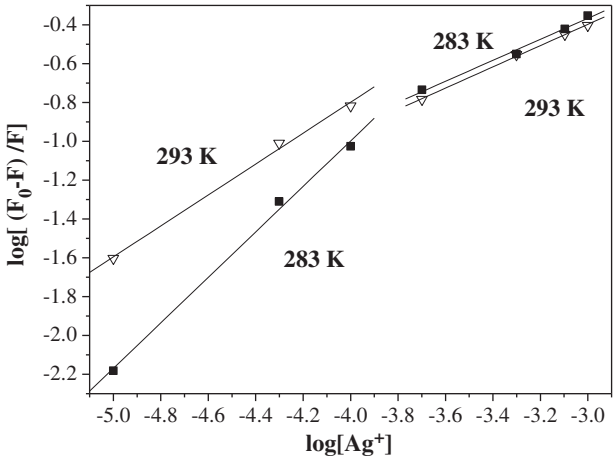

Fig. 3. Plot of lg [$(F_0-F)/F$] as a function of lg [Ag^+] at 283 K and 293 K.