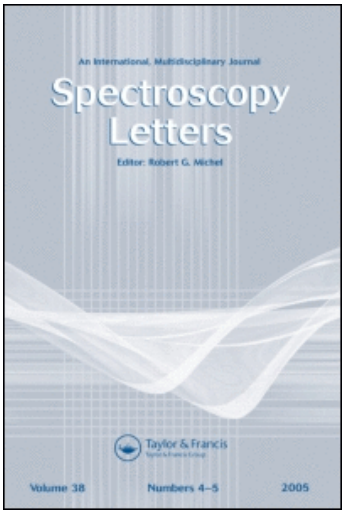

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Abstract: The interaction mechanism between trimethoxyphenylfluorone (TM-PF)–molybdenum [Mo(VI)] complex and human serum albumin (HSA) has been investigated using fluorescence method. The binding constants were measured at different temperature. Based on the theory of Förster energy transfer, the binding distance and the energy transfer efficiency between TM-PF–Mo(VI) complex and HSA were obtained. According to the thermodynamic parameters, the main sorts of binding force can be judged. The results indicate that HSA and TM-PF–Mo(VI)

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complex have strong interactions. The mechanism of quenching belongs to static quenching, and the main sorts of binding force are van der Waals force and H-bonding.

Keywords: Binding constant, energy transfer, fluorescence quenching, human serum albumin, trimethoxyphenylfluorone–Mo(VI) complex

INTRODUCTION

Human serum albumin (HSA) has a primary sequence of 609 amino acids in a single polypeptide chain with 17 disulfide bridges and one free cysteine residue.[1] It is the major transport protein for unesterified fatty acids and is also known to bind to metabolites, surfactants, drugs, organic compounds, and metal ions such as Ca^{2+}, Zn^{2+}, and Ni^{2+}.[2–10] Studies on the binding mechanism between protein and small molecules can give us a lot of useful information. For example, a detailed characterization of drug–protein binding properties is essential not only for understanding its key physiological function but also to help control its impact on drug delivery. Hence, interest in the mechanism of the interaction between them has stimulated much research using different methods in recent years.

Trimethoxyphenylfluorone (TM-PF), one of the triphenylmethane dyes, is a novel analytical reagent. Recently, we have done a series of works using triphenylmethane dye–Mo(VI) complex as a probe and determined protein via Rayleigh light scattering and spectroscopic methods.[11–14] So in continuation of our previous works, the interaction mechanism between TM-PF–Mo(VI) and human serum albumin (HSA) is studied by fluorescent method. In this paper, fluorescence spectroscopy and the theory of energy transfer are employed to investigate the binding mechanism of HSA with TM-PF–Mo(VI) complex. It may be conducive to our better understanding of their interaction on the biomolecular level. This system may be considered as a model for gaining general fundamental insights into drug–protein binding. Thus, studies on the interaction mechanism increasingly become an important research field in chemistry.

Fluorescence spectroscopy technique is the most accessible method because it has the advantages of high sensitivity, convenience in performance, and simplicity in apparatus. Tryptophan (trp) residues make major contribution to the intrinsic fluorescence of protein. The fluorescence of trp residues is extremely sensitive to its environment and can be monitored to reveal information regarding protein structure and conformation. The binding mode, binding constants, as well as binding distance between HSA and TM-PF–Mo(VI) complex at different temperatures are described in detail as below.

MATERIALS AND METHODS

Apparatus

Intensity and spectra of fluorescence were carried out on a Perkin-Elmer Model LS-55 luminescence spectrometer (USA) using 1.0-cm quartz cells and a thermostat bath. Absorption spectra were obtained with a Shimadzu model UV-3101PC spectrophotometer (Japan). A model PHS-3B pH meter was employed for pH measurements.

Reagents

Unless otherwise mentioned, all chemicals were of analytical reagents grade, and doubly distilled water was used throughout this experiment.

Human serum albumin (HSA, 1.0×10^{-4} mol $\cdot$ L^{-1}) was obtained from Sigma (Germany) and prepared by dissolving commercial products in doubly distilled water and stored at $0 \sim 4^{\circ}$C.

TM-PF solution (1.0×10^{-4} mol $\cdot$ L^{-1}) was purchased from the Institute of Changke Chemical Reagent (Shanghai, China) and prepared by dissolving 0.0041 g of TM-PF in ethanol containing several drops of 1 : 1 sulfuric acid and brought to 100 mL in a volumetric flask with pure ethanol. The structure of TM-PF is shown in Fig. 1.

