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Interaction of Cr(VI) Binding with Bovine Serum Albumin: Reaction Mechanisms and Thermodynamic Properties

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The interaction between Cr(VI) and bovine serum albumin (BSA) was investigated by spectroscopic method combined with thermodynamic experiment. Binding model of Cr(VI)-BSA system was found. The binding parameters were determined and the mechanism of interaction was interpreted. The effect of Cr(VI) acting on the bovine serum albumin's conformation was detected and the microenvironment change of tyrosine and tryptophan residues induced by Cr(VI) was explored. The enthalpy change ΔH and the entropy change ΔS of Cr(VI)-BSA system were calculated, which suggested that the main binding feature between Cr(VI) and bovine serum albumin is hydrophobic force.

Key Words: Cr(VI), Bovine serum albumin, Reaction mechanisms, Thermodynamic properties.

INTRODUCTION

Heavy metals are an increasing pollutant of the environment and there is a growing need to explorer their toxicity and develop effective methods for their detection. The heavy metal binding properties of biomolecules are linked to their toxicologic features, in particular their interaction with the protein molecule, has been attracting increasingly the interest of many research groups. Cr(VI) has a strong toxic effect, which can be enriched and accumulated to living organisms[1]. The study of interaction between Cr(VI) to protein plays a role in the prevention of poisoning and treatment of environmental pollution. Recently, some toxic interactions of heavy metal ions with proteins have been investigated, but seldom report on the interaction of serum albumin with Cr(VI). Bovine serum albumin (BSA) has been selected as protein model because of its structure homology with human serum albumin (HSA)[2,3]. This work focused on the interaction of BSA-Cr(VI) by spectra combined with thermodynamic experiment.

EXPERIMENTAL

0.1 mol L^{-1} *tris*-HCl buffer (pH 7.4, 0.10 mol L^{-1} NaCl was used to keep the ionic strength constant) was prepared. The working solution of bovine serum albumin (1×10^{-5} mol L^{-1}) and Cr(VI) stock solution (1 mmol L^{-1}) were prepared by dissolving them in *tris*-HCI buffer, respectively.

Bovine serum albumin were added to a 1 cm quartz cell and then adding Cr(VI) into the quartz cell gradually (total volume ≤ 200 L). Fluorescence quenching spectra of bull serum albumin were recorded from 250 to 500 nm with an excitation wavelength of 282 nm at 25 ºC and 37 ºC. The synchronous fluorescence spectra were recorded from 230 to 360 nm and 260 to 360 nm at $\Delta\lambda$ = 60 nm and $\Delta\lambda$ = 15 nm respectively. BSA-Cr(VI) solution absorption spectra was also determined.

RESULTS AND DISCUSSION

Fluorescence quenching mechanisms are usually classified into dynamic and static quenching and the dynamic fluorescence quenching data can be analyzed by Stern-Volmer equation[4]:

$$F_0/F = 1 + K_q\tau_0[D] = 1 + K_{SV}[D] \quad (1)$$

where F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively; K_q is the quenching rate constant of the biomolecule; τ_0 is the average fluorescence lifetime of the biomolecule without Cr(VI); [D] is the concentration of the quenchers, mol L^{-1}; K_{sv} is the Stern-Volmer quenching constant, L mol^{-1} ($K_q = K_{SV}/\tau_0$). The binding constant (K) and binding sites (*n*) are calculated by the double-logarithm equation[5]:

$$\log[(F_0 - F)/F] = \log K + n\log[D_t] \quad (2)$$

The fluorescence of BSA quenched by various concentrations of Cr(VI) at 25 ºC and 37 ºC were shown in Fig. 1. It was observed that the fluorescence intensity of BSA dropped

regularly with obvious blue shifts of fluorescence of maximum emission wavelength (λ_{em}, 10 nm at 25 ºC and 6 nm at 37 ºC) by adding Cr(VI), which indicated that interaction had happened between Cr(VI) and BSA. Bovine serum albumin had a higher levels of quenching at 25 ºC than 37 ºC.

