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Comparative Study on the Interaction Between Bovine Serum Albumin and Different Chlorophenols by Spectroscopic Approach

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The interaction of 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP) and pentachlorophenol (PCP), with bovine serum albumin (BSA) was investigated by means of fluorescence spectrometry under simulative physiological conditions. It was found that the intrinsic fluorescence of BSA was quenched uniformly at low PCP concentration or higher 2,4-DCP concentration, while the fluorescence quench of BSA was not observed with the addition of 4-CP. The fluorescence data analysis indicated that PCP and 2,4-DCP bind strongly to BSA by complex formation and the association constants determined by static quenching equation were calculated to be 3.44×10^5 and 1.82×10^4 L mol^{-1} at 298 K for PCP-BSA and 2,4-DCP-BSA interaction, respectively. The binding affinity order of the three chlorophenols is PCP > 2,4-DCP > 4-CP. The thermodynamic calculation implied that hydrophobic interaction and electrostatic interaction involved in the interaction process. Conformation investigation results confirmed BSA is predominantly α-helical although the microenvironment of BSA was partly changed with the addition of PCP or 2,4-DCP.

Key Words: Chlorophenol, Bovine serum albumin, Fluorescence quenching, Circular dichroism, FT-IR spectroscopy, Binding affinity.

INTRODUCTION

The serum albumin, as the major soluble protein constituent of the circulatory system, has many physiological functions. The most important property of this group of protein is that they serve as a depot protein and transport protein for a variety of compounds[1]. It plays an important role in the transport and disposition of a variety of endogenous and exogenous ligands presented in blood. These ligands include metal ions, fatty acids, amino acids, diverse drugs and toxicants[2,3]. The binding of toxicants to serum albumin has toxicological importance, since it controls their free, active concentration and affects duration and intensity of their effects[4].

Chlorophenols, a class of chlorine atoms substituted phenols, have been used in agriculture, industry and public health as bactericides, insecticides, herbicides, fungicides and wood preservative as well as intermediates of dyes since the 1920s[5,6]. The annual worldwide production of chlorophenols has been estimated to be 200,000 tons[7]. However, most chlorophenols are toxic to a wide range of organism[8,9]. Due to their high toxicity and potential carcinogenicity, many of them are included in the list of priority toxic pollutants by United States Environmental Protection Agency and China[10,11]. Because of their persistence in the environment, they are still commonly detected in soil, sediments, surface water and may do harm to people[6]. Seriously, recent investigations have shown that significant levels of several chlorophenols in the blood and urine of people through occupational exposure[12-14]. However, according to the available literature, the binding properties of chlorine atom substituted phenols towards plasma proteins have seldom been thoroughly studied and the contribution of chlorine atom in the binding has not yet been involved.

In order to gain a better understanding of the interactions between plasma proteins with chlorophenols, we investigated *in vitro* the binding properties of 4-chlorophenol (4-CP), 2,4-dichlorophenol (2,4-DCP) and pentachlorophenol (PCP) with bovine serum albumin (BSA) by fluorescence spectroscopy. The effect of the three chlorophenols on the conformational change of BSA was characterized by synchronous spectroscopy, circular dichroism (CD) and Fourier transform infrared (FT-IR) spectroscopy. Bovine serum albumin is selected as our protein model because of its long-standing interest in the protein community[15]. On the basis of this study, the interaction mechanisms and conformational change of chlorophenols on BSA were disclosed and the correlation between the binding affinities and the number of substituted chlorine atom on phenol was also discussed. The work can be benefit understanding the toxicity of chlorophenols and cast some light on the future study of the interaction between other environmental pollutants.

EXPERIMENTAL

4-Chlorophenol, 2,4-dichlorophenol and pentachlorophenol were purchased from Sinopharm Chemical Regent Co. Ltd. (China). BSA (Fraction V) was purchased from Merck (Germany)and used without further purification. NaCl solution (1.0 mol L^{-1}) was used to keep the ionic strength of simulative physiological condition at 0.1. Buffer solution consists of *tris* (0.2 mol L^{-1}) and HCl (0.1 mol L^{-1}) was used to keep the pH of the solution at 7.40. Three chlorophenols stock solutions were obtained by dissolving them in ethanol. Bovine serum albumin solution was prepared in the *tris*-HCl buffer solution (pH 7.40) and kept in the refrigerator at 4 ºC. All other reagents were of analytical reagent grade and ultra-pure water was used throughout the experiment.

General procedures: Fluorometric experiments were taken as follows: to 10 mL cuvettes, NaCl (1.0 mol L^{-1}, 1.0 mL), BSA (2.0×10^{-5} mol L^{-1}, 1.0 mL) and appropriate amounts of chlorophenols solution were added, diluted to 10 mL with *tris*-HCl buffer solution and mixed well. The resulting solutions were allowed to stand for 0.5 h at the constant temperature before measurement. The fluorescence spectra were then recorded on an F-4500 spectrofluorimeter (Hitachi, Japan) in a 1.0 cm quartz cell at 298, 308 and 318 K. Temperatures were maintained by a thermostat bath with recycling water throughout the experiments. The excitation wavelength and emission wavelength range were set as 295 and 310-500 nm, respectively. The widths of both the excitation and emission slit were set to 5.0 nm. In the meantime, the synchronous fluorescence spectra of BSA in the absence and presence of increasing amount of chlorophenols were recorded at λ_{ex}: 250-350 nm, with the wavelength interval ($\Delta\lambda$) between excitation and emission wavelength stabilized at 15 nm or 60 nm, respectively. To eliminate the inner filter effect of the three chlorophenols, the absorbance measurements were performed at excitation and emission wavelengths of BSA and the corrected fluorescence values were taken throughout the fluorescence analysis by the equation[16]:

$$F_{cor} = F_{obsd} \times e^{\frac{A_{295}+A_{350}}{2}} \quad (1)$$

Circular dichroism (CD) spectra were measured on a Jasco J-810 spectropolarimeter (Jasco, Japan) at room temperature, using a cylindrical quartz cell with the path length of 0.1 cm. The spectra were recorded over the wavelength range of 200-260 nm with a constant scanning speed (100 nm min^{-1}) and response time (0.5 s). The instrument was controlled by Jasco's Spectra Manager™ software. For the CD experiment, BSA concentration was kept at 1.0×10^{-5} mol L^{-1} and the ratios of chlorophenol to BSA are 0:1, 1:1 and 10:1. Blank buffer solution was used as a spectral reference and subtracted from the sample.