A Mo(VI) stock solution of 0.1 mol $\cdot$ L^{-1} was prepared by dissolving 1.4394 g of spectroscopically pure MoO_3 in 20 mL of concentrated HCl, under heating. After cooling, the solution was diluted to 100 mL and stored. Mo(VI) working solution (1.0×10^{-4} mol $\cdot$ L^{-1}) was prepared by the appropriate dilution of the stock solution.

A Tris-HCl buffer (pH 7.42) was obtained by mixing 0.1 mol $\cdot$ L^{-1} Tris and 0.1 mol $\cdot$ L^{-1} HCl and adjusting the pH to 7.42.

Procedures

The following solutions were successively added into a 10 mL colorimetric tube: 2.0 mL pH 7.42 Tris-HCl buffer, 1.0 mL of 1.0×10^{-4} mol $\cdot$ L^{-1}

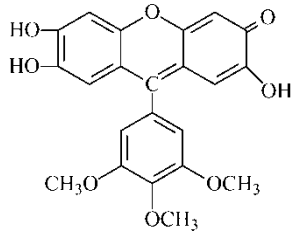

Figure 1. The structure of TM-PF.

protein solution, and various amount of 1.0×10^{-4} mol · L^{-1} TM-PF-Mo(VI) complex solution (the concentration ratio of TM-PF and Mo(VI) is 1 : 1). Then, the mixture was diluted to the mark with doubly distilled water and mixed thoroughly. The sample was excited at 285 nm, and the fluorescence intensity was monitored at $300 \sim 500$ nm after 15 min. Excitation and emission slit widths were set at 15/4 nm.

RESULTS AND DISCUSSION

Fluorescence Quenching Spectra

Figure 2 shows the emission spectra of HSA in the absence and presence of TM-PF–Mo(VI) complex at 20°C. TM-PF–Mo(VI) complex is observed to quench the fluorescence of HSA, and the maximum wavelength is shifted. All these indicate that TM-PF–Mo(VI) complex can interact with HSA.

Burstein[15] thought that the maximum fluorescence emission of trp residues is extremely sensitive to its environment. When they are exposed to the aqueous phase, the maximum wavelength is within $350 \sim 353$ nm. If they are completely in hydrophobic environment, the maximum wavelength is between 330 and 333 nm. As can be seen from Fig. 2, the maximum wavelength is shifted from 353 nm to 333 nm. So it is deemed that trp residues pass from the aqueous phase into hydrophobic environment gradually with the concentration of probe increasing.

Binding Constant of HSA and TM-PF–Mo(VI) Complex

Fluorescence quenching includes dynamic and static quenching. For the dynamic quenching, the equation is described by

$$F_0/F = 1 + k_q \tau_0 c_Q = 1 + K_{SV} c_Q$$

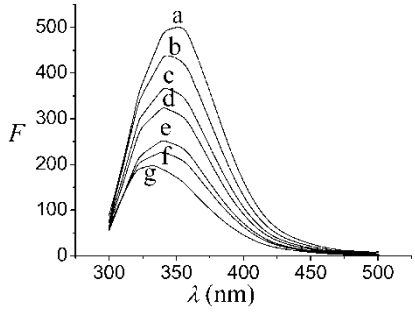

Figure 2. The fluorescence emission spectra of HSA (1.0×10^{-5} mol · L^{-1}) with different concentrations of TM-PF–Mo(VI) complex (from a to g): 0, 0.25, 0.50, 0.75, 1.0, 1.25, 1.5 (unit $\times 10^{-5}$ mol · L^{-1}), $t = 20$°C.

