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Investigation on the conformational changes of bovine serum albumin in a wide pH range from 2 to 12

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ABSTRACT

The conformation of bovine serum albumin largely depends on its microenvironment pH and affects its physical functions and applications. In this study, we investigated the effects of pH (wide range 2–12) on the conformation of bovine serum albumin based on spectroscopic signals by various spectroscopic means including fluorescence, synchronous fluorescence, resonance light scattering, UV-visible absorption, and circular dichroism. The changes in spectroscopic signals, such as peak position and intensity, showed that the structure of bovine serum albumin varied significantly with pH. The conformation of bovine serum albumin was compact at pH 6–7, as indicated by the largest peak position values and peak intensities in the fluorescence, synchronous fluorescence, and RLS spectra. The structure of bovine serum albumin became loose in the acidic or alkaline environment, as indicated by the decreased peak position values and peak intensities. The microenvironment of the amino acid residues of bovine serum albumin also varied with pH, as indicated by the changing peak position values. At pH 7, the hydrophobicity of the tyrosine and tryptophan residues was the weakest, as indicated by the minimum synchronous fluorescence signals. In addition, the secondary structure of bovine serum albumin, especially α-helix, varied with pH. The content of α-helix reached the maximum value of 68% at pH 6–8, whereas it decreased in the acidic or alkaline environment. The study provides valuable details for studying the physiological function and applications of serum albumins.

Introduction

Serum albumin is the principal extracellular protein and the most abundant protein in the vertebrate circulatory system and accounts for about 60% of the total plasma proteins.[1] As the most important transport protein in blood, it has important physical functions, such as transporting endogenous and exogenous substances, maintaining blood colloid osmotic pressure, scavenging free radical, and inhibiting platelet aggregation and blood clot.[2,3] The interactions between serum albumins and the ligands including various drugs, heavy metals, and organic pollutants have received wide attention in recent years. Due to the extraordinary capacity for binding ligands, serum albumins have a wide range of clinical, pharmaceutical, and biochemical applications. BSA (bovine serum albumin) is a single-peptide serum albumin consisting of 582 amino acid residues,[4] where two tryptophan (Trp) residues are located at the positions of 134 and 212.[5] BSA has the high structural homology to human serum albumin (76%) and is generally used as a substitute for human serum albumin.[6]

The spatial structure of a protein formed by spiraling and folding is the basis of its biological activity, whereas the conformational stability of a protein is the prerequisite for its activity stability.[7] Biomedical studies indicated that the abnormality of protein conformation caused diseases.[8,9] The behaviors and overall performance of proteins can be significantly altered by its microenvironment such as pH, salt, and surfactant.[10] pH is a significant influencing factor of protein conformation and its change results in the alteration of protein function.[11,12] Boulet et al. found that the aggregation of casein and soybean protein increased with the increase in pH.[13] Liu and Guo studied the effects of pH on structures and properties of casein micelles with fluorescent technique together with DLS and turbidity measurements and found that the conformation of casein was compact at low pH and loose at high pH and that the most compact structure occurred at pH 5.5.[14]

For the physical functions or the applications of serum albumins, the conformation of serum albumins is an important influencing factor.[15] The effects of pH on the structure of BSA has become a popular direction of research. Edri and Regev applied UV-vis spectroscopy to study the effects of pH on BSA-dispersed carbon nanotubes. At pH > 8, BSA shows the basic conformation; at pH 4–8, BSA is in a heart shape and become expanded with decrease in pH; at pH 3.5–4, the BSA adopts the faster migrating conformation; at pH < 3.5, it becomes the expanded conformation.[15] Wishnia and Pinder studied the conformational changes in
BSA below pH 5.\[16\] Xu and Grassian studied the structural changes of BSA at pH 7.4, 5.2, and 2.0 by ATR-FTIR.\[17\] He et al. found the conformation of BSA change from N to F under pH 4.3–8.0.\[18\] Givens, et al. discussed the conformation changes of BSA across the pH range of 2–8.\[19\] Cao et al. investigated pH-induced conformational changes of BSA by CD and FTIR, and found that the secondary structure of BSA changed from N to F below pH 5.\[20\] Qing et al. investigated the secondary structure changes of BSA in aqueous solution at two pH values (5 and 9) with FTIR spectroscopy.\[21\] Mei et al. considered the secondary structure of BSA at different pH values (4.3, 5.3, 10.1) by CD.\[22\]