FT-IR spectra were obtained using a ThermoNicolet 5700 spectrometer (USA). Samples of BSA solution were prepared using deuterated water and chlorophenol solution was prepared with DMSO so as to prevent interference by water in the amide region of protein spectra. The samples were placed in a liquid cell between two CaF_2 windows and all the spectra were taken with resolution of 4 cm^{-1} and 256-scan. For the spectra processing procedures, spectra of solvent were collected at the same condition. The FT-IR spectrum of free BSA was acquired by subtracting the absorption of deuterated water from the spectrum of the protein solution. The subtracting criterion was that the original spectrum of protein solution between 2200 and 1800 cm^{-1} was featureless[17]. For the experiment, the protein concentration was 40 mg mL^{-1} and the molar ratio of chlorophenol to protein was 1:1.

RESULTS AND DISCUSSION

Fluorescence quenching study: Bovine serum albumin molecule has two tryptophan residues that possess intrinsic fluorescence. The intrinsic fluorescence of albumins results mainly from tryptophan residues when excited at a wavelength of 295 nm[18]. A valuable feature of intrinsic fluorescence of proteins is the high sensitivity of tryptophan to its local environment. Changes in emission spectra of tryptophan are common in response to protein conformational transitions, subunit association, substrate binding or denaturation[19,20].

The effect of 4-CP, 2,4-DCP and PCP on the fluorescence intensity of BSA at pH 7.40 were shown in Fig. 1. As seen in Fig. 1, BSA shows a strong fluorescence emission with a peak at 350 nm when excited at 295 nm due to its tryptophan residue, while all the three chlorophenols were almost non-fluorescent under the same experimental conditions. At the low PCP concentration (2×10^{-6} mol L^{-1}), the fluorescence intensity of BSA was regularly decreased with the increasing PCP concentration (Fig. 1a), which indicates that PCP is an effective quencher for BSA. At the same low concentration, both 2,4-DCP and 4-CP did not cause noticeable fluorescence quenching of BSA (figure not shown). At higher concentration (2×10^{-5} mol L^{-1}), BSA fluorescence was observed decreased with the increase of 2,4-DCP (Fig. 1b), while the fluorescence intensity of BSA still keep unaffected for 4-CP (Fig. 1c). The results indicate the quenching ability of the three chlorophenols to BSA is PCP > 2,4-DCP > 4-CP.

Quenching mechanism and association constant: Quenching is the reduction of the intensity of fluorescence of a given substance in solution and results from the addition of a substance called quencher[21]. Fluorescence quenching can be dynamic, resulting from collisional encounters between the fluorophore and quencher or static, resulting from the formation of a ground-state complex between the fluorophore and quencher. In order to establish the fluorescence quenching mechanism of BSA by chlorophenols, the data from the fluorescence experiment at different temperatures (298, 308 and 318 K) can be analyzed using the classical Stern-Volmer equation[22]:

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{SV}[Q] \quad (2)$$

where F and F_0 are the fluorescence intensities with and without quencher. K_q, K_{SV}, τ_0 and [Q] are bimolecular quenching constant, the Stern-Volmer quenching constant and the average fluorescence lifetime of biomolecule without quencher and concentration of quencher, respectively. $K_{SV} = K_q\tau_0$, $K_q = K_{SV}/\tau_0$. Hence, eqn. 2 was applied to determine K_{SV} by linear regression of a plot of F_0/F against [Q].

