J Fluoresc (2011) 21:1857–1864
DOI 10.1007/s10895-011-0880-9

ORIGINAL PAPER

Fluorescence Enhancement Effect for the Determination of Polychlorinated Biphenyls with Bovine Serum Albumin

Fengju Zhang · Xia Wu · Jinhua Zhan

Received: 17 January 2011 / Accepted: 23 March 2011 / Published online: 30 March 2011

Abstract A sensitive and selective method for the trace determination of 3, 3', 4, 4'-tetrachlorobiphenyl (PCB77) by using bovine serum albumin (BSA) as a fluorescence probe was introduced. Under optimum conditions, the enhanced fluorescence intensity was proportional to the concentration of polychlorinated biphenyls in the range of 8.9×10^{-8}–5.0×10^{-6} mol L^{-1} for PCB77, and 5.0×10^{-7}–5.0×10^{-6} mol L^{-1} for 2, 2', 5, 5'-tetrachlorbiphenyl (PCB52). The detection limits (*S/N = 3*) of PCB77 and PCB52 were 2.6×10^{-8} mol L^{-1} and 2.9×10^{-7} mol L^{-1}, respectively. Furthermore, the fluorescence enhancement mechanism was discussed in detail. Results indicated that fluorescence enhancement of the system originated from the formation of BSA-PCBs complexes. In addition, PCBs were mainly bound to the tyrosine residues in BSA molecules.

Keywords Fluorescence · Polychlorinated biphenyls · Bovine serum albumin · Determination

Introduction

Polychlorinated biphenyls (PCBs) were widely used in dielectric, hydraulic, heat transfer fluids and plasticizers, which has led to ubiquitously distributed in the environment [1, 2]. This, together with their environmental persistence and chemical resistance, means that they have become an environmental problem worldwide. Due to their hydrophobicity and persistence, PCBs readily accumulated through the food chain of ecological environment. Animal experiments and clinical diagnosis show that PCBs have adverse biological effects on the mammals including endocrine effect on thyroid and steroid hormone [3, 4], neurotoxicity [5], embryotoxicity and oncogenicity [6]. They also pose threats to the ecosystem and human health [7, 8]. Thus, the determination of PCBs appears to be important in studying on potential toxicity and degradation mechanism of them. Recently, the trace detection of PCBs has become a hotspot of environmental science and analytical chemistry, which attracts extensive attention.

Various methods have been developed for the determination of PCBs including chromatogram, mass spectrometry [9–13], immunoassay analysis [14–17] and biologic analysis [18, 19]. Advantages such as high separation efficiency, wide range of applications, high sensitivity and less sample consumption will be arrived in the above approaches, while they suffer from complex sample preparation, limited specificity and cross-reaction and so on. Therefore, it is of significance to establish convenient and selective methods for the detection of PCBs. Due to its simplicity, high sensitivity and selectivity [20, 21], the fluorimetric technique is widely applied in the determination of coplanar polychlorinated biphenyls [22], biomacromolecules [23, 24] and drugs [25, 26].

In this paper, bovine serum albumin (BSA) was used as a fluorescence probe to selectively determine the trace of 3, 3', 4, 4'-tetrachlorobiphenyl (PCB77). Results demonstrated that there existed the interaction between BSA and PCBs, resulting in fluorescence enhancing of the system. Based on this phenomenon, a novel and sensitive determination method was proposed. This approach has been applied to the detection of PCB77 in synthetic sample with satisfactory results. The mechanism of fluorescence enhancement in this system

F. Zhang · X. Wu (✉) · J. Zhan
Key Laboratory of Colloid and Interface Chemistry (Shandong University), Ministry of Education, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, People's Republic of China
e-mail: wux@sdu.edu.cn

investigated by resonance light scattering (RLS), UV absorption spectrometry, synchronous fluorescence and circular dichroism (CD) spectroscopies.

Experiment

Materials

Stock solutions of PCBs (1.0×10^{-4} g mL^{-1}) were prepared by dissolving commercial 3, 3', 4, 4'-tetrachlorobiphenyl (PCB77) or 2, 2', 5, 5'-tetrachlorbiphenyl (PCB52) or 2, 4, 4'-trichlorbiphenyl (PCB28) (0.1000 g, J&K Scientific Ltd, China) in absolute ethanol and diluted to 100 mL with the same solvent. A stock standard solution of bovine serum albumin (1.0×10^{-4} g mL^{-1}) was prepared by dissolving commercial BSA (0.0100 g, Shanghai Bio Life Co., China) in ultra-pure water and diluted to 100 mL. NaAc-HAc buffer solution was prepared by dissolving NaAc (4.103 g) in 250 mL ultra-pure water and adjusting the pH to 5.02 with HAc solution (0.2 mol L^{-1}). All solutions were stored at 0–4 °C. Water for all reactions and solution preparation was ultra-pure water (18.25 MΩ cm^{-1}). All reagents and solvents used were of analytical reagent grade without further purification.

