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Interaction of Surface-active Fluorescence Probes with Bovine Serum Albumin

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Abstract: The binding between three surface-active substituted 3H-indole fluorescence probes and bovine serum albumin (BSA) in aqueous solution was studied using fluorescence quenching. The binding constants of 3H-indole molecules with BSA were obtained. According to the Förster resonance energy transfer theory, the distances between 3H-indole molecules and tryptophan of BSA were calculated. The results show that the oligoethyloxyethylene chain of 3H-indole molecules is longer, the binding between them is stronger, the energy transfer efficiency is higher, and the distance between tryptophan and 3H-indole is nearer.

Keywords: Substituted 3H-indole quaternary ammonium molecule, bovine serum albumin, fluorescence resonance energy transfer.

The interaction between surfactants and proteins has been extensively studied. A lot of studies have been devoted to study the interaction of bovine serum albumin (BSA) with surfactants, in particular, anionic surfactants, such as sodium dodecylsulfate[1-3]. But only a few literatures reported on the effect of cationic surfactants association to proteins[4]. In general, the cationic surfactants interact less strongly with proteins, mainly as a consequence of a smaller relevance of electrostatic interactions at the pHs of interest. In this paper, we studied the interaction between three surface-active substituted 3H-indole fluorescence probes and bovine serum albumin (BSA) in aqueous solution, and found that their Stern-Volmer binding constants (K_{sv} values) are more than 10^4 L/mol, on the other hand, the quenching of BSA fluorescence strongly depends upon the length of the oligoethyloxyethylene chain. And this is also consistent with the result of distance between tryptophan and 3H-indole gained by the method of Förster energy transfer.

Experimental

The synthesis and purification for compound **1**, **2**, **3** (see **Scheme 1**) have been carried out according to the methods of Popowycz[5] and Xu[6]. BSA (fraction V) is gained from

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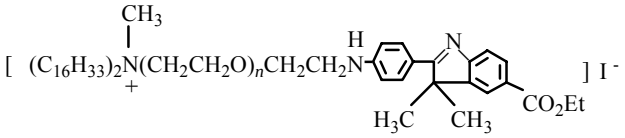
Scheme 1



compound **1**, *n*=1; compound **2**, *n*=2; compound **3**, *n*=3

Beijing Shuangluo Weishengwu Peiyangji Zhipinchang (electrophoresis grade). All other chemical reagents used in this study were analytical grade. Triply distilled water was used throughout the experiments. Tris-HCl buffer solution was used for the pH 7.4 adjustment. Absorption spectra were recorded on an U-3010 (Hitachi, Japan) spectrophotometer using 1 cm-path quartz cells. The slit was 5 nm. Fluorescence spectra (λ_{ex}= 280 nm, λ_{em}= 343 nm) were measured on a FL-4500 (Hitachi, Japan) spectrofluorimeter, and the band-passe was 5 nm. A mixture of 3H-indole solution, BAS standard solutions, and 1 mL 0.5 mol·L^{-1} Tris-HCl buffer (pH 7.4) was diluted to 5 mL with 0.9% NaCl solution.

Results and Discussion

Binding constant of 3H-indole with BSA

In order to study the binding constant of 3H-indole with BSA, the fluorescence quenching method was used. According to the Stern-Volmer equation, in an appropriate concentration range, the relationship between the quenching efficiency (F_0/F) and the concentration of quencher ([Q]) obeys the following equation:

$$F_0/F = 1 + k_{sv}[Q] \qquad (1)$$

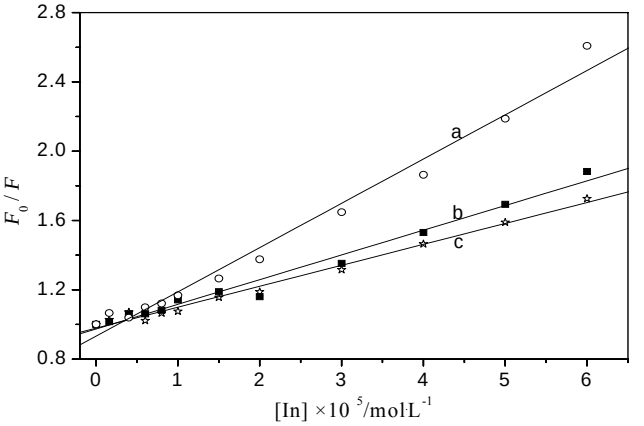
where F_0 and F are the fluorescence intensities of the fluorophore in the absence and presence of the quencher, respectively, and k_{sv} is the Stern-Volmer quenching constant, corresponding to the slope of the plot of F_0/F *vs*. [Q]. The intrinsic fluorescence of BSA at 343 nm (excitation at 280 nm) is strongly quenched by 3*H*-indole. A reasonable linear relationship between the quenching efficiency and the concentration of 3H-indole in the range 0-6.0 × 10^{-5} mol·L^{-1} at room temperature is obtained (see **Figure. 1**). The results indicate that k_{sv} of compound **1**, **2**, **3** with BSA is 1.20 × 10^4 L·mol^{-1}, 1.42 × 10^4 L·mol^{-1}, 2.56 × 10^4 L·mol^{-1} respectively. So the oligoethyloxyethylene chain is longer, the binding constant is larger, and the binding between them and BSA is stronger.

Förster energy transfer

Bound 3*H*-indole at ground state absorbs light emitted from BSA and converts to excited state, and then the rapid Förster energy transfer occurs to the nearby tryptophan. According to the Förster theory[7-8], the efficiency of energy transfer is given by

$$E = 1 - F/F_0 = \frac{R_0^6}{R_0^6 + r^6} \qquad (2)$$

Figure 1 Stern volmer plot of F_0 / F *vs* [In].



a is compound **3**, b is compound **2**, c is compound **1**

where *r* is the distance between the donor and acceptor, and R_0 is the Förster or critical transfer distance at which the energy transfer rate is 50%. R_0, a function of the spectral properties of a donor-acceptor pair, can be represented as:

$$R_0^6 = \frac{8.8 \times 10^{-25} \kappa^2 \phi_D}{n^4} \frac{\int F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d(\lambda)}{\int F_D(\lambda) d(\lambda)} \qquad (3)$$

where κ^2 is the orientation factor related to the geometry of the donor-acceptor diploes and $\kappa^2 = 2/3$ for random orientation as in fluid solution, n is the refractive index of the medium, ϕ_D is the quantum yield of the donor in the absence of acceptor, $F_D(\lambda)$ is the spectral distribution of the donor emission, $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor.

It is showed in **Table 1** ([BSA] = 1.0×10^{-6} mol•L^{-1}) that the oligoethyloxyethylene chain is longer, the energy transfer efficiency is higher, and the distance between tryptophan and 3H-indole is nearer. This regulation is also consistent with the trend of their correspond to binding constants.

Table 1 Resonance energy transfer date for compound **1**, **2** , **3** and tryptophan of BSA

Compd.	J values / 10^{-14} cm^3•mol/L	critical transfer distance (nm)	energy transfer efficiency (%)	Distance (nm)
1	1.8	2.70	7.0	4.16
2	2.1	2.77	12.4	3.84
3	2.1	2.77	14.6	3.72

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References

1. S. Deep, J. C. Ahluwalia, *Phys. Chem. Chem. Phys.*, **2001**, *3*, 4583.
2. P. Hazra, D. Chakrabarty, A. Chakraborty, N. Sarkar, *Biochem. and Biophys. Res. Commun.*, **2004**, *314*, 543.
3. T. Xu, X. Shen, H. Gao, *Spectroscopy and Spectra Analysis*, in press.
4. X. Diaz, E. Abuin, E. Lissi, *J. Photochem. Photobio. A: Chem.,* **2003**, *155*, 157.
5. A. M. Sc Popowycz, Thesis, University, Montreal, **1991**.
6. T. Xu; X. Shen; H. Gao, *Chin. Chem. Lett.*, **2004**, *15* (1), 26.
7. J. H. Kleinschmidt, L. K. Tamm, *Biochemistry,* **1999**, *38* (16), 4996.
8. A. C. Ribou, J. Vigo, P. Viallet, JM. Salmon, *Biophys. Chem.*, **1999,** *81*, 179.

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