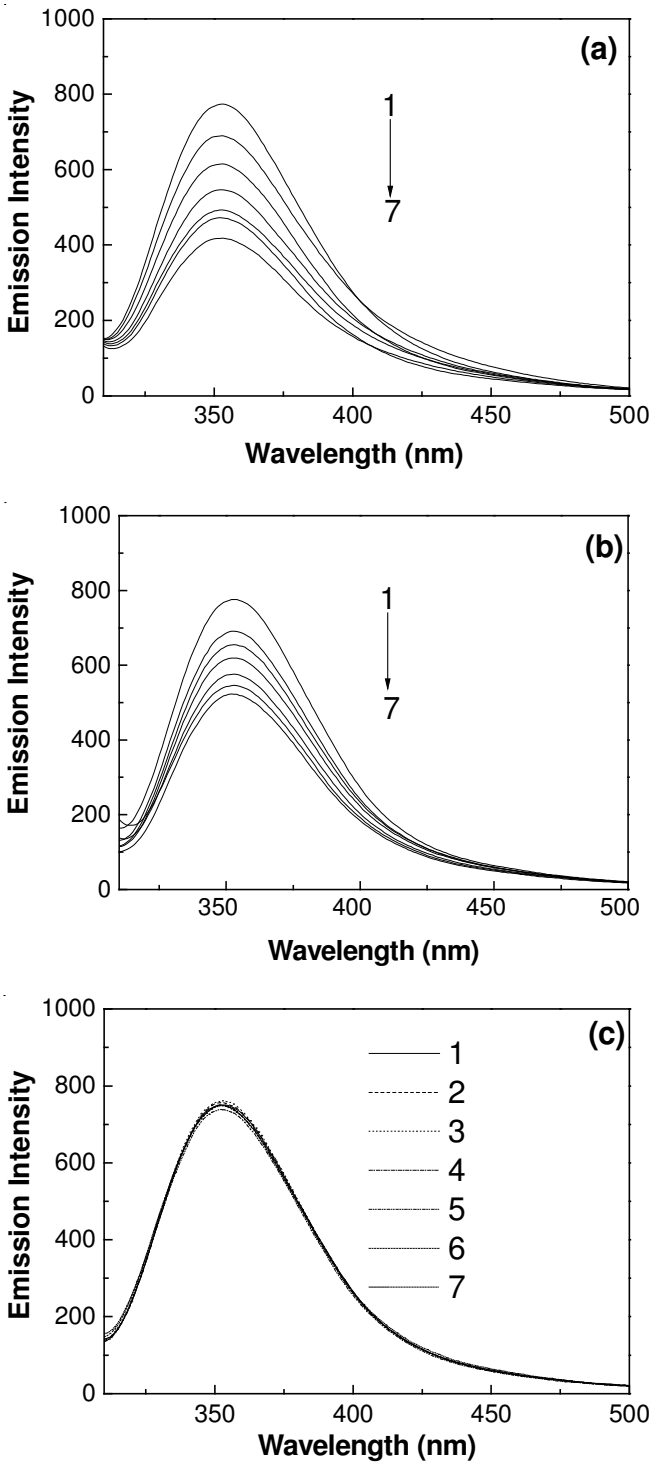


Fig. 1. Bovine serum albumin fluorescence emission spectra at λ_{ex} = 295 nm (pH 7.4) in the presence of different concentrations of chlorophenols. [BSA] = 2.0 × 10^{-6} mol L^{-1} (a) [PCP] from 1-7: 0, 0.5, 1, 1.5, 2.0, 2.5, 3.0 × 10^{-6} mol L^{-1}; (b) [2,4-DCP] and (c) [4-CP] from 1-7: 0, 0.5, 1, 1.5, 2.0, 2.5, 3.0 × 10^{-5} mol L^{-1}

A linear Stern-Volmer plot is generally indicative of a single class of fluorophores in a protein, all equally accessible to the quencher. This also means that only one mechanism (dynamics or static) of quenching occurs[23]. Because the fluorescence lifetime of the biopolymer is 10^{-8} s[24], quenching constant can be obtained by the slope (Figs. 2 and 3). If quenching is initiated by dynamic collision, the maximum scatter collision quenching constant of various kinds of quenchers to biopolymer

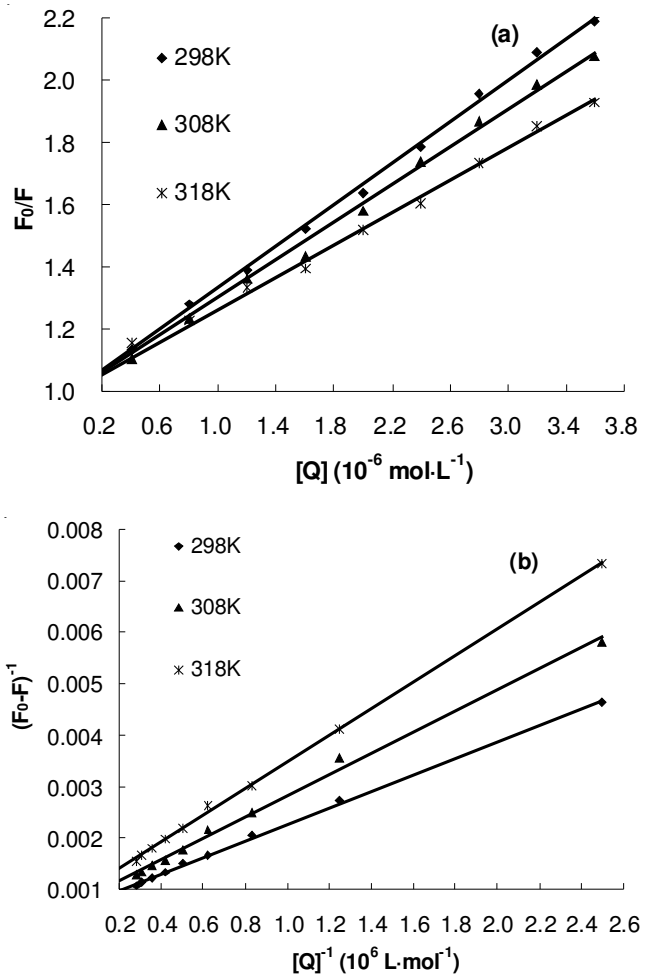


Fig. 2. Stern-Volmer plots (a) and Lineweaver-Burk plots (b) for the fluorescence quenching of BSA by PCP at different temperatures, pH = 7.4

is 2.0 × 10^{10} L mol^{-1} s^{-1} [25]. The rate constants of protein quenching procedure initiated by PCP and 2,4-DCP are greater than the K_q of the scatter procedure (Table-1). This shows that above quenching should be initiated by static quenching. For 4-CP, fluorescence quenching was not observed, the quenching mechanism was not discussed here.

TABLE-1
STERN-VOLMER CONSTANTS OF PCP AND 2,4-DCP TO BSA AT DIFFERENT TEMPERATURES

T (K)	K_{SV} (L mol^{-1})	K_q (L mol^{-1} s^{-1})	R*	SD**
298 (PCP)	3.35 × 10^5	6.70 × 10^{13}	0.9989	0.0094
308 (PCP)	3.05 × 10^5	6.10 × 10^{13}	0.9976	0.0181
318 (PCP)	2.64 × 10^5	5.28 × 10^{13}	0.9972	0.0138
298 (2,4-DCP)	1.63 × 10^4	3.26 × 10^{12}	0.9979	0.0126
308 (2,4-DCP)	1.46 × 10^4	2.92 × 10^{12}	0.9964	0.0225
318 (2,4-DCP)	1.34 × 10^4	2.68 × 10^{12}	0.9925	0.0178

*R represents the regression coefficient of the Stern-Volmer equation.
**SD represents the standard deviation for K_{SV}.