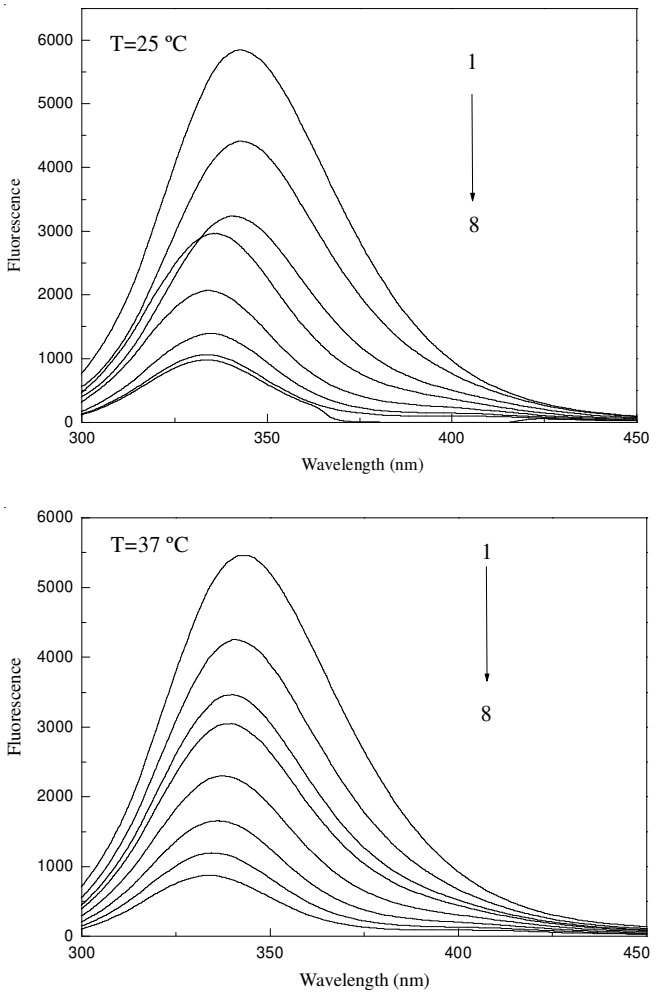


Fig. 1. Effect of Cr(VI) on quenching of BSA fluorescence (25 ºC and 37 ºC), $c_{(BSA)} = 1.0 \times 10^{-5}$ mol L^{-1}, $c_{(Cr(VI))}/10^{-4}$ mol L^{-1}, 1 to 8: 0, 0.4, 0.8, 1.0, 1.6, 2.4, 3.2, 4.0

The values of F_0/F for BSA had good linear relation with [D] (Fig. 2). The values of K_q (Table-1) were far greater than the expected maximum dynamic quenching constant (2×10^{10} L mol^{-1} s^{-1}) and the value of K_q drop significantly with the increase of temperature, which proved that the quenching between BSA and Cr(VI) was not initiated by dynamic collision but from the formation of a stable complex.

The binding constant (K) and number of binding sites (n) were calculated by eqn. (2) and the corresponding linear curves were shown in Fig. 3. The results were listed in Table-2.

From Table-2, the values of K of BSA-Cr(VI) were in the range of 10^4-10^7 L·mol^{-1}, which agreed with the common affinity of ligand for albumin. Both the K and n at 25 ºC were smaller compared with those at 37 ºC, which indicated that Cr(VI) may be got nearer to tryptophan residues or tyrosine residues at 37 ºC. The changed binding parameters indicated that the temperature plays important roles in BSA-Cr(VI) binding.