Apparatus

The fluorescent measurements were performed on a LS-55 spectrofluorimeter (PE, USA) with a quartz cell (1 cm× 1 cm). All absorption spectra were measured on a U-4100 spectrophotometer (Hitachi, Japan). The Circular Dichroism (CD) spectra were obtained by using a JASCO J-810 Circular Dichroism Spectrophotometer (Japan). The pH of solution was adjusted by Model pHS-3C pH meter (Shanghai, China).

Procedure

To a 10 mL colorimetric tube, solutions were added in the following order: NaAc-HAc, BSA and PCBs. The mixture was thoroughly mixed and diluted to 5 mL with ultra-pure water, and its fluorescence spectrum was measured at λ_{ex}=260 nm and λ_{em}=362 nm with the slit widths at 10 nm for excitation and emission. The extent of fluorescence enhancement was defined as $\Delta I_f = I_f - I_f^0$, where I_f and I_f^0 were the fluorescence intensities of the system with and without PCBs, respectively.

Results and Discussion

Fluorescence Spectra

Figure 1 depicted the excitation and emission spectra of the system developed in this research. The maximum excitation and emission peak of BSA occurred at 284 nm and at 362 nm in aqueous solution (data not shown). As could be seen from Fig. 1 that with addition PCB77 or PCB52 to BSA solution, the fluorescence intensity of BSA enhanced, and the maximum excitation wavelength had an obvious blue shift (from 280 nm to 260 nm). Furthermore, the extent of fluorescence enhancement of BSA-PCB77 system is further larger than that of BSA-PCB52 system, whereas PCB28 had little effect on the fluorescence intensity of BSA. At the same concentrations, PCB77, PCB52 and PCB28 could enhance the fluorescence intensity ratio of BSA solution (λex/λem=260 nm/362 nm) was 125:4.6:1.0. The experimental results demonstrated that there existed strong interactions between BSA and tetrachlorbiphenyls, and the extent of fluorescence enhancement depended on the structure of PCBs.

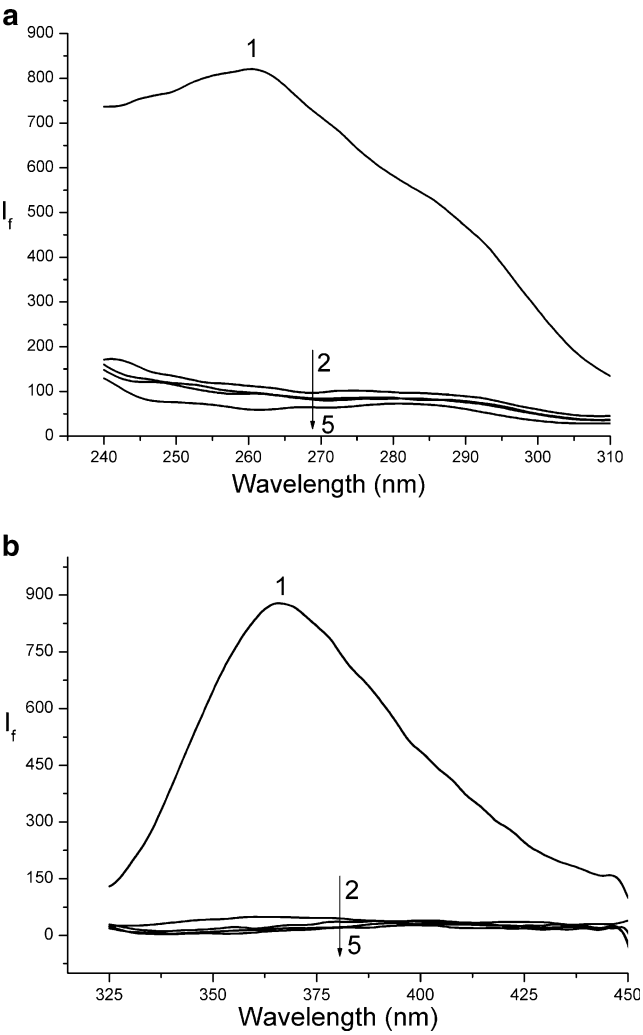

Fig. 1 Fluorescence spectra. **a** Excitation spectra (λ_{em}=362 nm) **b** Emission spectra (λ_{ex}=260 nm) *1*, BSA-PCB77; *2*, BSA-PCB52; *3*, BSA-PCB28; *4*, BSA-C_2H_5OH; *5*, BSA. Conditions: BSA: 9.0×10^{-7} g mL^{-1}; PCB77: 2.1×10^{-6} mol L^{-1}; PCB52: 2.1×10^{-6} mol L^{-1}; PCB28: 2.1×10^{-6} mol L^{-1}; NaAc-HAc: 0.02 mol L^{-1} (pH=5.02)

