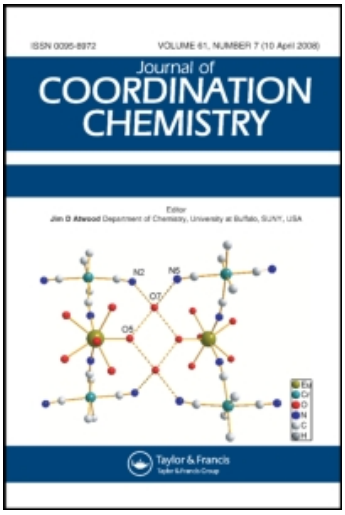

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A spectroscopic study of the interaction of octacarboxylic metal phthalocyanine with bovine serum albumin

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The interaction of octacarboxylic metal phthalocyanines ($MPc(COOH)_8$, M = Al(III) and Co(II) with bovine serum albumin (BSA) has been studied. From the binding isotherm based on spectrophotometric titration, the association constant and a number of ligands per binding site were calculated at 25°C. By using the well studied Hemin chloride (HE), Ibuprofen(IB) and L-tryptophan (TRP) as competitive ligands, the binding sites of $AlPc(COOH)_8$ were found to be on domain I and II of BSA, while on domain I for $Co(COOH)_8$.

Keywords: Octacarboxylic metal phthalocyanine; Bovine serum albumin; Competitive binding method; Spectroscopic method

1. Introduction

Phthalocyanine (Pc) and its substituted derivatives have been intensively studied as photosensitizers for photodynamic therapy (PDT) of cancer and macular degeneration [1]. It was found that the presence of hydrophilic groups in the Pc ring is beneficial for transportation of photosensitizers to malignant tissue by serum albumin and accumulating selectively in the connective tissue which surrounded the tumor cells [2]. The water-soluble octacarboxylic metal phthalocyanine has eight carboxylic groups on the periphery of the benzene ring of the bulk moiety, so it is negatively charged and easy to combine with proteins or other bio-macromolecules.

Serum albumin, the dominant protein of plasma, has a high affinity for negatively charged molecules and determines the distribution of many drugs through the circulatory system [3, 4]. Albumin has been used as effective protein carriers for photosensitizers and anticancer drugs to improve selectivity by receptor-mediated uptake [5], but use of serum albumin as carrier for octacarboxylic phthalocyanine photosensitizers remains little reported. In this article, the binding of $MPc(COOH)_8$

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to BSA was investigated using spectrophotometric titration and the competitive binding approach.

2. *Experimental*

$MPc(COOH)_8$ (M = Al and Co) were synthesized by condensation of benzene-1,2,4,5-tetracarboxylic dianhydride in the presence of urea and the corresponding metal chloride. The crude products were purified by silica gel chromatography (DMF) and characterized by elemental analysis, IR, HPLC, UV/vis and TG-DTA.

The standard solution of BSA was $1.0 \times 10^{-3}\,mol\,L^{-1}$ in buffer solution. Stock solutions of IB and TRP were prepared in buffer solution at $2 \times 10^{-3}\,mol\,L^{-1}$ and $0.1\,mol\,L^{-1}$ respectively, whereas HE was dissolved in $0.01\,mol\,L^{-1}$ NaOH in $2 \times 10^{-3}\,mol\,L^{-1}$. UV/Vis spectra was recorded on Perkin-Elmer Lambda 9 UV/Vis/IR spectrometers. Fluorescence spectra were recorded on the FL900/FS920 steady-state florescence spectrometers.

3. Results and discussion

3.1. *Absorption spectra of $MPc(COOH)_8$ and $MPc(COOH)_8$/BSA conjugate*

The interactions of $MPc(COOH)_8$ (M = Al and Co) with BSA were investigated in different buffer solutions by fluorescence spectroscopy. Figure 1 shows the changes of fluorescence spectra of BSA titrated with $AlPc(COOH)_8$ in different buffer solutions. With increasing pH, the intrinsic emission decreased in intensity and red shifted. The maximal fluorescent quenching of $AlPc(COOH)_8$/BSA conjugate was found in pH = 5 buffer indicating that at pH = 5, there was maximal interaction of $AlPc(COOH)_8$ with BSA.

