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Study on the interaction of La^{3+} with bovine serum albumin at molecular level

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ABSTRACT

The interaction of La^{3+} to bovine serum albumin (BSA) has been investigated mainly by fluorescence spectra, UV–vis absorption spectra, and circular dichroism (CD) under simulative physiological conditions. Fluorescence data revealed that the quenching mechanism of BSA by La^{3+} was a static quenching process and the binding constant is 1.75×10^4 L mol^{-1} and the number of binding sites is 1 at 289 K. The thermodynamic parameters ($\Delta H=-20.055$ kJ mol^{-1}, $\Delta G=-23.474$ kJ mol^{-1}, and $\Delta S=11.831$ J mol^{-1} K^{-1}) indicate that electrostatic effect between the protein and the La^{3+} is the main binding force. In addition, UV–vis, CD, and synchronous fluorescence results showed that the addition of La^{3+} changed the conformation of BSA.

1. Introduction

Serum albumins have been one of the most studied proteins for many years. They are the most abundant of the proteins in blood plasma, accounting for about 60% of the total protein corresponding to a concentration of 42 g L^{-1} and provide about 80% of the osmotic pressure of blood [1]. Serum albumins have been used as a model protein for many and diverse biophysical, biochemical, and physicochemical studies [2–4]. As a kind of serum albumin, bovine serum albumin (BSA) has the advantages of medical importance, low cost, ready availability, and unusual ligand-binding properties. Bovine and human serum albumin tertiary structures are 76% similar, and the results of all studies are consistent with the fact that human and bovine serum albumin are homologous proteins [5,6].

Lanthanum is a rare-earth trace metal that naturally occurs in monazite sand and coal. The commercial use of lanthanum in manufacturing and industry is increasing. Lanthanum is a byproduct in the nuclear industry, and it is used in optical and semiconductor applications, in the agricultural sector, in metal manufacturing, and as a component in fluorescent lights and rechargeable batteries [7–9].

Considerable attention has been paid to interaction of lanthanum to organisms [10–13]. As the lanthanum level of exposure increased, a corresponding increase of lanthanum occurred in the tissues [14,15]. The effect of lanthanum on distributions and accumulations in various tissues, especially in the liver, bone, kidney, and brain of people and animals has been reported [7,16,17], but few papers have focused on the interactions of La^{3+} with serum albumin through multi-spectroscopic techniques [18]. In this paper, we use fluorescence, UV–vis absorption and CD spectra to explore the interactions between La^{3+} and BSA under simulative physiological conditions, and determine their binding constants, binding sites, and the impact on the conformational structures of BSA. This report provides a new approach to explore the biological interaction of lanthanum on the functional macrobiomolecular level.

2. Experimental

2.1. Reagents

BSA (electrophoretic pure, water content: 10%) is obtained from Sinopharm Chemical Reagent Beijing Co., Ltd. Its stock solution (1.0×10^{-5} mol L^{-1}) is prepared by directly dissolving in ultrapure water and then storing in the dark at 0–4 °C. $LaCl_3$ is acquired from Shanghai Reagent Company (China). It is the source of La^{3+} and [La^{3+}] ranges from 1×10^{-5} to 1×10^{-4} mol L^{-1}.

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Physiological buffer (Tris–saline buffer) was 0.1 mol L^{-1} Tris–HCl with 0.1 mol L^{-1} NaCl, pH 7.4.

The other common chemicals were obtained from commercial sources. Ultrapure water was used throughout.

2.2. Apparatus

Fluorescence spectra were recorded on an F-4500 Spectrofluorimeter (Hitachi, Japan) equipped with a xenon lamp light source and 1 cm quartz cells. The absorption spectra were collected at room temperature on a UV-2450 Spectrophotometer (SHIMADZU, Kyoto, Japan) in 1 cm quartz cells. Circular dichroism (CD) spectra were measured by a J-810 Spectropolarimeter (Jasco, Tokyo, Japan) at room temperature under constant nitrogen flush. The pH was measured with a pHs-3C acidometer (Shanghai Pengshun Scientific Instrument Co., Ltd.).

2.3. Methods

For the fluorescence measurement, 0.5 mL Tris–HCl (0.1 mol L^{-1}, pH 7.4), 0.5 mL BSA (1×10^{-5} mol L^{-1}) and various amounts of La^{3+} solution were added to a 5 mL colorimetric tube in sequence, then diluted with ultrapure water to 5 mL and incubated for 30 min to equilibrate the system. The equilibrated solution was transferred into the quartz cells for detection. The corresponding fluorescence emission spectra were recorded from 290 to 500 nm using an excitation wavelength of 280 nm. The excitation and emission slit widths were set at 5 nm. Scan speed was 1200 nm/min. Photo multiplier tube (PMT) voltage was 400 V.

The synchronous fluorescence spectra were obtained through simultaneous scanning of the excitation (λ_{ex}=280 nm) and emission monochromators while maintaining a constant wavelength interval between them ($\Delta\lambda$, 15 and 60 nm). The excitation and emission slit widths were set at 5 nm. Scan speed was 1200 nm/min. PMT voltage was fixed at 400 V.