Optimization of the Analytical Procedure

Effect of pH and the Choice of Buffer Solution

As shown in Fig. 2 that the maximum ΔI_f occurred at pH=5.02. The influences of different buffers on the ΔI_f of the system were also tested at the same pH (pH=5.02± 0.05). The ΔI_f for Na_2HPO_4-citrate acid, HMTA-HCl, NaAc-HAc, Briton-Robinson and sodium citrate-citrate acid were 748.8, 715.4, 795.2, 726.4 and 752.4, respectively. The results indicated that different kinds of buffers had some influences on the system, and NaAc-HAc (pH=5.02, 0.50 mL) was selected as the most suitable buffer for this study.

Effect of BSA Concentration

The influence of BSA concentration was presented in Fig. 3. It could be seen that the ΔI_f of the system reached a maximum value when the concentration of BSA was 9.0×10^{-7} g mL^{-1}. Therefore, 9.0×10^{-7} g mL^{-1} was subsequently utilized for further experiments.

Effect of Ethanol

In this experiment, PCBs were dissolved in ethanol, and the effect of the volume fraction of ethanol on the fluorescence intensity of the system was investigated and shown in Fig. 4. Under optimized conditions, we varied the volume fraction of ethanol from 10% to 100%. The result indicated that the fluorescence intensity of BSA increased with increasing of volume fraction of ethanol, while the fluorescence intensity of BSA-PCBs system had a little

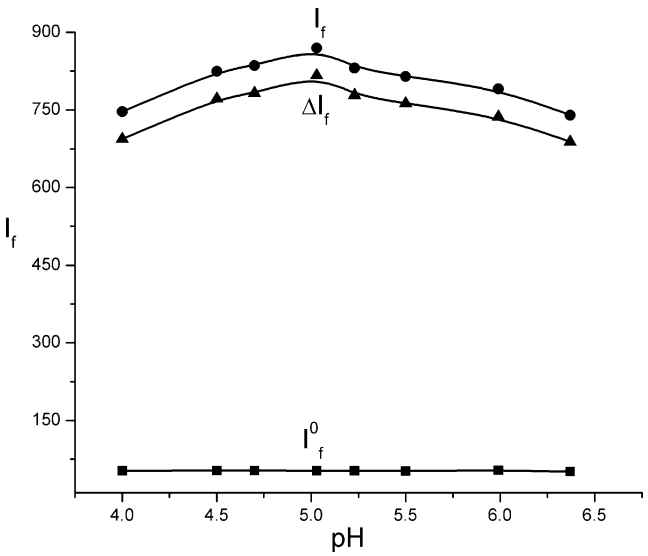

Fig. 2 Effect of pH. Conditions: BSA: 9.0×10^{-7} g mL^{-1}; PCB77: 2.1×10^{-6} mol L^{-1}; NaAc-HAc: 0.02 mol L^{-1}

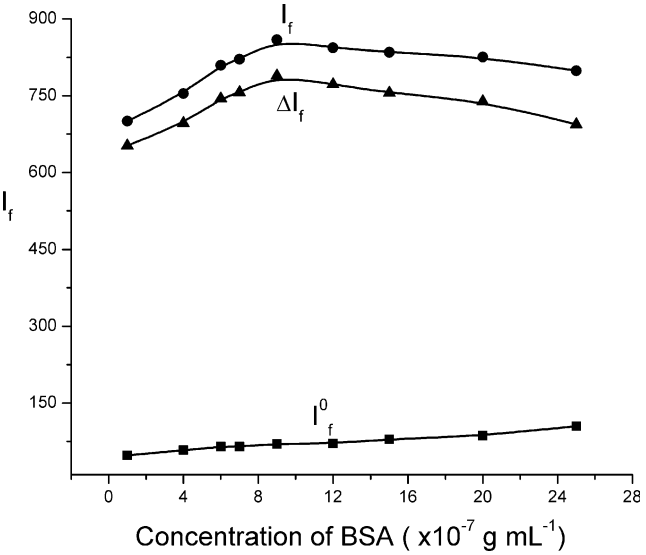

Fig. 3 Effect of the concentration of BSA. Conditions: PCB77: 2.1×10^{-6} mol L^{-1}; NaAc-HAc: 0.02 mol L^{-1} (pH=5.02)

change, and the extent of fluorescence enhancement ΔI_f decreased. Therefore, during measurement process, the influence of ethanol on fluorescence intensity of BSA was deducted. To deduct the effects of ethanol on the fluorescence intensity of the system, the same amount of ethanol as PCBs solution was added in the blank solution.