The absorption spectra of $AlPc(COOH)_8$ and $AlPc(COOH)_8$/BSA conjugate are shown in figure 2. The maximal absorption wavelength of $AlPc(COOH)_8$ was at 694 nm, and its shape indicated that it mainly existed as a monomer. When titrated

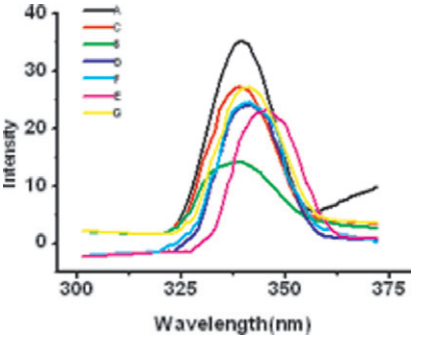

Figure 1. Change in fluorescence spectra of $AlPc(COOH)_8$/BSA conjugate (excited at 280 nm) in different buffer solutions. $C_{BSA} = 1.0 \times 10^{-5}\,mol\,L^{-1}$, $C_{AlPc\ (COOH)\ 8} = 1.0 \times 10^{-5}\,mol\,L^{-1}$, A. BSA B. $AlPc(COOH)_8$. C -G, pH = 3, 4, 6, 5 and 7.

with BSA, the intensity of maximal absorption wavelength of the $AlPc(COOH)_8$/BSA conjugate decreased and shifted to 702 nm, indicating that the binding with BSA caused a virtual monomerization of $AlPc(COOH)_8$.

The absorption spectra of $CoPc(COOH)_8$ and $CoPc(COOH)_8$/BSA conjugate are shown in figure 3. $CoPc(COOH)_8$ mainly existed as a dimer, whose monomer was at 682 nm and the dimer at 630 nm. When titrated with BSA, the intensity of dimer decreased and the monomer increased and shifted to 692 nm. This indicated that binding with BSA caused a partial monomerization of $CoPc(COOH)_8$. A new absorption at 850 nm may be attributed to ligands coordinated with Co^{2+} in $CoPc(COOH)_8$.

Binding isotherm calculated from the combined data of titrations was analyzed by the Scatchard model, as described in detail elsewhere [5]. From the linear Scatchard graph, it was concluded that a protein was associated with more

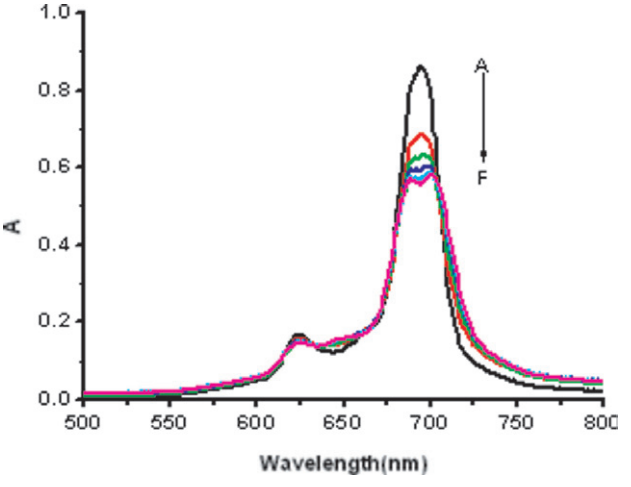

Figure 2. Absorption spectra of AlPc(COOH) and $AlPc(COOH)_8$/BSA conjugate, 25°C, pH = 5, $C_{AlPc(COOH)8}$:1×10^{-5} mol L^{-1}, C_{BSA} ($\times 10^{-5}$ mol L^{-1}) A.0; B.0.2; C.0.4; D.0.6; E.0.8; F.1.0.