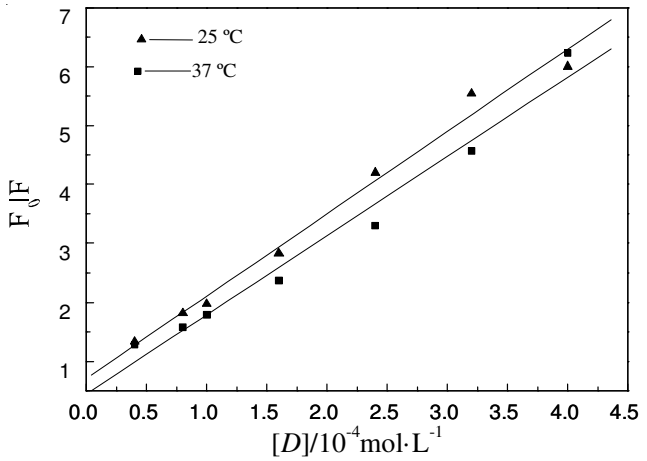


Fig. 2. Stern-Volmer polts of BSA quenched by Cr(VI)

TABLE-1
QUENCHING CONSTANTS OF Cr(VI) to BSA

Sample	T (ºC)	K_{SV} (L mol^{-1})	K_q (L mol^{-1} s^{-1})	R
BSA-Cr(VI)	25	1.40×10^4	1.40×10^{12}	0.9935
	37	1.34×10^4	1.34×10^{12}	0.9879

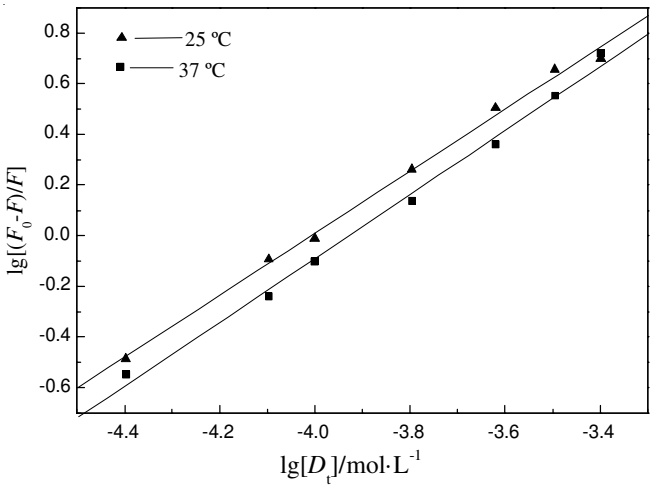


Fig. 3. Double-logarithm curves of BSA quenched by Cr(VI)

TABLE-2
BINDING PARAMETERS OF Cr(VI)-BSA SYSTEM

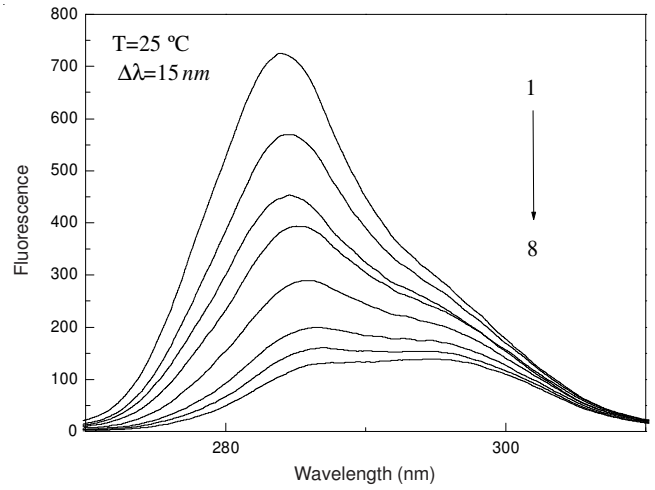
T (ºC)	K (L mol^{-1})	n	R
25	8.01×10^4	1.22	0.9980
37	9.06×10^4	1.26	0.9971