Although various techniques are available for studying the conformational change of serum albumins, spectroscopic means are the most important methods due to their high sensitivity.\[23\] Detailed information about the protein conformation can be deduced from a mass of information accumulated by spectroscopic studies.\[24\] However, the previous spectroscopic studies were only focused on the conformational transition of BSA in the small pH range of 2–9, the studies of conformational changes in BSA is not comprehensive. In this study, we established the relationship between pH and spectroscopic signals indicating BSA conformation in a broad pH range of 2–12 through spectroscopic assays including fluorescence, synchronous fluorescence, resonance light scattering (RLS), UV-visible absorption, and circular dichroism. Compared with others research, this study can provide valuable information for the research and applications of serum albumins under different pH conditions. The study can also help to clarify protein conformational changes at different pH and understand the physiological function of serum albumins.

**Materials and methods**

**Reagents**

BSA (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) was dissolved in ultrapure water to form a 1.0 × 10⁻⁵ mol L⁻¹ stock solution and then stored at 0–4°C and 0.2 mol L⁻¹ phosphate buffer (mixture of NaH₂PO₄·2H₂O and Na₂HPO₄·12H₂O) was used to adjust the pH. Na₂HPO₄·2H₂O and Na₃HPO₄·12H₂O (analytically pure) were purchased from Tianjin Damao Chemical Reagent Factory, Tianjin, China. Ultrapure water was used throughout the experiments.

**Experimental instruments**

The experimental instruments adopted in the study include pHs-3C pH meter (Shanghai Pengshun Scientific Instrument Co., Ltd., Shanghai, China), F-4600 spectrofluorometer (Hitachi, Tokyo, Japan), UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan), and JASCO J-810 CD spectrometer (Jasco, Tokyo, Japan).

**Experimental methods**

**Fluorescence measurements**

All fluorescence spectra were recorded on an F-4600 spectrofluorometer (Hitachi, Japan). Before the measurements, 1.0 mL of 0.2 mol L⁻¹ phosphate buffer (pH 7.4) and 1.0 mL of 1.0 × 10⁻⁵ mol L⁻¹ BSA were successively added into each of a series of 10 mL test tubes. After 20 min equilibration, the fluorescence spectra were then measured (excitation wavelength at 278 nm and emission wavelengths of 290–450 nm). The excitation and emission slit widths were set at 5.0 nm. The scan rate and photomultiplier tube (PMT) voltage were 1200 nm/min and 700 V, respectively. The synchronous fluorescence spectra were measured at λₑₓ= 250 nm, Δλ = 15 nm, and Δλ = 60 nm.

**Resonance light scattering (RLS) measurements**

The RLS spectra were obtained by simultaneously scanning the excitation and emission monochromators with the slit width of 5.0 nm (Δλ = 0.0 nm) from 200 to 800 nm. The scan rate was 1200 nm/min.

**UV-visible absorption measurements**

The absorption spectra were recorded on a UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan) equipped with 10 nm quartz cells. Slit width was set at 2.0 nm. The wavelength range was 200–450 nm.

**Circular dichroism (CD) measurements**

CD spectra were collected from 200 to 260 with an interval of 0.2 nm on a JASCO J-810 CD spectrometer (Jasco, Tokyo, Japan) with a quartz cell with a path length of 10 mm. The scan rate was set as 200 nm/min. Three scans were made and averaged for each CD spectrum.

**Results and discussion**

**Spectra signals of BSA**

Typical spectroscopic signals of BSA solution at pH 7 shown in Fig. 1 were recorded by fluorescence spectrometer, ultraviolet-visible spectrophotometer, and CD spectrometer. The fluorescence emission spectra of BSA exhibit a peak at 338 nm. The peaks of the synchronous fluorescence spectra appear at 285.8 nm (Δλ = 15 nm) and 278.7 nm (Δλ = 60 nm). When Δλ was set as 15 and 60 nm, the synchronous fluorescence gives the characteristic information of tyrosine (Tyr) residues or Trp residues, respectively. The peak of RLS spectrum of BSA is located at 275.5 nm. The ultraviolet-visible absorption spectra possess a major peak at 205.8 nm, which is ascribed to the transition of π→π* of BSA’s characteristic polypeptide backbone structure C=O.\[25\]

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In the CD spectra of BSA, two negative peaks at 208 and 222 nm are the characteristic peaks of an α-helical structure of proteins.\[20\]

The structural changes of BSA can cause the alterations in the spectroscopic signals. We investigated the
conformational changes of BSA with pH in a broad range (pH 2–12) by using the above spectrometers.