Adding Sequence and Signal Stability

The effect of the adding sequence of reagents on the ΔI_f of the system was studied. The results showed that the value of ΔI_f (%) for the systems of buffer-BSA-PCB77, buffer-PCB77-BSA, BSA-PCB77-buffer, PCB77-BSA-buffer, BSA-buffer-PCB77 and PCB77-buffer-BSA were 100, 91.5, 64.6, 44.2, 92.5 and 47.3, respectively, and

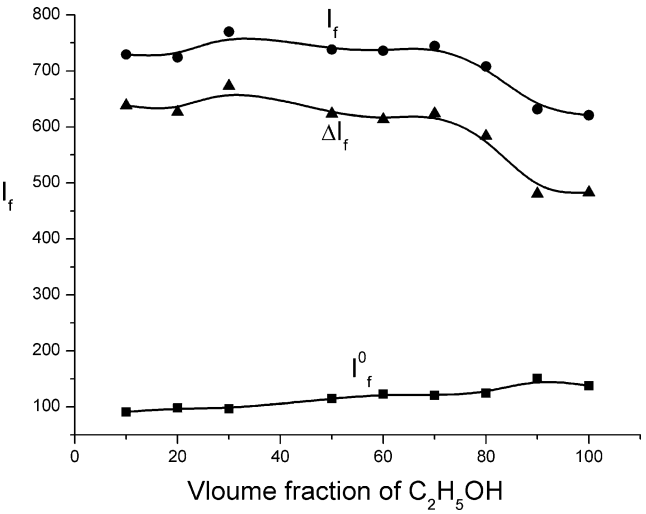

Fig. 4 Effect of ethanol. Conditions: BSA: 9.0×10^{-7} g mL^{-1}; PCB77: 2.1×10^{-6} mol L^{-1}; NaAc-HAc: 0.02 mol L^{-1} (pH=5.02)

the related as the % of the value obtained for the system of buffer-BSA-PCB77. Thus, the best adding sequence of the reagents was found to be buffer, BSA and PCB77.

Under optimized conditions, the value of ΔI_f reached a maximum in 20 min after all reagents were added and remained stable for about 120 min.

Effect of Foreign Substances

To assess the selectivity of the proposed method, the effect of foreign substances on the fluorescence intensity of the BSA-PCBs system was evaluated and the results were shown in Table 1. It was found that some organic compounds and most metal ions except Fe^{3+} had little effect on the determination of PCBs within ±5% relative error.

Analytical Application

Calibration Curve and Detection Limits

Under optimized conditions, the analytical parameters of this method were listed in Table 2. It showed that there existed a good linear relationship between ΔI_f and the concentrations of PCB77 and PCB52. The relative standard deviations of the slope of the linear equations for PCB77 and PCB52 were 2.8% and 6.9% separately. The determination results indicated that this method had high sensitivity and good reproducibility. As could be seen form Table 2, this method for the determination of PCB77 had a lower detection limits and a wider linear range than that of PCB52.

Table 1 Interference from foreign substance

Foreign substance	Concentration coexisting ($\times10^{-6}$ mol L^{-1})	Change of I_f (%)
K^+, Cl^-	2.0	−5.1
NH_4^+, Cl^-	4.0	−3.4
Fe^{3+}, Cl^-	0.1	+1.0
Zn^{2+}, Cl^-	4.0	−1.3
Ba^{2+}, Cl^-	6.0	−2.4
Al^{3+}, NO_3-	1.0	+1.5
Na^+, CO_3^{2-}	10	−4.6
Mg^{2+}, SO_4^{2-}	6.0	−2.0
o-chlorphenol	1.0	−3.1
p-chlorphenol	2.0	−2.8
chlorobenzene	1.0	−1.0

Conditions: BSA: 9.0×10^{-7} g mL^{-1}; PCB77: 2.1×10^{-6} mol L^{-1}; NaAc-HAc: 0.02 mol L^{-1} (pH=5.02)

To demonstrate the selectivity of the determination method, we studied the influences of different concentrations of PCB52 and PCB28 on the fluorescence intensity of BSA-PCB77 system. The experimental results showed that the molar concentration ratio of PCB52 and PCB77 was 1.5:1; the relative standard deviation of ΔI_f of BSA-PCB77 system was 2.1%. When the molar concentration ratio of PCB28 and PCB77 was 3:1, the relative standard deviation of ΔI_f of BSA-PCB77 system was 4.4%. The results showed that the existence of PCB52 or PCB28 had little interference with the determination of PCB77 within ±5% relative error.

