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Study on Interaction Between Cefixime and Bovine Serum Albumin by Modified Fluorescence Spectroscopy

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The reaction mechanism between cefixime (CFX) and bovine serum albumin (BSA) at different temperature was investigated by the classical fluorescence spectroscopy with focus on the fluorescence change of protein, as well as the elastic scattering fluorescence spectroscopy with focus on the fluorescence change of drug. The results indicated that cefixime could quench the intrinsic fluorescence of bovine serum albumin strongly through a static quenching process. The electrostatic force played an important role on the conjugation reaction between cefixime and bovine serum albumin, the number of binding site (*n*) in the binary system was approximately equal to 1. The binding constant obtained from elastic scattering fluorescence spectroscopy was larger than the one obtained from classical fluorescence spectroscopy by two orders of magnitude for the CFX-BSA system. At last the correctness of elastic scattering fluorescence spectroscopy method was verified by UV-visible absorption spectroscopy. It is also speculated that "point to side" interaction between drugs and peptides was existed.

Keywords: Fluorescence spectroscopy, Elastic scattering fluorescence, Cefixime, Bovine serum albumin, Interaction.

INTRODUCTION

The classical fluorescence spectroscopy (CFS) studies the reaction mechanism of small molecule drugs and proteins, by studying the change of fluorescence intensity of protein at the maximum emission wavelength before and after adding the drugs as well as the derived binding constants, binding sites and the donor-to-acceptor distance, *etc*. between proteins and drugs[1,2]. Elastic light scattering is a kind of light scattering, which the radiation light wavelength is the same with incident light wavelength[3]. In classical fluorescence spectroscopy, the main source of bovine serum albumin fluorescence is Trp-212. Classical fluorescence spectroscopy does not reflect interaction of the other non-fluorescence-emitting residues with drugs[4] and the fluorescence spectrogram only reflects partial information of the interaction of bovine serum albumin with drug, the obtained information is thus of insufficient accuracy. Fluorescence changes of small molecule drugs will reflect the whole information of interaction between drugs and proteins. As a result, the fluorescence of small molecule drug reflects the overall fluorescence in the interaction. Because of this, a new method by taking the drug as the object of detection is applied, to study the interaction between drugs and proteins in order to cover the shortcomings of classical fluorescence spectroscopy.

Cefixime (CFX) is an oral third-generation cephalosporin and has some advantages such as a broad antimicrobial generation, strong antimicrobial effects and strong stability to β-lactamases[5]. In this study, the binding mechanism of cefixime and bovine serum albumin is investigated by utilizing classical fluorescence quenching method, elastic scattering fluorescence spectroscopy and UV-visible absorption spectroscopy, respectively. The binding constant obtained from elastic scattering fluorescence spectroscopy was larger than the one obtained from classical fluorescence spectroscopy by two orders of magnitude, which indicated that elastic scattering fluorescence spectroscopy with focus on the fluorescence change of drug was a more accurate and more reasonable method, followed by UV-visible to verify it. The new method is a useful supplement to the application of fluorescence spectroscopy on the research of combination mechanism of proteins and drugs and helps to further properly understand about the interaction mechanism of drug-protein system.

EXPERIMENTAL

All fluorescence spectra were recorded with a Shimadzu RF-5301PC. Absorption was measured with an UV-visible recording spectrophotometer (UV-265 Shimadzu, Japan). All pH measurements were made with a pHS-3C precision acidity

meter (Leici, Shanghai). All temperatures were controlled by a CS501 super-heated water bath (Nantong Science Instrument Factory).

Cefixime was purchased from Shanghai Boyle Chemical Co.,Ltd. Bovine serum albumin (BSA) was purchased from Sigma Company. Stock solutions of bovine serum albumin (1×10^{-5} M) and cefixime (2×10^{-3} M) were prepared. All the stock solutions were further diluted as working solutions prior to use. *tris*-HCl buffer solution (0.05 M of *tris*, 0.15 M of NaCl) was used to keep the pH of the solution at 7.40 and NaCl solution was used to maintain the ionic strength of the solution. All other reagents were analytical grade and all aqueous solutions were prepared with newly double-distilled water and stored at 277 K.