If fluorescence quenching is resulting from static quenching, the data can be also analyzed according to Lineweaver-Burk static quenching formula[26]:

$$\frac{1}{F_0 - F} = \frac{1}{F_0} + \frac{1}{K_{LB}} \cdot \frac{1}{F_0} \cdot \frac{1}{[Q]} \quad (3)$$

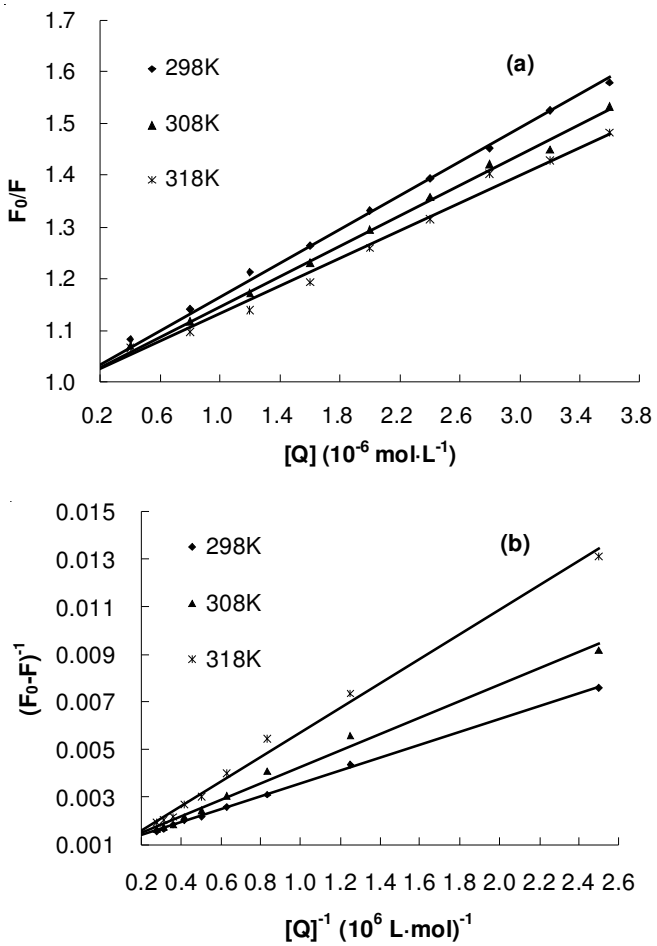


Fig. 3. Stern-Volmer plots (a) and Lineweaver-Burk plots (b) for the fluorescence quenching of BSA by 2,4-DCP at different temperatures, pH = 7.4

where K_{LB} = association constant. For static quenching, fluorophore and quencher form compound. As the temperature increases, the stability of compound is decreased. The Lineweaver-Burk plot of PCP and 2,4-DCP with BSA are shown in Figs. 2 and 3, respectively and the association constants are calculated as shown in Table-2. The results confirmed that the quenching mechanisms of PCP and 2,4-DCP with BSA are static quenching. In addition, the association constant values seen in Table-2 are great, which indicates that 2,4-DCP and PCP had high affinity to BSA and the binding affinity order of the three chlorophenols is PCP > 2,4-DCP > 4-CP.

Binding mode from thermodynamic parameters: In general, small molecules are bound to the macromolecule by four types of interactions: hydrogen bonds, van der Waals force, electrostatic and hydrophobic interactions. The thermodynamic parameters, free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) of interaction are important to interpret the binding mode. For this purpose, the temperature dependence of the association constant was studied. The temperatures chosen for experiment were 298, 308 and 318 K. From the temperature dependence of association constants, it is possible to calculate values for the thermodynamic functions involved in the binding process. The thermodynamic parameters were evaluated using the equations:

$$\ln K_{LB} = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (4)$$

$$\Delta G = \Delta H - T\Delta S \quad (5)$$

where R = gas constant, T = experimental temperature and K_{LB} = association constant calculated from the Lineweaver-Burk static quenching equation at corresponding temperature T. Then the enthalpy change (ΔH) and entropy change (ΔS) can be calculated based on the slope of the van't Hoff relationship (enq. 4), free energy change (ΔG) is estimated according to eqn. 5.

The thermodynamic values for the interaction of PCP and 2,4-DCP with BSA are summarized in Table-2. It is observed that the formation of PCP/BSA complex is accompanied by a negative enthalpy change and positive entropy change, the same sign of enthalpy change and entropy change was observed for 2,4-DCP/BSA complex formation. In the present study, both the negative values of ΔG at various temperatures imply the tendency of spontaneous binding of chlorophenols to BSA. Moreover, it is worth mentioning that for PCP-BSA interaction, the main source of free energy is derived from a large contribution of entropy term (account for 75 %) with small contribution from the enthalpy factor, so the main interaction is hydrophobic contact, but the electrostatic interaction cannot be excluded[27]. While for 2,4-DCP-BSA interaction, only 40 % of the total free energy can be attributing to the entropy change. It is obvious that the binding of PCP to BSA was markedly more entropy-driven and less enthalpy-driven than that of 2,4-DCP. Large positive entropy value in the case of PCP showed a major contribution from hydrophobic interaction. The rational explanation could be that benzene ring is the primary hydrophobic portion of the chlorophenols[28]; with the increase of substituted chlorophenol atom on benzene ring, the hydrophobicity of PCP is more than 2,4-DCP. There are many hydrophobic residues Arg 218, Trp 214, His 288, Tyr 319, *etc*., in domain II of BSA. Trp-214 is near or within the