Recovery Test of Synthetic Sample

The standard addition method was used for both recovery test and the determination of PCB77 in synthetic samples which included PCB77 1.0×10^{-7} mol L^{-1}, $ZnCl_2$ 1.0×10^{-8} mol L^{-1}, $MgSO_4$ 6.0×10^{-8} mol L^{-1} and PCB28 1.0×10^{-9} mol L^{-1}. The detectable concentration of PCB77 was 0.97×10^{-7} mol L^{-1}±4.8% (n=3) and the recovery ratio for PCB77 was 93–102%. The result indicated that BSA could be used as a fluorescence probe to selectively determine PCB77.

Interaction Mechanism of the System

Formation of BSA-PCBs Complex

According to the RLS theory [27], the intensity of the RLS should sensitively depend on the size of the aggregate. The influences of PCBs on the intensity of resonance light scattering of BSA were presented in Fig. 5. It showed that after adding PCBs to BSA solution, the RLS intensity of BSA was strongly enhanced. The experimental phenomena demonstrated that BSA and PCBs had interacted during the process, and a large BSA-PCBs complex was formed in this system. Moreover, the extent of RLS enhancement depended on the structure of PCBs in the order of PCB77 > PCB52.

Absorption Spectra of the System

From the absorption spectra of the system (shown in Fig. 6), it can be seen that after PCB77 was added into the protein solution, the absorbance of BSA increased and the absorption peak moved to shorter wavelength (from 280 nm to 263 nm). It is well known that the peak at about 280 nm can reflect the absorption spectra of the aromatic amino residues of protein. The results indicated that there existed the interaction between PCB77 and aromatic amino residues of protein.

Table 2 Analytical parameters of this method

PCBs	Linear range (mol L^{-1})	Linear regression equation (mol L^{-1})	r[a]	Limit of detection (10^{-8} mol L^{-1})
PCB77	8.9×10^{-8}–5.0×10^{-6}	$\Delta I_f=1.65\times10^{8}C+32.84$	0.996	2.6
PCB52	5.0×10^{-7}–5.0×10^{-6}	$\Delta I_f=1.04\times10^{7}C+0.68$	0.999	29

Conditions: BSA: 9.0×10^{-7} g mL^{-1}; PCB77: 2.1×10^{-6} mol L^{-1}; PCB52: 2.1×10^{-6} mol L^{-1}; NaAc-HAc: 0.02 mol L^{-1} (pH=5.02)

[a] Correlation coefficient

Binding Site of BSA Molecules and PCB77

Fluorescence Quantum Yield of BSA and BSA-PCB77 Complex

Under different excitation wavelengths, L-tryptophan (fluorescence quantum yield is 0.14) was used as a standard substance to measure the fluorescence quantum yield of BSA and BSA-PCB77 complex [28]. According to previous research [29], when the excitation wavelength of BSA was at 260 nm, the fluorescence of protein was mainly attributed to tyrosine residues. When it was at 295 nm, the fluorescence of protein was mainly contributed by the tryptophan residues. When the excitation wavelength was 260 nm, the fluorescence quantum yield of BSA increased from 0.091 to 0.307 in the presence of PCB77, while the excitation wavelength was 295 nm, the fluorescence quantum yield of BSA increased from 0.248 to 0.339 with adding PCB77. The augmentation of fluorescence quantum yield of BSA-PCB77 complex at the excitation wavelength of 260 nm was larger than that at the excitation wavelength of 295 nm. We speculate that PCB77 mainly interacted with the tyrosine residues in BSA molecules.