To measure the UV absorption spectra, 1 ml Tris–HCl (0.1 mol L^{-1}, pH 7.4), 2 mL BSA (1×10^{-5} mol L^{-1}) and various amounts of La^{3+} solution were added to 10 ml colorimetric tubes in sequence, then diluted with ultrapure water to the mark. After 30 min, the equilibrated solution was poured into the quartz cells and the spectrum was recorded in the range of 200–300 nm.

CD spectra were collected from 190 to 260 at 0.2 nm intervals on a JASCO J-810 CD spectrometer using a quartz cell with a path length of 10 mm. The scanning speed was set at 200 nm/min. Three scans were made and averaged for each CD spectrum.

3. Results and discussion

3.1. The influence of La^{3+} on BSA structure evaluated by fluorescence spectra

BSA has three intrinsic fluorophores: tryptophan, tyrosine, and phenylalanine. Because the quantum yield of phenylalanine is very low and the fluorescence of tyrosine is almost totally quenched, the intrinsic fluorescence of BSA is almost entirely due to tryptophan [19]. By analyzing the fluorescence spectrum, we can obtain information about the interactions between BSA and La^{3+} such as the quenching mechanism, binding constant, and binding sites.

We measured the fluorescence emission spectra of BSA at a series of concentrations of La^{3+} in 0.01 mol L^{-1} Tris–HCl buffer solution (pH 7.4) by fixing the excitation wavelength at 280 nm (Fig. 1). The fluorescence intensity of BSA at the emission peak decreased regularly and the maximum emission wavelength underwent a slight blue shift from 338.6 to 336.2 nm when La^{3+} was added. This suggests that interactions occur between BSA and La^{3+}.

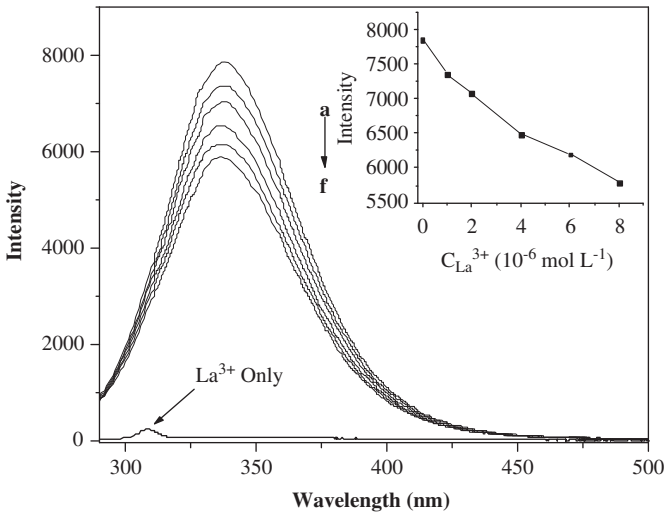

Fig. 1. The influence of La^{3+} on the fluorescence emission spectra of BSA. The inset shows the fluorescence intensities of the peak vs the La^{3+} concentration. Conditions: BSA 1×10^{-6} mol L^{-1}; La^{3+} (a) 0, (b) 1×10^{-6}, (c) 2×10^{-6}, (d) 4×10^{-6}, (e) 6×10^{-6}, (f) 8×10^{-6} mol L^{-1}, (g) La^{3+} only, 4×10^{-6} mol L^{-1}; Buffer Tris–HCl, 0.01 mol L^{-1}, pH 7.4; (T=289 K, λ_{ex}=280 nm).

There are two tryptophans within a BSA molecule, which are located at amino acid positions 134 and 213. Trp-134 is located on the surface of the protein, and the other tryptophan residue (Trp-213) is located in a hydrophobic environment, which is similar to the interior of a micelle. According to the principle of micelle sensitization in spectrophotometric determinations, the fluorescence intensity of the tryptophans in BSA is much stronger than tryptophan in aqueous solution at the same concentration. Therefore, Trp-213 contributes more to the fluorescence intensity than Trp-134, which is located on the surface of BSA [20,21].

3.2. The influence of La^{3+} on BSA structure evaluated by UV–vis absorption spectra

UV–vis absorption spectroscopy technique can be used to explore the structural changes of protein and to investigate protein–ligand complex formation. BSA has two main absorption bands. One is located in the range of 200–220 nm, which is the skeleton absorption peak, and the other is in the range of 260–300 nm, which is the absorption band of the aromatic amino acids (Trp, Tyr, and Phe). Fig. 2 shows that upon adding La^{3+}, the BSA skeleton absorption intensity around 210 nm decreased and red shifted. At the same time, the absorption peak at 260–300 nm is higher than before. Both of the results indicate that the hydrophobicity decreased and the peptide strands of BSA became more extended. Therefore, the binding between La^{3+} and BSA leads to changes in the BSA conformation [22,23].

3.3. The influence of La^{3+} on BSA structure evaluated by CD spectroscopy

CD spectroscopy is a quantitative technique to investigate the conformation of proteins in aqueous solution. To further investigate the conformation of BSA, CD spectroscopy was performed. The CD spectra (Fig. 3) displayed the characteristic peaks (intensive positive peak at around 190 nm and two negative double humped peaks at 208 and 222 nm) of a high α-helical content in native BSA.

The CD results are expressed in terms of mean residue ellipticity (MRE; in deg cm^2 $dmol^{-1}$) according to the following