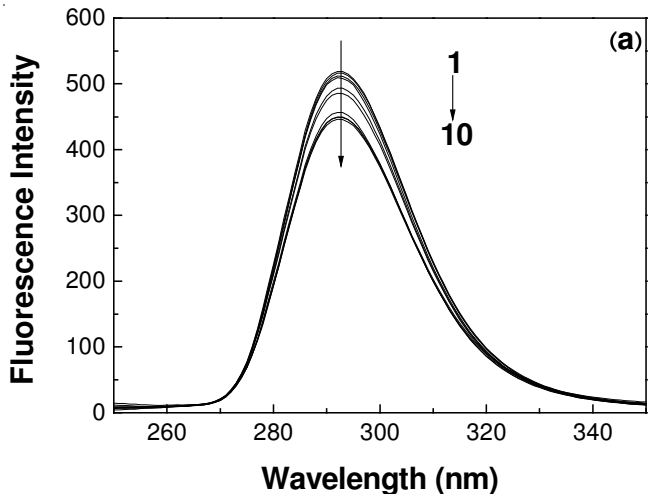
TABLE-2
ASSOCIATION CONSTANTS AND THERMODYNAMIC PARAMETERS FOR THE BINDING OF PCP AND 2, 4-DCP TO BSA AT DIFFERENT TEMPERATURES

T (K)	K_{LB} (L mol^{-1})	R_1*	SD**	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	R_2***
298 (PCP)	3.44×10^5	0.9984	0.0109	–	-31.60	–	–
308 (PCP)	3.17×10^5	0.9962	0.0254	-7.81	-32.40	79.83	0.9927
318 (PCP)	2.82×10^5	0.9989	0.0233	–	-33.20	–	–
298 (2,4-DCP)	1.82×10^4	0.9969	0.0188	–	-24.33	–	–
308 (2, 4-DCP)	1.55×10^4	0.9919	0.0296	-14.77	-24.65	32.07	0.9948
318 (2, 4-DCP)	1.25×10^4	0.9944	0.0227	–	-24.97	–	–

*R_1 represents the regression coefficient of the Lineweaver-Burk equation. **SD represents the standard deviation for K_{LB}. ***R_2 represents the regression coefficient of Van't Hoff equation.

binding site. Based on the hydrophobicity of the amino acid residues in domain II, it is tempting to speculate a direct hydrophobic interaction between the aromatic ring and the hydrophobic amino acid residue via stacking of their aromatic chromophores[29]. Furthermore, both PCP and 2,4-DCP are partially ionized at physiological pH, ionic interactions have also been suggested. Specific electrostatic interaction between ionic species in a aqueous solution is also characterized by a positive value of ΔS and a negative ΔH[30]. They may interact with some of the positive charged amino acid residue by electrostatic interaction. Thus it is not possible to account for the thermodynamic parameters of PCP-BSA or 2,4-DCP-BSA interaction with a single intermolecular force model. It's probably that hydrophobic and electrostatic interaction both involved in the formation of PCP-BSA and 2,4-DCP-BSA compound.

Conformational investigation: Spectroscopy is a useful tool to observe conformational changes in proteins because it allows non-intrusive measurements of substances at low concentrations under physiological conditions[31]. To explore the structural change of BSA by addition of chlorophenols, we measured synchronous fluorescence spectra of BSA with various amounts of chlorophenols. When the wavelength intervals ($\Delta\lambda$) between excitation and emission wavelength are stabilized at 15 or 60 nm, the synchronous fluorescence gives the characteristic information of tyrosine or tryptophan residues[32]. The effect of 2,4-DCP and PCP on BSA synchronous fluorescence spectroscopy are shown in Figs. 4 and 5, respectively. It can be seen from Fig. 4 that a stronger fluorescence quenching effect of tryptophan residues compared with tyrosine residues is observed upon addition of 2,4-DCP, whereas the emission maximum of both tryptophan and tyrosine keep the position. The results indicate that the binding site of 2,4-DCP is nearer to tryptophan than that of the tyrosine residues and the microenvironment of tryptophan and tyrosine residues were not influenced. For PCP-BSA interaction, a hypsochromic shift can be observed for the emission maximum of tyrosine residue and the emission maximum of tryptophan keeps unchanged (Fig. 5). The hypsochromic shift effect expresses the change in conformation of BSA and it indicated that the polarity around the tyrosine residues was decreased and the hydrophobicity was increased[32,33].



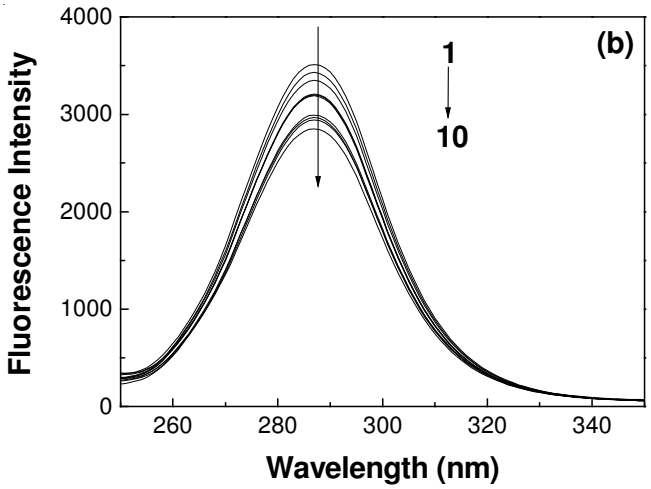
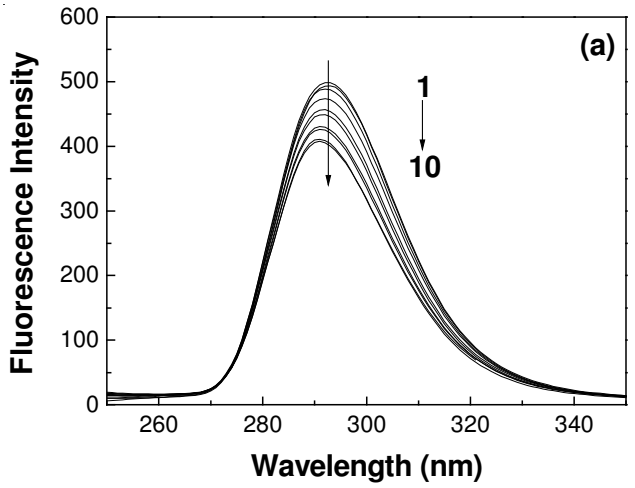