Fluorescence emission spectra of BSA under different pH conditions

BSA contains three kinds of intrinsic fluorophores: Trp, Tyr, and Phenylalanine (Phe).

Figure 1. The spectroscopic signals of bovine serum albumin. (A) fluorescence spectrum; (B) synchronous fluorescence spectrum (Δλ = 15 nm); (C) synchronous fluorescence spectrum (Δλ = 60 nm); (D) resonance light scattering (RLS) spectrum; (E) UV-Vis spectrum; (F) circular dichroism spectrum. The fluorescence emission spectrum of bovine serum albumin exhibits a peak at 338 nm. The peaks of the synchronous fluorescence spectra appear at 285.8 nm (Δλ = 15 nm) and 278.7 nm (Δλ = 60 nm). The peak of RLS spectrum is located at 275.5 nm. The UV-Vis absorption spectrum possesses a major peak at 205.8 nm. Conditions: bovine serum albumin: (A–D) 1.0 × 10^{-6} mol L^{-1}; (E) 2.0 × 10^{-6} mol L^{-1}; (F) 2.0 × 10^{-7} mol L^{-1}; pH = 7; 298 K.

Figure 2. The fluorescence spectra of bovine serum albumin under different pH. The peak position values rise gradually from 324 nm to 338 nm (red shift), then decline (blue shift), and finally decrease to 332 nm, showing that the hydrophobicity of amino acid residues reduces or increases, respectively. The peak intensities increase from 4028 to 7748 and then decrease sharply to 2297, revealing the high or low level of energy transfer among the aromatic amino acid residues of bovine serum albumin. Conditions: bovine serum albumin: 1.0 × 10^{-6} mol L^{-1}; 298 K.

Trp residues located at positions 134 and 212 (mainly the one at position 212) contribute most to the fluorescence intensity (about 95%) of BSA. By analyzing the fluorescence emission spectra of BSA, we obtained its structure and dynamic information.

As shown in Fig. 2, both the positions and intensities of the fluorescence peaks of BSA vary with pH and show the similar tendency. The changes in peak positions of fluorescence emission spectra of BSA solution indicate the
Alternations in protein hydrophobicity. The peak intensity reflects the level of energy transfer among the aromatic amino acid residues of BSA. The peak position values rise gradually from 324 nm to 338 nm, then decline, and finally decrease to 332 nm, indicating the first red shift and subsequent blue shift. The red or blue shift of the peak position shows that the hydrophobicity of amino acid residues reduces or increases, respectively. The peak intensities increase from 4028 to 7748 and then decrease sharply to 2297. The increased or decreased peak intensities revealed the high or low level of energy transfer among the aromatic amino acid residues of BSA. The highest values for the peak position values and intensities appear at pH \( \approx 6-7 \) (near neutral pH), which is close to the BSA isoelectric point (4.6–5.8), indicating that the energy transfer among the amino acid residues of BSA is effective. Therefore, the protein structure is compact and the hydrophobicity of the protein is the weakest. When the pH environment of BSA tends to high acidity or alkalinity, the peak position values and intensities decrease, indicating that the structure of BSA extends and that the level of energy transfer among aromatic amino acid residues decreases. In summary, the phenomena suggested that pH could change the micro-environment of fluorophores and the structure of BSA.

**Synchronous fluorescence spectra of BSA under different pH conditions**

Synchronous fluorescence spectroscopy can effectively distinguish the characteristic peaks of Tyr and Trp residues. The effects of different pH values on the peak position values and intensities in the synchronous fluorescence spectra of Tyr (\( \Delta \lambda = 15 \text{ nm} \)) and Trp (\( \Delta \lambda = 60 \text{ nm} \)) residues are shown in Figs. 4 and 5.