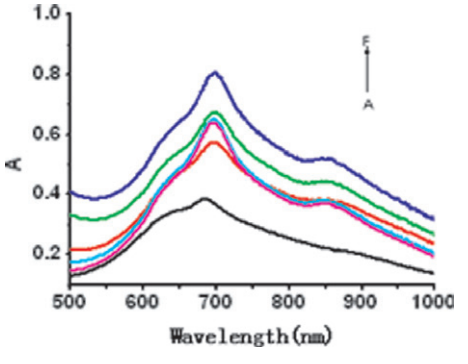

Figure 3. Absorption spectra of $CoPc(COOH)_8$ and $CoPc(COOH)_8$/BSA conjugate, 25°C, pH = 5, $C_{CoPc(COOH)8}$ 1×10^{-5} mol L^{-1}, C_{BSA} ($\times 10^{-5}$ mol L^{-1}) A.0; B.0.2; C.0.4; D.0.6; E.0.8; F.1.0.

than one $MPc(COOH)_8$. By solving the system of parametic equation [5], the association constants K_{sv} as well as the number of ligand molecules per binding site n were calculated. The parameters of the interaction of $MPc(COOH)_8$ with BSA were listed in table 1.

3.2. *Fluorescence spectra of $MPc(COOH)_8$ and $MPc(COOH)_8$/BSA conjugate*

Figure 4 shows the changes of fluorescence emission of BSA upon titration with $MPc(COOH)_8$ in pH 5.0 buffer solution. With increasing concentration of $MPc(COOH)_8$, the intrinsic emission band of protein at 357 nm decreased in intensity and shifted gradually to 361 nm. The fluorescence emission of BSA was quenched by two $MPc(COOH)_8$ in a similar manner, perhaps due to, in acid medium, amino acids of proteins molecules were highly protonated, thus positive protein molecules easily interacted with the negative $MPc(COOH)_8$. The quenching data followed the Stern–Volmer equation (1)

$$I_0/I = 1 + K_{sv}[Q] = 1 + K_q\tau_s[Q] \quad (1)$$

Table 1. The parameters of $MPc(COOH)_8$ interaction with BSA.

Compounds	$AlPc(COOH)_8$		$CoPc(COOH)_8$	
Association constants K_{sv} ($\times 10^5\,mol^{-1}L^{-1}$)	5.74 ± 0.82*	3.51 ± 0.04#	3.24 ± 0.48*	6.90 ± 0.08#
Number of binding sites n	3.54 ± 0.42*		1.9 ± 0.15*	
Dissociation constant K_D ($\times 10^{-6}\,mol^{-1}L^{-1}$)	2.85 ± 0.03#		1.40 ± 0.01#	
Apparent quenching coefficient K_q ($\times 10^{13}\,mol^{-1}L^{-1}\,s^{-1}$)	4.91 ± 0.09#		4.55 ± 0.07#	
Shift in emission position $\Delta\lambda$ (nm)	+4		−3	

The value with * was calculated by absorption spectroscopic method. The value with # was calculated by fluorescence spectroscopic method.