Fig. 4. Synchronous fluorescence spectrum of BSA in the present of 2,4-DCP (T = 298 K, pH = 7.40). [BSA] = 2.0×10^{-6} mol L^{-1}; [2,4-DCP] from 1-10: 0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6×10^{-5} mol L^{-1}, respectively. (a) $\Delta\lambda$ = 15 nm, (b) $\Delta\lambda$ = 60 nm



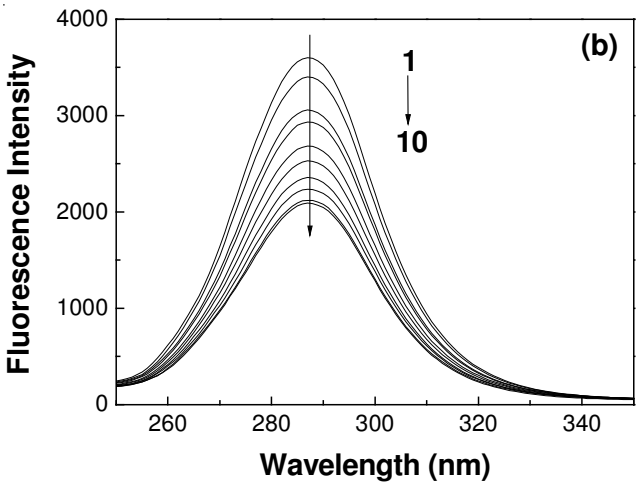


Fig. 5. Synchronous fluorescence spectrum of BSA in the present of PCP (T = 298 K, pH = 7.40). [BSA] = 2.0×10^{-6} mol L^{-1}; [PCP] from 1-10: 0, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6×10^{-6} mol L^{-1}, respectively. (a) $\Delta\lambda$ = 15 nm, (b) $\Delta\lambda$ = 60 nm

In order to gain more information on the conformation change of BSA by addition of 2,4-DCP and PCP, we measured the FT-IR and CD spectra of BSA. In the infrared region, the frequencies of bands due to the amide I band ≈1653 cm^{-1}

(mainly C=O stretch) and amide II band ≈ 1548 cm^{-1} (C-N stretch coupled with N-H bending mode) both have a relationship with the secondary structure of the protein[34]. Fig. 6 showed the FT-IR spectra of free BSA and PCP (or 2,4-DCP)/BSA complexes in *tris*-HCl buffer solution at 298 K. The peak position of the amide I band has no remarkably changed for 2,4-DCP-BSA interaction, while for PCP-BSA interaction it slightly moved from to 1650 cm^{-1} to 1648 cm^{-1}. In the amide II region, the peak position moved from 1548 cm^{-1}, 1560 cm^{-1} and 1562 cm^{-1} for 2,4-DCP-BSA and PCP-BSA interaction, respectively. The spectra shift indicated that chlorophenol interact with the C=O, C-N and N-H groups in the protein polypeptides and cause the microenvironment change of BSA. Pentachlorophenol has high affinity to BSA compared to 2,4-DCP, so spectra change in amide I and amide II caused by complex formation is relatively evident. The CD spectra of BSA with the absence or presence of chlorophenol are shown in Fig. 7. Bovine serum albumin exhibits two negative bands at 208 and 222 nm, which is typical of the α-helix structure of the class proteins[35]. However, no obvious change in band intensity and positions of the peaks could be observed even though the ratio of chlorophenol (2,4-DCP or PCP) to BSA is up to 10:1. This indicates that the structure of BSA is also predominantly α-helical with the presence of 2,4-DCP or PCP.

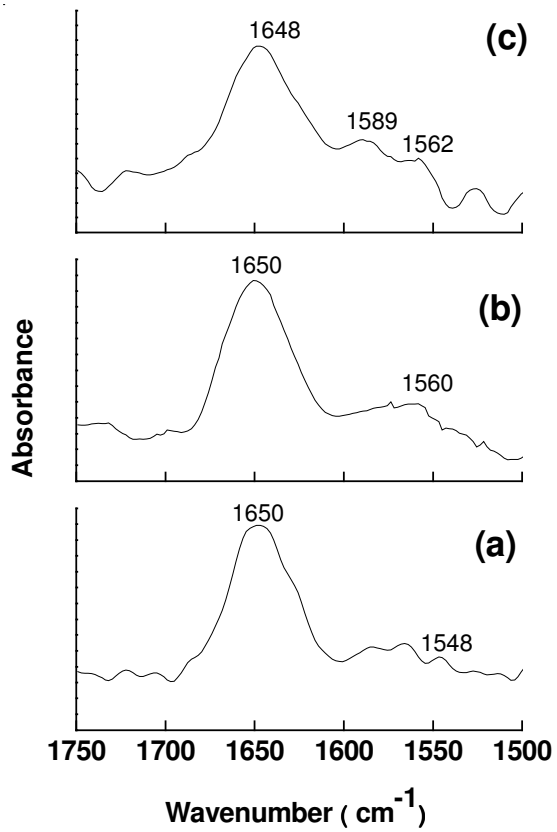


Fig. 6. FT-IR spectra and difference spectra of BSA in the region 1750-1500 cm^{-1}: (a) free BSA; (b) 2,4-DCP-BSA complex; (c) PCP-BSA complex. [BSA] = 40 mg mL^{-1} and molar ratio of chlorophenol to BSA is 1:1