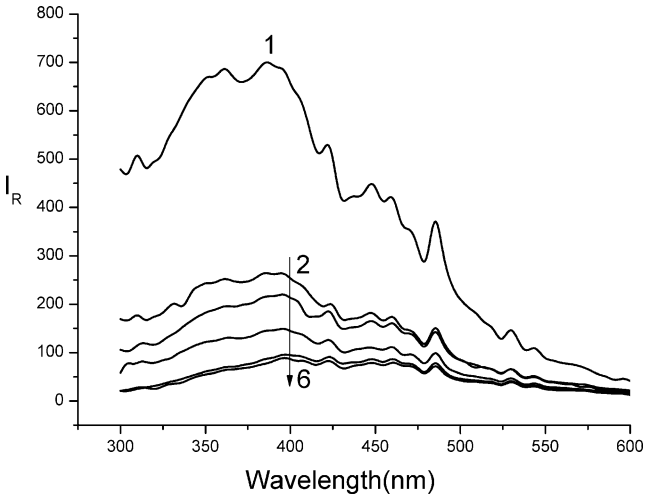

Fig. 5 Resonance light scattering spectra of the system. *1*, BSA-PCB77; *2*, PCB77; *3*, BSA-PCB52; *4*, PCB52; *5*, BSA-C_2H_5OH; *6*, BSA. Conditions: BSA: 9.0×10^{-7} g mL^{-1}; PCB77: 2.1×10^{-6} mol L^{-1}; PCB52: 2.1×10^{-6} mol L^{-1}; NaAc-HAc: 0.02 mol L^{-1} (pH=5.02)

Fluorescence Spectra of Tryptophan and Tyrosine

The fluorescence spectra of tryptophan (Trp) and tyrosine (Tyr) were shown in Fig. 7. The maximum emission peaks of tryptophan (Fig. 7a) and tyrosine (Fig. 7b) occurred at 358 nm and 305 nm, respectively. It shown in Fig. 7b that after PCB77 was added to tyrosine solution, the fluorescence intensity of tyrosine obviously enhanced and the maximum emission wavelength showed an obvious red shift (from 305 nm to 365 nm). In addition, there existed the interaction between tryptophan and PCB77, and its extent of fluorescence enhancement was far less than that of tyrosine-PCB77. The experimental results further suggested that PCB77 mainly interacted with the tyrosine residues in BSA molecules.

Synchronous Fluorescence Spectra of the System

Figure 8 depicted the synchronous fluorescence spectra of the system. When the constant wavelength interval between

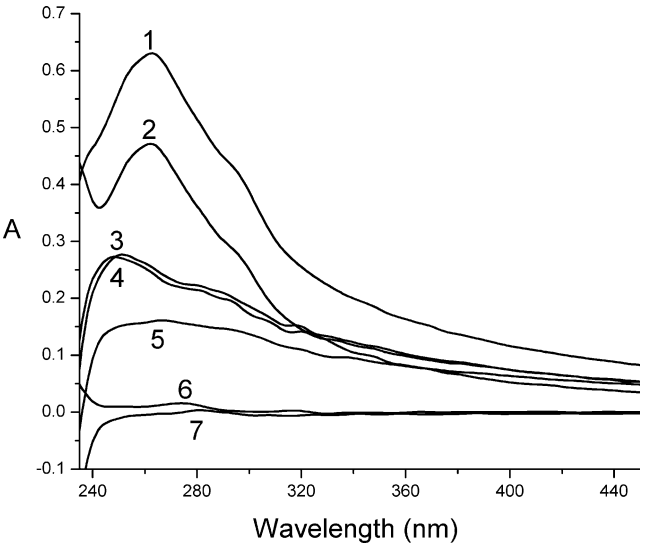

Fig. 6 Absorption spectra of the system. *1*, BSA-PCB77; *2*, BSA-PCB77 (*vs* PCB77); 3, BSA-PCB52; 4, PCB52; 5, PCB77 (1–5 *vs* buffer-C_2H_5OH); 6, BSA (*vs* H_2O); 7, BSA-C_2H_5OH (*vs* buffer-C_2H_5OH). Conditions: BSA: 2.0×10^{-5} g mL^{-1}; PCB77: 2.1×10^{-5} mol L^{-1}; PCB52: 2.1×10^{-5} mol L^{-1}; NaAc-HAc: 0.02 mol L^{-1} (pH=5.02)