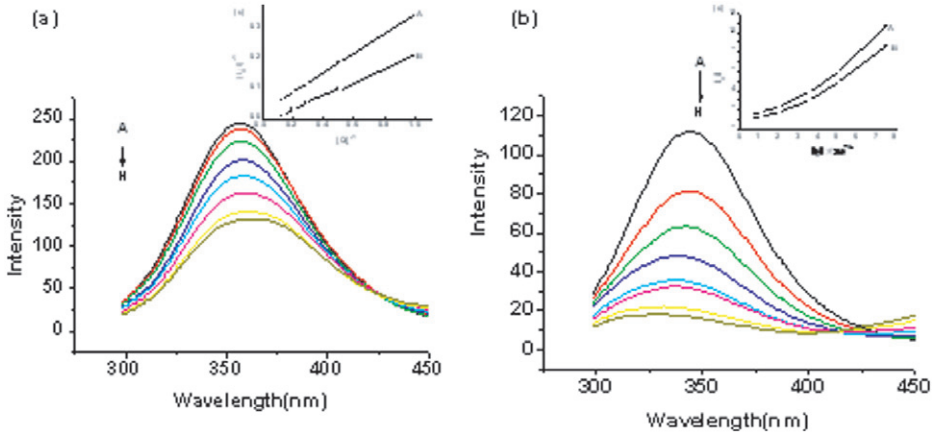

Figure 4. Changes in fluorescence spectra of BSA ($1.0 \times 10^{-6}\,mol\,L^{-1}$, excited at 280 nm) in buffer upon titration with different concentration of $MPc(COOH)_8$ from A to H, the concentration of $AlPc(COOH)_8$ and $CoPc(COOH)_8$ were 0, 0.5, 1.0, 2.0, 3.5, 5.0, 7.5 and 10 ($\times 10^{-6}\,mol\,L^{-1}$). The inset (c) is the Stern–Volmer plot of $MPc(COOH)_8$ (d) Lineweaver–Burk plot of $MPc(COOH)_8$ with BSA. (a)$AlPc(COOH)_8$, (b) $CoPc(COOH)_8$.

where I_0 and I were the intensity fluorescence in the absence and presence of $MPc(COOH)_8$ respectively, and K_{sv} was the association constant (Stern–Volmer quenching constant) which was the product of bimolecular quenching rate constant K_q and the lifetime of the fluorescing species in the absence of $MPc(COOH)_8$ (τ_s), [Q] was the concentration of $MPc(COOH)_8$.

Equation (2) can be deduced from equation (1)

$$(I_0 - I)^{-1} = I_0^{-1} + I_0^{-1} K_D [Q]^{-1} \tag{2}$$

where K_D was diassociation constant. K_{sv} can be deduced from K_D.

The quenching data follow the Stern–Volmer equation (equation (1)) and lineweaver-Burk equation (equation (2)) as show in the inset of figure 4. The corresponding K_{sv}, K_D, n values, and shift of band were compiled in table 1.

The K_{Sv} values, which reflect the degree of interaction between BSA and $MPc(COOH)_8$, were comparable to these for systems involving sulfonated metallophthalocyanines as quencher ($2 \times 10^4\,mol^{-1}L^{-1}$-$1.1 \times 10^5\,mol^{-1}L^{-1}$) [6]. Since the fluorescence lifetime of BSA is on the order of 10^{-8} s [7], the apparent quenching coefficients K_q for $MPc(COOH)_8$ are on the order of $10^{13}\,mol^{-1}L^{-1}\,s^{-1}$, much higher than the normal value for dynamic quenching (*ca* $10^{10}\,mol^{-1}L^{-1}\,s^{-1}$ [8]. This strongly suggested that the fluorescence quenching of BSA by $MPc(COOH)_8$ was mainly through a static quenching mechanism [9].

3.3. *Binding sites of MPc(COOH)$_8$ (M = Al, Co) on BSA*

BSA consists of three similar structural domains I, II and III. Each domain contains two subdomains, A and B. Six domain regions of ligand binding to protein were distinguished [10]. To identify the binding sites of $MPc(COOH)_8$ (M = Al, Co), the competitive binding with well studied BSA ligands (HE, IB and TRP) were used [11]. The binding site of HE ($K_1 = 5 \times 10^7\,mol\,L^{-1}$) was on domain I, and IB ($K_2 = 2.2 \times 10^5\,mol\,L^{-1}$) on domain II, and TRP ($K_2 = 2.5 \times 105\,mol\,L^{-1}$) on the domain III [11]. In order to identify the binding sites of $MPc(COOH)_8$ (M = Al, Co), the interaction of $MPc(COOH)_8$ (M = Al, Co)/BSA conjugate with the various competitive binding BSA ligands including HE, IB and TRP were first investigated by absorption and fluorescence spectroscopy. The concentration of competing ligands to be added were calculated using known association constants in order to provide complete displacement of the bound $MPc(COOH)_8$.