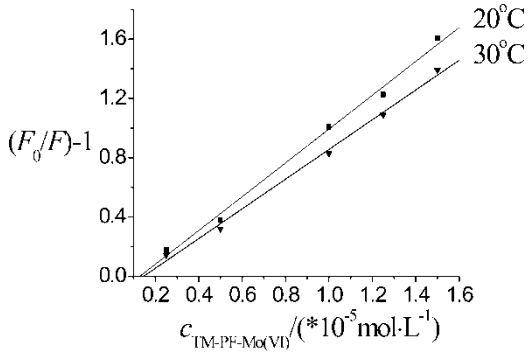

Figure 3. Stern–Volmer quenching plot of HSA with increasing concentration of TM-PF–Mo(VI) complex.

where F_0 and F are the fluorescence intensities in the absence and in the presence of TM-PF–Mo(VI) complex, respectively, K_{sv} is the Stern–Volmer fluorescence quenching constant, c_Q is the concentration of quencher, τ_0 *is* average lifetime of molecule without quencher, and k_q is quenching rate constant of bimolecule. For the static quenching, the equation is described by

$$(F_0 - F)^{-1} = F_0^{-1} + K_{LB}^{-1} F_0^{-1} c_Q^{-1}$$

where F_0, F, and c_Q are the same as formerly, and K_{LB} is formation constant.

In order to judge the type of quenching, the procedure was first assumed to be dynamic quenching. The plot of $F_0/F-1$ versus c_Q is constructed (Fig. 3), and k_q and K_{sv} are shown in Table 1.

In general, the fluorescence lifetime biomacromolecule is 10^{-8} s. Maximum scatter collision quenching constant of quencher to biomacromolecule is $2.0 \times 10^{10}\,L \cdot mol^{-1} \cdot s^{-1}$.[16,17] Obviously, the rate constant given in Table 1 is greater than k_q ($2.0 \times 10^{10}\,L \cdot mol^{-1} \cdot s^{-1}$) of scatter procedure. Therefore, the quenching mentioned above is not initiated by dynamic collision.

Table 1. Binding parameters of HSA with TM-PF–Mo(VI) at different temperatures

	t	K_{sv}	k_q	K_{LB}
Protein	(°C)	($L \cdot mol^{-1}$)	($L \cdot mol^{-1} \cdot S^{-1}$)	($L \cdot mol^{-1}$)
HSA	20	1.140×10^5	1.140×10^{13}	3.59×10^4
HSA	30	1.002×10^5	1.002×10^{13}	2.32×10^4

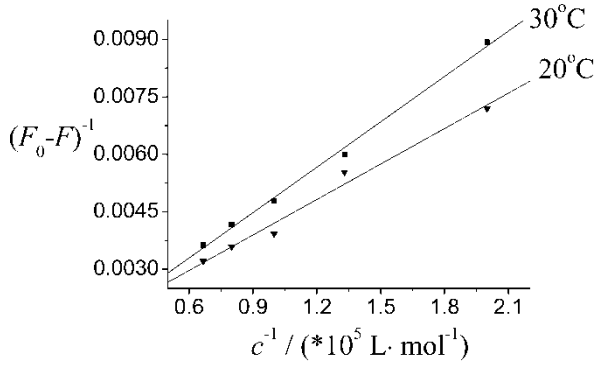

Figure 4. Double-reciprocal curves of fluorescence intensity with concentration of TM-PF–Mo(VI) complex.

Because it is static quenching, the quenching constant is considered as the formation constant of TM-PF–Mo(VI) and HSA. The double-reciprocal plot is made by $(F_0 - F)^{-1}$ versus c_Q^{-1} (Fig. 4), and K_{LB} is shown in Table 1.

It is found that there is a strong interaction between TM-PF–Mo(VI) complex and HSA. The interaction is weakened when the temperature rises.

Energy Transfer Between HSA and TM-PF–Mo(VI) Complex

According to Förster's theory,[18,19] energy transfer depends mainly upon the following factors: (1) spectral overlap between the donor emission and the acceptor absorption; (2) the distance r between the acceptor [TM-PF–Mo(VI) complex] and the donor (HSA) is within 7 nm; (3) a donor has high fluorescence quantum yield.

Figure 5 is the overlap of the fluorescence spectra of HSA and the absorption spectra of TM-PF–Mo(VI) complex when the molar ratio is 1 : 1. From Fig. 5, we have calculated the overlap integral J

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda}{\sum F(\lambda)\Delta\lambda}$$

where $F(\lambda)$ is the fluorescence quenching when the wavelength is λ, and $\varepsilon(\lambda)$ is the molar absorbance coefficient at the wavelength of λ.