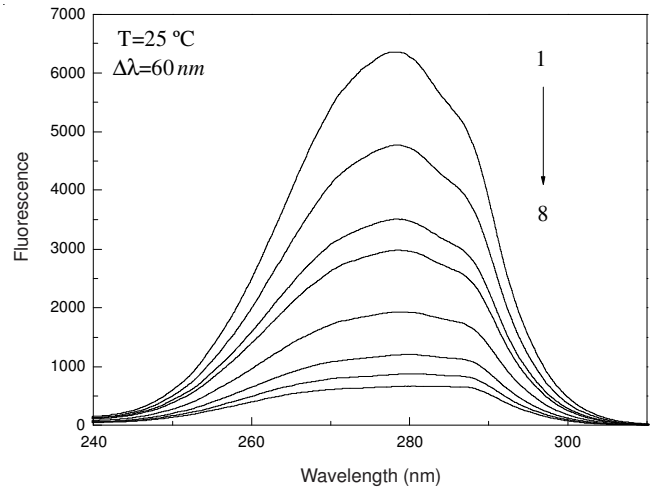
The maximum emission wavelength of amino acid residues can be affected by their chemical environment, therefore, the changes in the maximum emission wavelength of synchronous fluorescence spectra can be uesd as a index to evaluate the microconformational changes of proteins. The synchronous fluorescence spectra were shown in Fig. 4, where $\Delta\lambda = 60$ nm only indicates the fluorecence emitted by tryptophan (Trp) residues and $\Delta\lambda = 15$ nm only indicates the fluorecence emitted by tyrosine (Tyr)[6] residues.

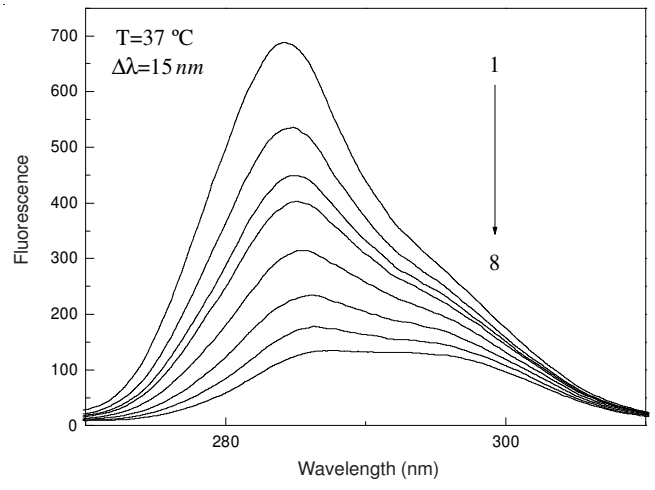
From Fig. 4, the synchronous fluorescence intensity of BSA dropped regularly with obvious red shifts (λ_{em}, 3 nm at 25 ºC and 4 nm at 37 ºC) of the maximum emission wavelength, which indicated that Cr(VI) had changed the microconformation of BSA, making the microenvironments

of tyrosine and tryptophan residues more hydrophilic. In addition, the fluorescence intensity reduction of tryptophan residues was greater than that of tyrosine residues, which indicated that the binding sites of BSA-Cr(VI) is nearer to tryptophan residues than tyrosine residues[7]. Bovine serum albumin had a higher levers of red shifts at 25 ºC than 37 ºC indicated that the temperature also plays important roles in changes of microconformation of BSA.

UV-visible absorption is an effective method to explore the structural change[8]. Fig. 5 showed the UV-visible absorption







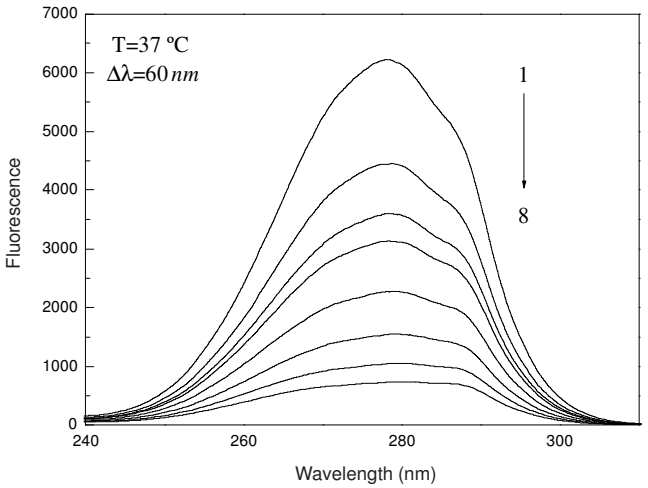


Fig. 4. Effect of the Cr(VI) on the synchronous fluorescence spectrum of BSA, $c_{(BSA)} = 1.0 \times 10^{-5}$ mol L^{-1}, $c_{(Cr(VI))}/10^{-4}$ mol L^{-1}, 1 to 8: 0, 0.4, 0.8, 1.0,1.6, 2.4, 3.2, 4.0