With the increase in pH, the peak position values increase from 284 nm (pH = 2) to 285.8 nm (pH = 7) and finally decreased to 284.7 nm (pH = 12) (Fig. 3). When the pH increases from 2 to 7, the hydrophobicity of Tyr residues decreases and Tyr residues buried in nonpolar hydrophobic cavities move to a more hydrophilic environment. As pH increases from 7 to 12, the reverse process happens. The hydrophobicity of Tyr residues of BSA is the weakest at pH 7 in the range of 2–12. In the pH range of 2–7, the peak intensities increase from 1647.67 (pH = 2) to 1866.33 (pH = 3) and then decrease finally to 1570.67 (pH = 7). With the increase in pH from 7 to 12, the peak intensities increase from 1570.7 (pH = 7) to 1629.7 (pH = 9) and finally decrease to 779 (pH = 12).

With the increase in pH from 2 to 7, the peak position values increase from 275.4 nm to 278.7 nm (Fig. 4). When pH is greater than 7, the peak position values start to decline and finally decrease to 276.5 nm at pH = 12. The increase of pH from 2 to 7 can decrease the hydrophobicity of the Trp residues and thus the Trp residues buried in nonpolar hydrophobic cavities move to a more hydrophilic environment. The opposite changing process occurs as pH increases from 7 to 12. The weakest hydrophobicity of Trp residues of BSA arises at pH 7 in the range of 2–12. From pH 2–4.5, the peak intensities increase from 3693.7 to 7320.7. When pH continues to increase from 4.5 to 12, the peak intensities decrease, and finally reach 2206.

In brief, the micro-environment (hydrophobicity) of Tyr and Trp residues of BSA can be changed by changing pH values. The hydrophobicity of Tyr and Trp residues are the weakest at pH = 7 in the range of 2–12.

**RLS spectra of BSA under different pH conditions**

The resonance light scattering (RLS) is a highly sensitive method for studying identification, assembly, and aggregation of biological macromolecules depending on the sensitive and abundant light scattering signals. The RLS spectra of
BSA in various pH solutions are shown in Fig. 5. As the pH increases, the peak position values and intensities of RLS spectra increase at first and then decreases. At pH = 6 (near the isoelectric point of BSA), the peak position values and intensities reach the maximum, showing a relatively compact BSA structure. In a strong acidic/alkaline environment, BSA molecules are positively charged or negatively charged due to protonation or deprotonation, thus resulting in the decline in the compact BSA structure followed by the decline in RLS signals (peak position values and intensities).

**Figure 4.** The synchronous fluorescence spectra of bovine serum albumin ($\Delta \lambda = 60$ nm) under different pH. The peak position values increase from 275.4 nm to 278.7 nm with increasing pH values from 2 to 7 (indicating decreased hydrophobicity of Tyr residues). When pH is greater than 7, the position values start to decline and finally decrease to 276.5 nm at pH = 12 (showing increased hydrophobicity of Tyr residues). Conditions: bovine serum albumin: $1.0 \times 10^{-6}$ mol L$^{-1}$; 298 K.

**Figure 5.** The RLS spectra of bovine serum albumin under different pH. The peak position values and intensities of RLS spectra increase at first and then decreases with increasing pH values, revealing the changes of bovine serum albumin structure. At pH = 6 (near the isoelectric point of bovine serum albumin), the peak position values and intensities reach the maximum, showing a relatively compact BSA structure. Conditions: bovine serum albumin: $1.0 \times 10^{-6}$ mol L$^{-1}$; 298 K.

**UV-visible absorption spectra of BSA under different pH conditions**

The structural changes of protein can be analyzed by the UV-visible absorption spectroscopic technique. From the analysis of the UV-vis absorption spectra of BSA under different pH conditions (Fig. 6), it can be seen that with the increase of pH, the intensities of the major peak decreases from 2.5 to 1.8, the position of the major peak displaying a red shift from 199 nm to 206 nm. The phenomena indicate the dramatic changes in BSA conformation (the loosening and unfolding of the protein skeleton) under varying
pH conditions.\textsuperscript{[15]} However, the spectral signals (especially the peak intensity) vary slowly within the pH range of 5–9, indicating that the BSA molecular conformation (“heart” type) is more stable.\textsuperscript{[15]}