The Förster distance R_0 at which the efficiency of energy transfer is 50% is calculated by

$$R_0^6 = 8.8 \times 10^{-25} K^2 n^{-4} \varphi J$$

where K^2 is the orientation factor $(2/3)$, n is the refractive index (1.336), and ϕ is the average fluorescence quantum yield of donor (0.15).[19]

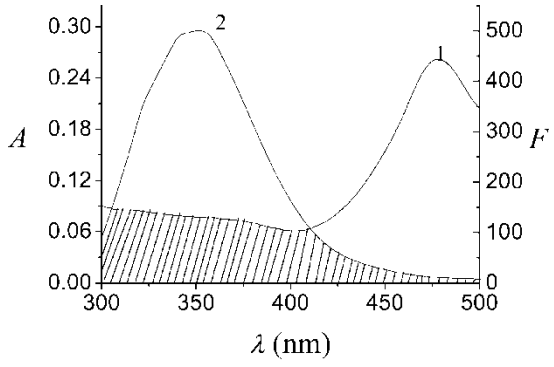

Figure 5. Overlap spectra of TM-PF–Mo(VI) complex spectra (1) and HSA fluorescence spectra (2).

Energy transfer efficiency (E) is defined by

$$E = R_0^6/(R_0^6 + r^6) = 1 - F/F_0$$

According to the equations mentioned above, J, E, and R_0 can be calculated, so the value of r also can be calculated. All of these parameters are displayed in Table 2. It is obvious that with increasing temperature, the distance and energy transfer efficiency are found to increase and decrease, respectively. The results further indicate that the binding reaction of TM-PF–Mo(VI) complex to HSA is through energy transfer, which gives rise to the fluorescence quenching of the protein ($r < 7$ nm).

Analysis of Binding Forces

The interaction of small molecule with protein belongs to intermolecular forces including van der Waals, hydrophobic, electrostatic, and hydrogen-bonding forces. According to the view of Ross,[20] the main sorts of binding force can be judged by thermodynamic parameters (ΔH and ΔS).

Table 2. All parameters of TM-PF–Mo(VI)-HSA at different temperature

Protein	t (°C)	E	J $cm^3/(mol \cdot L^{-1})$	R_0 (nm)	r (nm)
HSA	20	0.502	1.459×10^{-14}	2.61	2.61
HSA	30	0.453	1.428×10^{-14}	2.60	2.68

Table 3. Thermodynamic parameters of HSA with TM-PF–Mo(VI)

Protein	t (°C)	ΔG (KJ · mol^{-1})	ΔH (KJ · mol^{-1})	ΔS (J · mol^{-1} · K^{-1})
HSA	20	−25.55	−32.22	−22.77
HSA	30	−25.32		

If the temperature changes little, ΔH can be regarded as a constant. By the equations

$$\ln\frac{K_2}{K_1} = \frac{\Delta H}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \qquad \Delta G = -RT\ln K \qquad \Delta G = \Delta H - T\Delta S$$

ΔG, ΔH, and ΔS can be obtained, as shown in Table 3.

The negative value of ΔG reveals that the interaction process is spontaneous. The negative values found for ΔH and ΔS seem to suggest that the acting forces are mainly van der Waals force and H-bonding. But it is clear that owing to the complexity of HSA, many forces exist in the reaction between HSA and small molecules synchronously.

CONCLUSIONS

Studies on the interaction of HSA with TM-PF–Mo(VI) complex by fluorescent method have been presented. This method is an important tool for the investigation of the interaction between dye and protein. The results show that TM-PF–Mo(VI) complex is a strong quencher and binds to HSA with high affinity. This study also indicates that TM-PF–Mo(VI) complex quenches the intrinsic fluorescence of HSA, and the binding distance between them is 2.61 nm at 20°C. TM-PF–Mo(VI) complex probably binds to HSA mainly through van der Waals force and H-bonding.