3.3.1. Binding sites of AlPc(COOH)$_8$ on BSA. $AlPc(COOH)_8$ ($1 \times 10^{-5}\,mol\,L^{-1}$)/BSA ($1 \times 10^{-5}\,mol\,L^{-1}$) conjugate were titrated by HE ($2 \times 10^{-4}\,mol\,L^{-1}$), IB ($2 \times 10^{-4}\,mol\,L^{-1}$) and TRP ($1 \times 10^{-2}\,mol\,L^{-1}$), respectively. Titration of $AlPc(COOH)_8$/BSA conjugate by HE and IB revealed gradual changes in the absorption spectra (figure 5) corresponding to transition of the bound $AlPc(COOH)_8$ into the free state; the fluorescence of $AlPc(COOH)_8$ quenched by BSA increased again in intensity when titrated by HE and IB (figure 6). Total displacement of $AlPc(COOH)_8$ with HE and IB was observed at the $AlPc(COOH)_8$/HE and $AlPc(COOH)_8$/IB molar rate of 1/20.

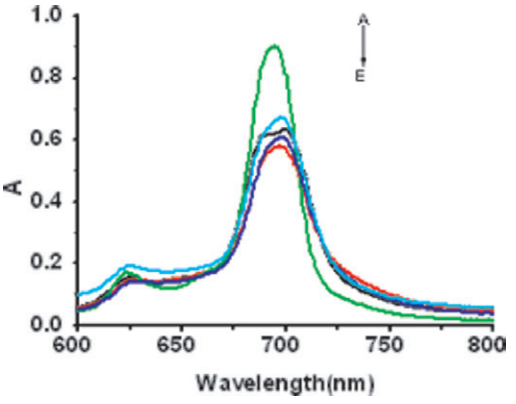

Figure 5. Effects of ligands on absorption spectra of $AlPc(COOH)_8$-BSA conjugate (1 : 1) A. $AlPc(COOH)_8$ (1×10^{-5} mol L^{-1}) B. $AlPc(COOH)_8 + BSA + HE(1:1:20)$ C. $AlPc(COOH)_8 + BSA + IB(1:1:20)$ D. $AlPc(COOH)_8 + BSA(1:1)$ E. $AlPc(COOH)_8 + BSA + TRP(1:1:1000)$.

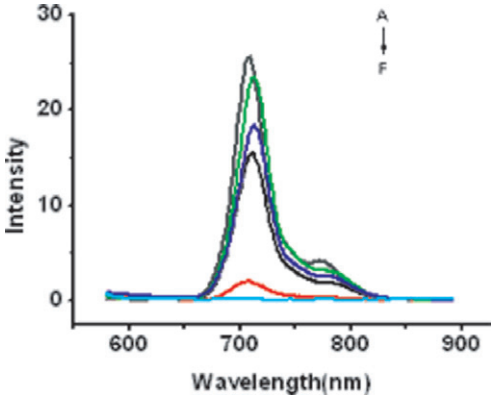

Figure 6. Effects of ligands on fluorescence spectra of $AlPc(COOH)_8$/BSA conjugate (1 : 1) (excited at 560 nm) A. $AlPc(COOH)_8$ (1×10^{-5} mol L^{-1}) B. $AlPc(COOH)_8 + BSA + HE(1:1:20)$ C. $AlPc(COOH)_8 + BSA + IB(1:1:20)$ D. $AlPc(COOH)_8 + BSA(1:1)$ E. $AlPc(COOH)_8 + BSA + TRP(1:1:1000)$ F. $BSA(1 \times 10^{-5}$ mol $L^{-1})$.