**Circular dichroism spectra of BSA under different pH conditions**

CD measurements can effectively detect the secondary structure of proteins. The \( \alpha \)-helical content of BSA under different pH values was calculated from Eqs. [1] and [2].\textsuperscript{[36]}

\[
\text{MRE} = \frac{\text{Observed CD(mdeg)}}{C_p n l \times 10}
\]

where \( C_p \) is the molar concentration of the protein; \( n \) is the number of amino acid residues (574); \( l \) is the path length of the cell (1 cm).

\[
\alpha\text{-Helix(\%)} = \frac{-\text{MRE}_{208} - 4000}{33,000 - 4000} \times 100
\]

where \( \text{MRE}_{208} \) is the observed MRE value at 208 nm; 4000 is the MRE of the \( \beta \)-form and random coil conformation cross at 208 nm; 33,000 is the MRE value of a pure \( \alpha \)-helix at 208 nm.

As shown in Fig. 7, when pH increases from 2 to 4.5, the \( \alpha \)-helix content of BSA increases from 43% (pH = 2) to 62% (pH = 3.5) and finally decrease to 55% (pH = 4.5). When pH increases from 4.5 to 6, the content of \( \alpha \)-helix increases from 55% to 68%. In the pH range of 6–8, the \( \alpha \)-helix content reaches the maximum value and remains constant. As pH continues to increase from 8 to 11, the \( \alpha \)-helix content starts to decrease from 68% to 60%. In the strong alkaline environment (pH 11–12), the \( \alpha \)-helix content slightly increases from 60% to 63%. BSA has the most compact conformation under near-neutral pH environment 6–8 (near the isoelectric point of BSA, pH 5.8), indicating the highest \( \alpha \)-helix content of BSA (68%). While at the alkaline environment or the strong acidic environment, the structure of BSA becomes loose with the decreasing \( \alpha \)-helix content. In a word, the content of \( \alpha \)-helix in BSA varies with pH. BSA has the highest \( \alpha \)-helix content (68%) in the near-neutral environment (6–8), followed by the alkaline environment, and the strong acidic environment.

**Conclusions**

In summary, we explored the changes of spectroscopy signals (peak position values and intensities) of BSA in a wide pH range of 2–12 with different spectroscopic methods such as

![Figure 6.](image-url) The UV-Vis spectra of bovine serum albumin under different pH. The decrease of the intensity (from 2.5 to 1.8) and the red shift of the position (from 199 nm to 206 nm) of the major peak with increasing pH values, indicating the dramatic changes in bovine serum albumin conformation (loosening and unfolding of the protein skeleton). Conditions: bovine serum albumin: \( 2.0 \times 10^{-4} \text{ mol L}^{-1} \); 298 K.

![Figure 7.](image-url) Effect of pH on the \( \alpha \)-helix conformation of bovine serum albumin. Bovine serum albumin has the highest \( \alpha \)-helix content (68%) in the near-neutral environment (6–8), followed by the alkaline environment, and the strong acidic environment. Conditions: bovine serum albumin: \( 2.0 \times 10^{-7} \text{ mol L}^{-1} \); 298 K.
fluorescence, synchronous fluorescence, RLS, UV-visible absorption, and CD. With the change in pH, the conformation of BSA, especially the secondary structure, changed and the microenvironment of amino acid residues varied as well. At pH 6–7, all the fluorescence, synchronous fluorescence, and RLS spectra showed the largest peak position values and peak intensities, indicating that the structure of the BSA was compact and that the hydrophobicity of the fluorophores was the weakest. The weakest hydrophobicity of Tyr and Trp resides appeared at pH 7. In acidic or alkaline environments, the fluorescence, synchronous fluorescence, and RLS spectra showed the decreased peak position values and intensities, revealing that BSA conformation became loose and unfolded. When pH increases from 2 to 12, the peak position values of UV-visible absorption spectra increased from 199.3 nm to 205.8 nm, and the peak intensities decreased. However, at pH 5–9, the peak intensities changed slowly due to the relatively stable conformation of BSA. The CD spectra indicated that the α-helix content had the maximum value of 68% at pH 6–8. The α-helix content in the alkaline environment was higher than that in the strong acidic environment. Our results can provide valuable information for the research and applications on serum albumins under different pH conditions and help to understand the physiological function of serum albumins.

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