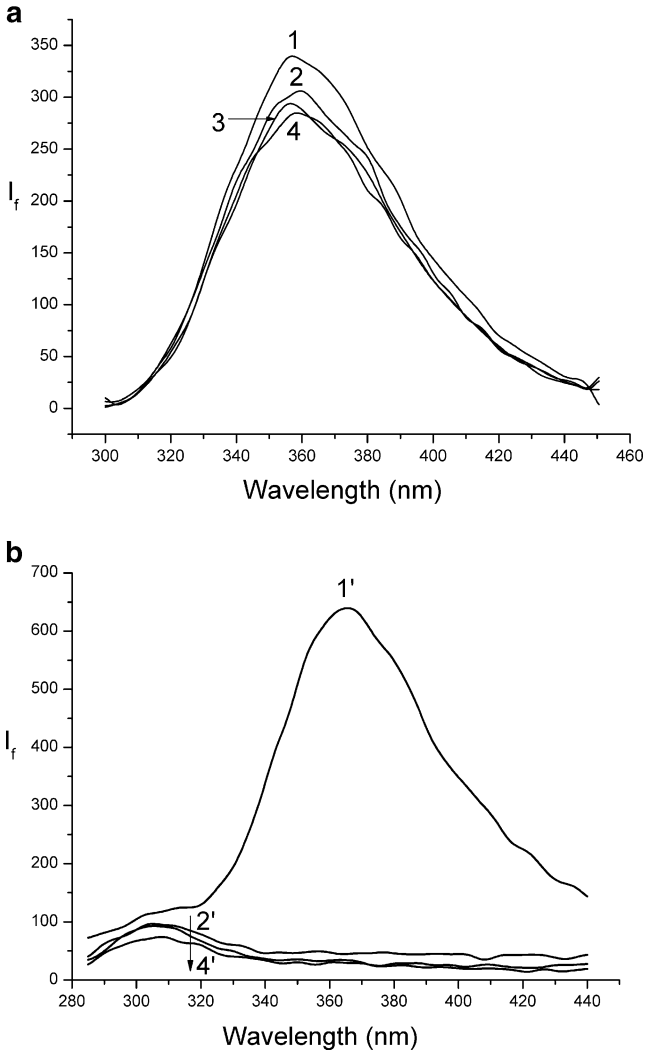

Fig. 7 Fluorescence spectra of tryptophan and tyrosine. **a** Fluorescence spectrum of tryptophan **b** Fluorescence spectrum of tyrosine. *1*, tryptophan-PCB77; *2*, tryptophan-PCB52; *3*, tryptophan-C_2H_5OH; *4*, tryptophan. *1*', tyrosine-PCB77; *2*', tyrosine-PCB52; 3', tyrosine-C_2H_5OH; 4' tyrosine. Conditions: tryptophan: 9.0×10^{-7} mol L^{-1}; tyrosine: 9.0×10^{-7} mol L^{-1}; PCB77: 2.1×10^{-6} mol L^{-1}; PCB52: 2.1×10^{-6} mol L^{-1}; NaAc-HAc: 0.02 mol L^{-1} (pH=5.02)

emission wavelength and excitation wavelength (Δλ) is stabilized at 20 nm or 60 nm, the synchronous fluorescence spectrum gives the characteristic information of tyrosine residues or tryptophan residues [30, 31]. As shown in Fig. 8, the fluorescence intensity of tyrosine residues was much weaker than that of tryptophan residues of BSA molecules. When Δλ was 60 nm, PCB77 with the same concentration could increase the intensity of fluorescence of BSA to 8.6-fold of its initial value, while 5.3-fold enhancement could be obtained when Δλ was 20 nm. In BSA-PCBs system, the extent of fluorescence enhancement of Tyr residues was relatively weaker than that of Trp residues mainly due to the energy transfer between Tyr residues and Trp residues [32].

Secondary Structural Change of BSA

Far-CD spectroscopy can give qualitative information in details on the secondary structure changes of proteins. A double-negative peak in the CD spectra is a symbol of α-helical secondary structure. The peak at 220 nm is the contribution of the n−π* transition of peptide bond, and the peak at 208–209 nm is due to the π−π* transition of peptide bond [33]. CD spectra (Fig. 9) were analyzed by the Yang principle to obtain structural information from the spectral changes. In the BSA-PCB77 system, the CD spectra showed a larger molar ellipticity at 209 nm and at 220 nm, indicating an augmentation of the α-helix content in BSA structure, i.e. the α-helix content of BSA-PCB77 increased from about 21.6 % of the native BSA to 36.5%,