In contrast, the absorption spectra of the $AlPc(COOH)_8$/BSA conjugate was not disturbed by addition of TRP at different molar ratios. The $AlPc(COOH)_8$/TRP ratio changed from 1/10 to 1/1000. The experiment indicated that the binding site of domain III was not occupied by $AlPc(COOH)_8$. Therefore, one can conclude that the binding sites of $AlPc(COOH)_8$ overlapped those of HE and IB and were located on domains I and II.

In attempting to identify the primary binding site of $AlPc(COOH)_8$, the concentration ratio of $AlPc(COOH)_8$/BSA was 1 : 10, thus the Pc binding to the secondary site was insignificant. Titration of $AlPc(COOH)_8$/BSA conjugate by HE revealed changes in the absorption spectra (figure 7) and fluorescence spectra (figure 8) corresponding to transition of bound $AlPc(COOH)_8$ into free. No evidence for displacement of bound $AlPc(COOH)_8$ with IB and TRP was observed, indicating that the primary binding site of $AlPc(COOH)_8$ was located on domain I.

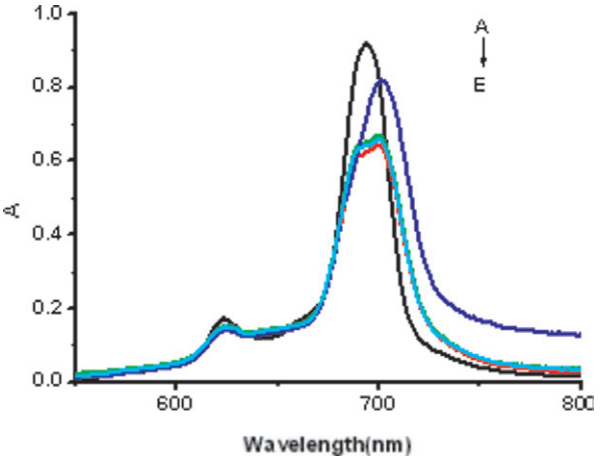

Figure 7. Effects of ligands on absorption spectra of $AlPc(COOH)_8$/BSA conjugate (1 : 10) A. $AlPc(COOH)_8$ (1×10^{-5} mol L^{-1}) B. $AlPc(COOH)_8$ + BSA + HE(1 : 1 : 20) C. $AlPc(COOH)_8$ + BSA + IB(1 : 1 : 20) D. $AlPc(COOH)_8$ + BSA(1 : 10) E. $AlPc(COOH)_8$ + BSA + TRP(1 : 1 : 1000).

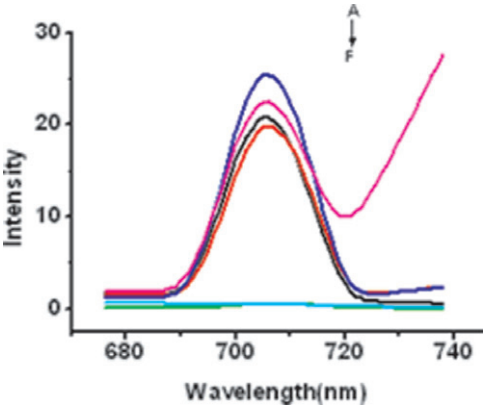

Figure 8. Effects of ligands on fluorescence spectra of $AlPc(COOH)_8$/BSA conjugate (1 : 10) (excited at 560 nm) A. $AlPc(COOH)_8$ (1×10^{-5} mol L^{-1}) B. $AlPc(COOH)_8$ + BSA + HE(1 : 1 : 20) C. $AlPc(COOH)_8$ + BSA + IB(1 : 1 : 20) D. $AlPc(COOH)_8$ + BSA(1 : 10) E. $AlPc(COOH)_8$ + BSA + TRP(1 : 1 : 1000) F. BSA (1×10^{-4} mol L^{-1}).