Correlation between combination and structure of chlorophenols: Chlorophenols are a class of compound with the common benzene ring and different number of chlorine atom. With the chlorine atoms on the benzene ring increased,

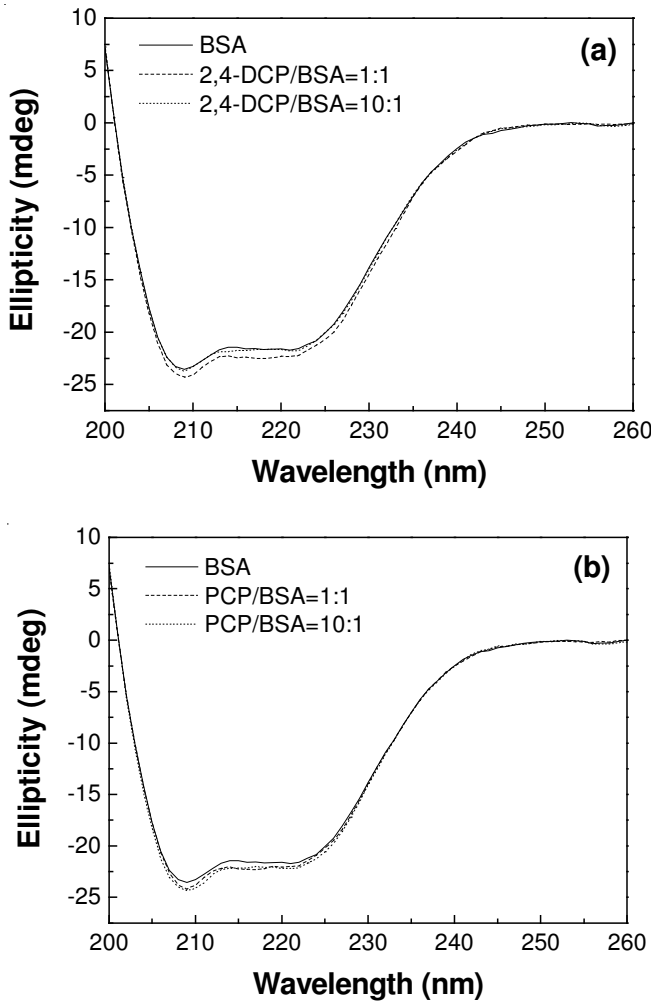


Fig. 7. Circular dichroism spectra of BSA and chlorophenol-BSA system at 298 K. Bovine serum albumin concentration was fixed at 1.0 × 10^{-5} mol L^{-1} (a) 2,4-DCP/BSA system, (b) PCP/BSA system

the electron flow from hydroxyl oxygen atom to benzene ring will be weaken, namely, the p-π conjugation between hydroxyl oxygen and benzene ring will be reduced. This means that the dissociation constant (K_a) of chlorophenol increases with the increasing number of substituted chlorine atoms on benzene ring. Depending on the value of pK_a, chlorophenols dissociate totally or partially. The pK_a values of 4-CP, 2,4-DCP and PCP are 9.1-9.4, 7.5-8.1 and 4.7-4.9, respectively according to the reference[6], so the binding affinity between dissociated chlorophenol and the positive charged amino acid residue of BSA by electrostatic interaction can be PCP > 2,4-DCP > 4-CP. On the other hand, the octanol-water partition coefficients (K_{ow}) strongly increased with the number of chlorine atoms and hydrophilicity inversely decreased, this may make contribution to the hydrophobic interaction between chlorophenol and BSA and the hydrophobic binding affinity is also PCP >2,4-DCP > 4-CP[6,36]. Further, molecular size of chlorophenol may also play a significant role in the binding of chlorophenol to BSA. Since the accessibility of interacting species varies with the nature and size of its molecules, it appears that the large size chlorophenol molecule probably has large hydrophobic area, which can interact with hydrophobic surface on the protein molecule by a more favourable spatial orientation[28]. In other words, steric effects also play a role. The above reasons can be best

explanation of the difference between three different chlorophenol on the fluorescence quenching of BSA.

Toxicological significance: The binding of toxicants to serum albumin has toxicological importance. Recently, quantitative structure activity relationships (QSARs) have been widespread used in the toxicology[37,38]. According to QSAR view[36,39], with the increase of substituted chlorine atom, the toxicity of the chlorinated compound is greater. In present work, the affinity order of three chlorophenols to BSA is PCP > 2,4-DCP > 4-CP. Since chlorophenols are toxic to a wide range of organisms, the higher affinity demonstrated that chlorophenol can be transported and released to the special target organ faster by transport protein, which results in greater toxicity. The experimental results are in accord with QSAR theory. These measurements supplied us with a new approach in ecotoxicology and environmental risk assessment.

Conclusion

The interaction of three chlorophenols compounds with BSA was investigated by fluorescence spectroscopy, CD spectroscopy and FT-IR spectroscopy. The fluorescence data analysis indicated that PCP and 2,4-DCP bind strongly to BSA by complex formation. The association constants determined by static quenching equation were calculated to be 3.44×10^5 and 1.82×10^4 L mol^{-1} at 298 K for PCP-BSA and 2,4-DCP-BSA interaction, respectively. The binding affinity order of the three chlorophenols is PCP > 2,4-DCP > 4-CP. The thermodynamic calculation implied that hydrophobic interaction and electrostatic interaction involved in the interaction process. Circular dichroism and FT-IR spectroscopic results confirmed BSA is predominantly α-helical although the microenvironment of BSA was partly changed with the addition of PCP or 2,4-DCP. The research also disclosed the number of chlorine atom on the benzene ring affect the binding affinity of chlorophenol to BSA, which is of particular interest in the toxicological comparison of homologous compound.