spectra of BSA and BSA-Cr(VI), from which we could see that the presence of Cr(VI) caused the increasing of the absorption band at 278 nm of BSA. All the spectra suggested that Cr(VI) induces the peptide chains of BSA more extension and changes the microconformation and making the tryptophan and tyrosine residues which were buried within the hydrophobic cavities more exposed to the polar environment. Meanwhile the hydrophobic effect among hydrophobic groups in BSA may be attenuated by Cr(VI). Thus the blue shifts ($\Delta\lambda = 2$ nm) of the absorption band at 278 nm of BSA occurred.

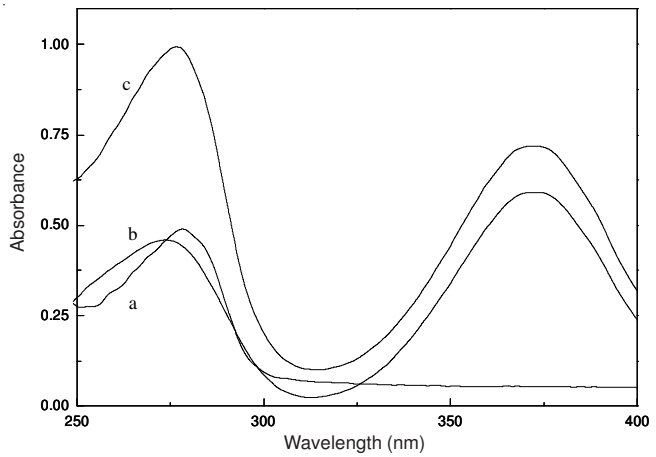


Fig. 5. UV-visible spectra of BSA (a), Cr(VI) (b) and BSA-Cr(VI) (c) ,$c_{(BSA)} = 1.0 \times 10^{-5}$ mol L^{-1}, $c_{(Cr(VI))} = 1.0 \times 10^{-4}$ mol L^{-1}

The molecular forces between small molecular and protein molecular may be hydrophobic interactions, electrostatic interactions, hydrogen bonds, van der Waals interactions, *etc*. The thermodynamic parameters for protein reactions are the main evidence for confirming the molecular forces, which can be calculated from the binding constant at two temperatures (298 and 310 K). The thermodynamic parameters were determined from the van't Hoff formula:

$$\ln(K_2 / K_1) = (\Delta H / R) \cdot (1/T_1 - 1/T_2) \quad (3)$$

The free energy change (ΔG) of the binding reaction at different temperature was calculated from:

$$\Delta G = \Delta H - T \cdot \Delta S \quad (4)$$

$$\Delta G = -RT \ln K \quad (5)$$

where K is the binding constant at the corresponding temperature T. ΔH is the enthalpy change and ΔS is entropy change. Table-3 gives the values of ΔH, ΔS and ΔG. The negative sign for ΔG means the spontaneity of the binding of Cr(VI) with BSA. From the positive ΔS value and the positive ΔH value observed in the present study, it could be concluded that hydrophobic interactions is the main molecular forces in the BSA-Cr(VI) system[9].

TABLE-3
THERMODYNAMIC PARAMETERS OF BSA-Cr(VI)

T (K)	ΔH (J mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔG (J mol^{-1})
298	7871.27	120.29	-27974.67
310	7871.27	120.29	-29418.13

Conclusion

The results of fluorescence quenching measurements and thermodynamic parameters suggested that Cr(VI) could bind to BSA through the hydrophobic interaction with high binding ability. The binding constant and the number of binding sites between Cr(VI) and BSA were obtained based on the fluorescence experiment results. UV-VIS absorption spectra also showed that the structure and microenvironment of BSA changed after Cr(VI) binding to BSA. The results provided important insight into the effect of strong oxidizing heavy metals on protein.

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