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REFERENCES

1. He, X. M.; Carter, D. C. Atomic structure and chemistry of human serum albumin. *Nature* **1992**, *358*, 209–215.
2. Sulkowska, A. Interaction of drugs with bovine and human serum albumin. *J. Mol. Struct.* **2002**, *614*, 227–232.
3. Tatikolov, A. S.; Costa, S. M. B. Complexation of polymethine dyes with human serum albumin: a spectroscopic study. *Biophys. Chem.* **2004**, *107*, 33–49.
4. Gharibi, H.; Javadian, S.; Hashemianzadeh, M. Investigation of interaction of cationic surfactant with HSA in the presence of alcohols using PFG-NMR and potentiometric technique. *Colloids Surf. A* **2004**, *232*, 77–86.
5. Ohyoshi, E.; Hamada, Y.; Nakata, K.; Kohata, S. The interaction between human and bovine serum albumin and zinc studied by a competitive spectrophotometry. *J. Inorg. Biochem.* **1999**, *75*, 213–218.
6. Gelamo, E. L.; Tabak, M. Spectroscopic studies on the interaction of bovine (BSA) and human (HSA) serum albumins with ionic surfactants. *Spectrochim. Acta A* **2000**, *56*, 2255–2271.
7. Guillaume, Y. C.; Peyrin, E.; Berthelot, A. Chromatographic study of magnesium and calcium binding to immobilized human serum albumin. *J. Chromatogr. B* **1999**, *728*, 167–174.
8. Martinez-Landeira, P.; Ruso, J. M.; Prieto, G.; Sarmiento, F.; Jones, M. N. The interaction of human serum albumin with dioctanoylphosphatidyl-choline in aqueous aolutions. *Langmuir* **2002**, *18*, 3300–3305.
9. Jiang, C. Q.; Gao, M. X.; Meng, X. Z. Study of the interaction between daunorubicin and human serum albumin and the determination of daunorubicin in blood serum samples. *Spectrochim. Acta A* **2003**, *59*, 1605–1610.
10. Sun, H. Z.; Szeto, K. Y. Binding of bismuth to serum proteins: implication for targets of Bi(III) in blood plasma. *J. Inorg. Biochem.* **2003**, *94*, 114–120.
11. Du, B.; Wu, D.; Wei, Q.; Duan, C. H.; Li, Y. Rapid and highly sensitive method for protein determination in biological samples with *m*-nitrophenylfluorone-Mo(VI) complex as a spectral probe. *Instrum Sci. Technol.* **2005**, *33*, 1–11.
12. Du, B.; Wu, D.; Zhang, H.; Li, Y.; Wei, Q.; Li, Z. J. Fast determination of ultra trace amounts of residual proteins in penicillin based on the enhancement in Rayleigh light scattering spectra of phenylfluorone-Mo(VI) complex. *Chem. Anal. (Warsaw)* **2005**, *50*, 795–806.
13. Wei, Q.; Wu, D.; Du, B.; Li, Z.; Huo, Y. Q. A spectrophotometric method for determination of total proteins in cow milk powder samples using the *o*-nitrophenylfluorone/Mo(VI) complex. *J. Food Compos. Anal.* **2006**, *19*, 76–82.
14. Wei, Q.; Li, Y.; Zhang, H.; Ding, Y. L.; Du, B. Rapid determination of ultra trace amounts of residual protein in penicillin based on its enhancement effects of Rayleigh light scattering on trimethoxyphenlyfluorone (TM-PF)–Mo(VI) complex. *J. Pharm. Biomed. Anal.* **2005**, *38*, 232–238.
15. Burstein, E. A.; Vedenkina, N. S.; Ivkova, M. N. Fluorescence and the location of tryptophan residues in protein molecules. *Photochem. Photobiol.* **1973**, *18*, 263–279.
16. Liu, J. Q.; Tian, J. N.; Zhang, J. Y.; Hu, Z. D.; Chen, X. G. Interaction of magnolol with bovine serum albumin: a fluorescence-quenching study. *Anal. Bioanal. Chem.* **2003**, *376*, 864–867.
17. Jiang, C. Q.; Gao, M. X.; He, J. X. Study of the interaction between terazosin and serum Albumin: synchronous fluorescence determination of terazosin. *Anal. Chim. Acta* **2002**, *452*, 185–189.

18. Clegg, R. M. Fluorescence resonance energy transfer. *Curr. Opin. Biotech.* **1995**, *6*, 103–110.
19. Cristobal, G.; Dos, R.; Pierre, D. J. M. Fluorescence resonance energy transfer spectroscopy is a reliable "ruler" for measuring structural changes in proteins: dispelling the problem of the unknown orientation factor. *J. Struct. Biol.* **1995**, *115*, 175–185.
20. Ross, P. D.; Subramanian, S. Thermodynamics of protein association reactions: forces contributing to stability. *Biochemistry* **1981**, *20*, 3096–3102.