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REFERENCES

1. A. Papadopoulou, R.J. Green and R.A. Frazier, *J. Agric. Food Chem*., **53**, 158 (2005).
2. X.M. He and D.C. Carter, *Nature*, **358**, 209 (1992).
3. D.C. Carter and J.X. Ho, *Adv. Protein Chem*., **45**, 153 (1994).
4. U. Kragh-Hansen, *Pharmacol. Rev*., **33**, 17 (1981).
5. E. Tamer, Z. Hamid, A.M. Aly, E.T. Ossama, M. Bo and G. Benoit, *Chemosphere*, **63**, 277 (2006).
6. M. Czaplicka, *Sci. Total Environ*., **322**, 21 (2004).
7. S.K. Zheng, Z.F. Yang, D.H. Jo and Y.H. Park, *Water Res*., **38**, 2315 (2004).
8. K. Fahr, H.G. Wetzstein, R. Grey and D. Schlosser, *FEMS Microbiol. Lett*., **175**, 127 (1999).
9. S. Olivier, A.H. Scragg and J. Morrison, *Enzym. Microb. Technol*., **32**, 837 (2003).
10. USEPA, Water Quality Criteria Summary, Ecological Risk Assessment Branch (WH-585) and Human Risk Assessment Branch (WH-550D). Washington, DC (1991).
11. Q. Xia and X.H. Zhang, Manual on Water Quality Standards, China Environ. Sci. Press, Beijing (1990).
12. M. Mari, M. Schuhmacher and J.L. Domingo, *Int. Arch. Occup. Environ. Health*, **82**, 519 (2009).
13. J.S. Park, A. Bergman, L. Linderholm, M. Athanasiadou, A. Kocan, J. Petrik, B. Drobna, T. Trnovec, M.J. Charles and I. Hertz-Picciotto, *Chemosphere*, **70**, 1676 (2008).
14. L. Meijer, J. Weiss, M. Van Velzen, A. Brouwer, A. Bergman and P.J. Sauer, *Environ. Sci. Technol*., **42**, 3428 (2008).
15. J.N. Tian, J.Q. Liu, X. Tian, Z.D. Hu and X.G. Chen, *J. Mol. Struct*., **691**, 197 (2004).
16. S.S. Shyamali, D.R. Lillian, L. Lawrence and B. Esther, *Biochemistry*, **18**, 1026 (1979).
17. A.C. Dong, P. Huang and W.S. Caughey, *Biochemistry*, **29**, 3303 (1990).
18. G.H. Xiang, C.L. Tong and H.Z. Lin, *J. Fluoresc*., **17**, 512 (2007).
19. J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Kluwer Academic/Plenum Publishers, New York, edn. 2 (1999).
20. A. Sulkowska, *J. Mol. Struct*., **616**, 227 (2002).
21. D. Silva, C.M. Cortez, J. Cunha-Bastos and S.R.W. Louro, *Toxicol. Lett*., **147**, 53 (2004).
22. M.R. Eftink and C.A. Ghiron, *Anal. Biochem*., **144**, 199 (1981).
23. S. Soares, N. Mateus and V.D. Freitas, *J. Agric. Food. Chem*., **55**, 6726 (2007).
24. J.R. Lakowicz and G. Weber, *Biochemistry*, **12**, 4161 (1973).
25. W.R. Ware, *J. Phys. Chem*., **66**, 455 (1962).
26. G.Z. Chen, X.Z. Huang, J.G. Xu, Z.B. Wang and Z.X. Zheng, Method of Fluorescent Analysis, Science Press, Beijing, edn. 2 (1990).
27. S.N. Khan, B. Islam, R. Yennamalli, A. Sultan, N. Subbarao and A.U. Khan, *Eur. J. Pharm. Sci*., **35**, 371 (2008).
28. B.P. Kamat and J. Seetharmappa, *J. Pharm. Biomed. Anal*., **35**, 655 (2004).
29. H.M. Zhang, T.T. Chen, Q.H. Zhou and Y.Q. Wang, *J. Mol. Struct*., **938**, 221 (2009).
30. P.D. Ross and S. Subramanian, *Biochemistry*, **20**, 3096 (1981).
31. Y.J. Hu, Y. Liu, R.M. Zhao, J.X. Dong and S.S. Qu, *J. Photochem. Photobiol. A*, **179**, 324 (2006).
32. R.W. Congdon, G.W. Muth and A.G. Splittgerber, *Anal. Biochem*., **213**, 407 (1993).
33. G.C. Zhang, Y.Q. Wang, H.M. Zhang, S.H. Tang and W.H. Tao, *Pestic. Biochem. Physiol*., **87**, 23 (2007).
34. J.N. Tian, J.Q. Liu, Z.D. Hu and X.G. Chen, *Bioorg. Med. Chem*., **13**, 4124 (2005).
35. W.Y. He, Y. Li, C.X. Xue, Z.D. Hu, X.G. Chen and F.L. Sheng, *Bioorg. Med. Chem*., **13**, 1837 (2005).
36. Z.L. Qin and C.J. Feng, *Chin. J. Org. Chem*., **23**, 654 (2003).
37. T.W. Schultz, M.T.D. Cronin and T.I. Netzeva, *J. Mol. Struct. (Theochem.)*, **622**, 23 (2003).
38. K.L.E. Kaiser, *J. Mol. Struct. (Theochem.)*, **622**, 85 (2003).
39. S. Sixt, J. Altschuh and R. Bruggemann, *Chemosphere*, **30**, 2397 (1995).