3.3.2. The binding sites of $CoPc(COOH)_8$ on BSA. Similar experiments were performed to study the binding sites of $CoPc(COOH)_8$ on BSA. $CoPc(COOH)_8$ (1×10^{-5} mol L^{-1})/BSA (1×10^{-5} mol L^{-1} conjugate was titrated by HE (2×10^{-4} mol L^{-1}), IB (2×10^{-4} mol L^{-1}) and TRP (1×10^{-2} mol L^{-1}), respectively. No disturbance of the absorption and fluorescence spectra change was observed for $CoPc(COOH)_8$/BSA conjugate titrated by HE and TRP at different molar ratios. A relatively small increase for the intensity of absorption spectra was found when $CoPc(COOH)_8$/BSA conjugate was titrated by IB. This indicated conversion of bound $CoPc(COOH)_8$ into free (figure 9), obvious when studied by fluorescence spectroscopy. The intensity of fluorescence of $CoPc(COOH)_8$ quenched by BSA increased (figure 10)

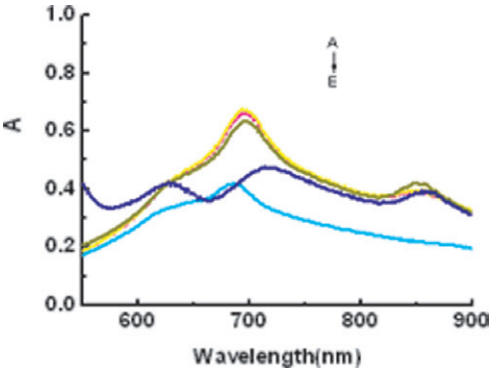

Figure 9. Effects of ligands on absorption spectra of $CoPc(COOH)_8$/BSA conjugate (1 : 1) A. CoPc(COOH)8 (1×10^{-5} mol L^{-1}) B.$CoPc(COOH)_8$ + BSA + IB(1 : 1 : 20) C. $CoPc(COOH)_8$ + BSA + HE(1 : 1 : 20) D. $CoPc(COOH)_8$ + BSA(1 : 1) E. $CoPc(COOH)_8$ + BSA + TRP(1 : 1 : 1000).

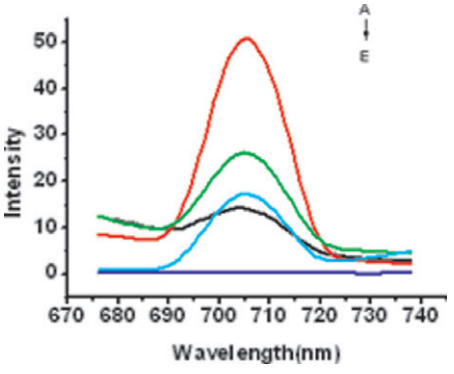

Figure 10. Effects of ligands on fluorescence spectra of $CoPc(COOH)_8$/BSA conjugate (1 : 1) (excited at 560 nm) A.$CoPc(COOH)_8$ (1×10^{-5} mol L^{-1}) B.$CoPc(COOH)_8$ + BSA + IB(1 : 1 : 20) C.$CoPc(COOH)_8$ + BSA + HE(1 : 1 : 20) D. $CoPc(COOH)_8$ + BSA(1 : 1) E. $CoPc(COOH)_8$ + BSA + TRP(1 : 1 : 1000).

when it was titrated by IB. Hence the binding site of $CoPc(COOH)_8$ was located on domain II.

Similarly for 1 : 10 $CoPc(COOH)_8$/BSA, only absorption (figure 11) and fluorescence spectra (figure 12) changes were observed when $CoPc(COOH)_8$/BSA conjugate was titrated by IB; thus the primary binding site of $CoPc(COOH)_8$ was domain II.

4. Conclusion

In summary, the interactions of $MPc(COOH)_8$, (M = Al and Co) with BSA were studied spectroscopically. The high-binding affinity and abundance of $MPc(COOH)_8$ on BSA indicated that BSA can be considered as the main endogenous carrier of $MPc(COOH)_8$.