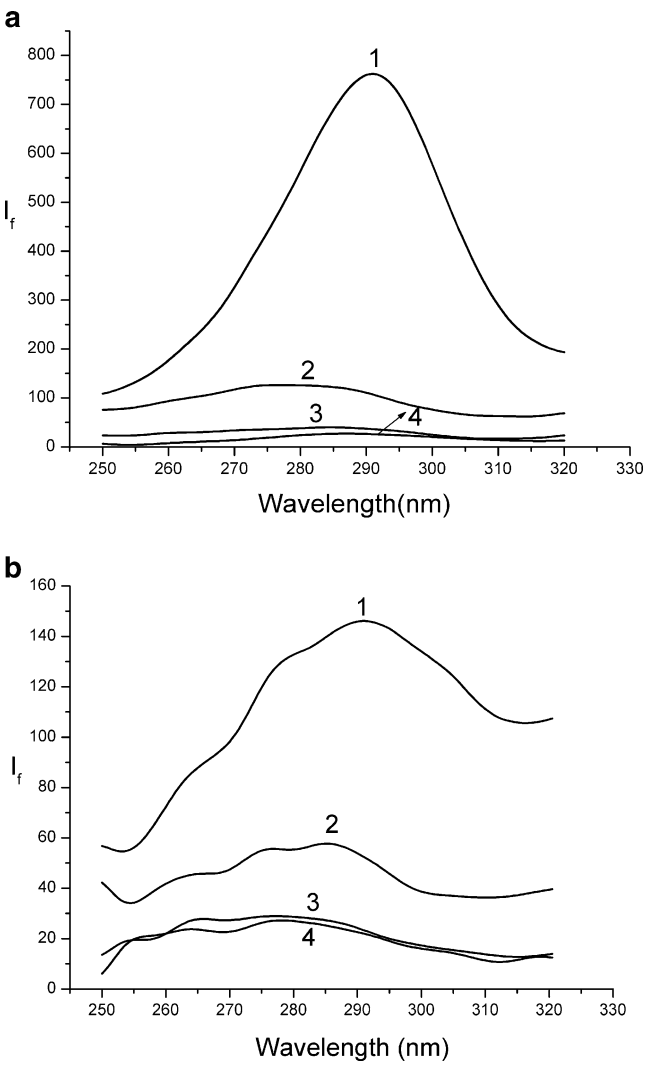

Fig. 8 Synchronous Fluorescence spectra of the system. **a** Δλ=60 nm **b** Δλ=20 nm. *1*, BSA-PCB77; *2*, BSA-PCB52; *3*, BSA-C_2H_5OH; *4*, BSA. Conditions: BSA: 9.0×10^{-7} g mL^{-1}; PCB77: 2.1×10^{-6} mol L^{-1}; PCB52: 2.1×10^{-6} mol L^{-1}; NaAc-HAc: 0.02 mol L^{-1} (pH=5.02)

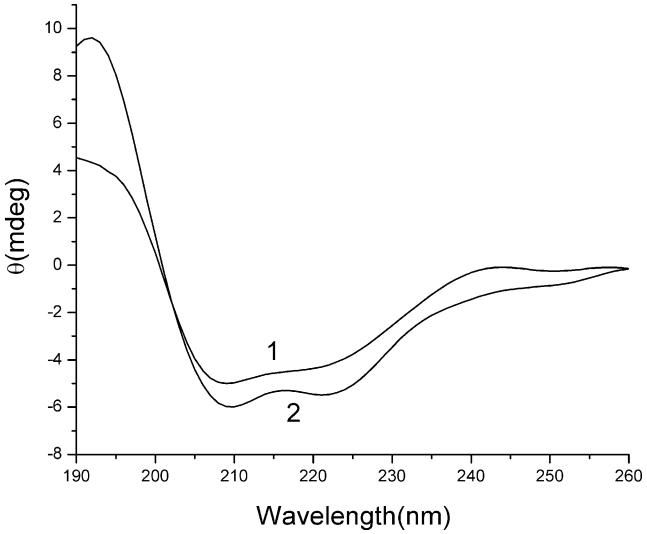

Fig. 9 Circular dichroism spectra of the system. *1*, BSA; *2*, BSA-PCB77. Conditions: BSA: $4.0\times10^{-}$ g mL^{-1}; PCB77: 9.2×10^{-5} mol L^{-1}; NaAc-HAc: 0.02 mol L^{-1} (pH=5.02)

while the β-sheet content reduced from 42.6% to 24.0%. We speculated that the increase of α-helix content of BSA molecules making the secondary structure of BSA more compact, and the decrease of the distance between Tyr residues and Trp residues was beneficial to the energy transfer between them, resulting in the fluorescence intensity of the system significantly enhanced.

Conclusions

In our research, we found that the binding site of PCB77 was closer to tyrosine residues in BSA molecules. Mechanism studies demonstrated that secondary structure of BSA molecules changed more compact in the presence of PCB77, which was beneficial to the energy transfer between Tyr residues and Trp residues, leading to the fluorescence of the system significantly enhanced. Based on this, a sensitive and selectively approach for the trace determination of PCB77 was proposed. This method possesses potential application in preliminary assessment of pollution levels of PCBs in water due to its good selectivity and high sensitivity. The mechanistic studies on the fluorescence enhancement in this system are helpful for the understanding of binding mode and reaction mechanism between protein and persistent organic pollutants, which is of theoretical significance and of applied value in pathological analysis and clinical testing.

Acknowledgments This work was financially supported by the National Basic Research Program of China (Grant No. 2007CB936602) and Natural Science Foundations of Shandong Province (Grant No. Z2008B04).

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