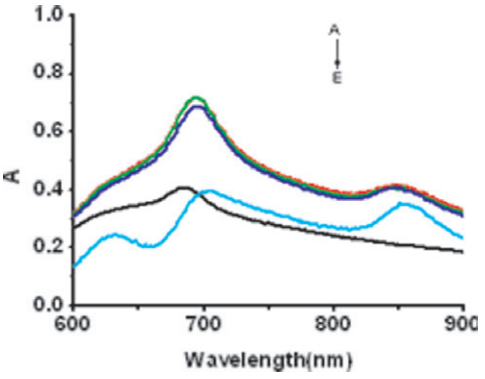

Figure 11. Effects of ligands on absorption spectra of $CoPc(COOH)_8$/BSA conjugate (1 : 10) A. $CoPc(COOH)_8$ (1×10^{-5} mol L^{-1}) B. $CoPc(COOH)_8$ + BSA + IB(1 : 1 : 20) C. $CoPc(COOH)_8$ + BSA(1 : 10) D. $CoPc(COOH)_8$ + BSA + HE(1 : 1 : 20) E. $CoPc(COOH)_8$ + BSA + TRP(1 : 1 : 1000).

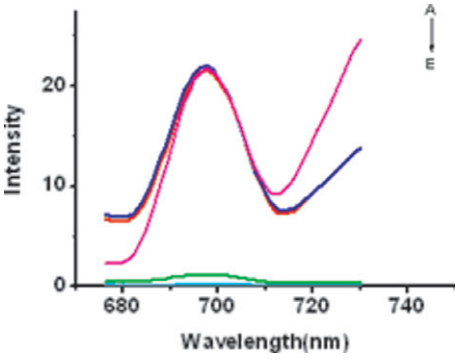

Figure 12. Effects of ligands on fluorescence spectra of $CoPc(COOH)_8$/BSA conjugator (1 : 10) (excited at 560 nm) A. $CoPc(COOH)_8$ (1×10^{-5} mol L^{-1}) B. $CoPc(COOH)_8$ + BSA + IB(1 : 1 : 20) C. $CoPc(COOH)_8$ + BSA(1 : 10) D. $CoPc(COOH)_8$ + BSA + HE(1 : 1 : 20) E. $CoPc(COOH)_8$ + BSA + TRP(1 : 1 : 1000).

Acknowledgments

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References

[1] D.E.J.G. Dolman, D. Fukumura, R.K. Jain. *Nat. Rev. Cancer*, **3**, 380 (2003).
[2] P.P.S. Lee, P.C. Lo, E.Y.M. Chan, W.P. Fong, W.H. Ko, D.K.P. Ng. *Tetra. Lett.*, **46**, 1551 (2005).
[3] W.M. Sharma, J.E. Van Lier. *Bioconjugate Chem.*, **16**, 1166 (2005).
[4] M.R. Detty, S.L. Gibon, S.J. Wagner. *J. Med. Chem.*, **47**, 3897 (2004).

[5] C.R. Cantor, P.R. Schimnel, *Biophysical Chemistry*, Vol. 3, Chap. 15, W.H. Freeman and Co, San Francisco (1985).
[6] A. Oguusipe, T. Nyokong. *Photochem. Photobiol. Sci.*, **4**, 510 (2005).
[7] I.E. Borisevitch, T.T. Tominaga, H. Imasato, M. Tabak. *J. Lumin.*, **69**, 65 (1996).
[8] U. Kragh-Hansen. *Dan. Med. Bull.*, **37**, 57 (1990).
[9] M. Dockal, D.C. Carter, F. Ruber. *J. Biol. Chem.*, **274**, 29303 (1999).
[10] T. Peters Jr., *All about Albumin: Biochemistry, Genetics and Medical Applications*, Academic Press, Orlando, FL (1996).
[11] A.I Filyasova, I.A. Kudelina, A.V. Feofanov. *J. Mol. Struct.*, **566**, 173 (2001).