Classical fluorescence spectroscopy measurements: At 293, 303 and 310 K, 1 mL of *tris*-HCl buffer, pH 7.40, 0.4 mL of 1×10^{-5} M bovine serum albumin solution and different concentrations of cefixime were added into 10 mL colourimetric tube successively. The samples were diluted to scaled volume with double-distilled water. The fluorescence spectra were measured (excitation at 280 nm and emission wavelengths of 290-450 nm). The widths of both excitation and emission slit were set to 5 nm. The fluorescent intensity F at the maximum fluorescent peaks was recorded.

Elastic scattering fluorescence spectroscopy measurements: At 293, 303 and 310 K, 1 mL of *tris*-HCl buffer, pH 7.40, 1 mL of 2×10^{-4} M cefixime solution and different concentrations of bovine serum albumin were added into 10 mL colourimetric tube successively. The samples were diluted to scaled volume with double-distilled water. The fluorescence spectra were measured ($\Delta\lambda$ at 0 nm and emission wavelengths of 220-700 nm). The widths of both excitation and emission slit were set to 5 nm. The fluorescent intensity I at 325 nm was recorded.

UV-visible measurements: The volume of cefixime was 2 mL, the others were the same as in section elastic scattering fluorescence spectroscopy measurements, with corresponding concentration of bovine serum albumin solution as the reference. The UV-visible absorption spectra of cefixime in the presence and absence of bovine serum albumin were scanned with 1 cm quartz cells in the range from 190 to 600 nm and the absorption intensity A at the maximum absorption peak was recorded.

RESULTS AND DISCUSSION

Fluorescence quenching spectra of BSA-CFX system: The fluorescence spectra of BSA-CFX system was shown in Fig. 1. As shown in Fig. 1, the fluorescence intensity of bovine serum albumin decreased gradually with the addition of cefixime with red shift of 4 nm. The result showed that cefixime could

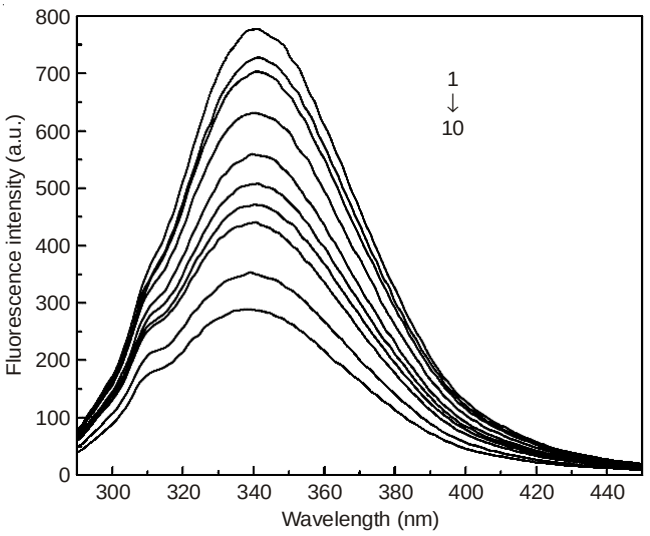

Fig. 1. Fluorescence spectra of BSA-CFX system (T = 310 K) $C_{BSA} = 4 \times 10^{-7}$ M; 1-10 C_{CFX} = (0, 2.0, 3.0, 4.0, 8.0, 20, 40, 60, 80, 100) $\times 10^{-7}$ M

quench the intrinsic fluorescence of bovine serum albumin significantly and there was an interaction between cefixime and bovine serum albumin, it could also reveal that a new complex is being formed, it further suggested that the quenching was a static process.

In order to confirm the quenching mechanism, the fluorescence quenching data are analyzed by the Stern-Volmer eqn. 6:

$$F_0/F = 1 + K_q\tau_0[L] = 1 + K_{sv}[L] \quad (1)$$

where, F_0 and F are the fluorescence intensities of bovine serum albumin before and after the addition of the cefixime, respectively. τ_0 is the average lifetime of fluorescence without ligand, which is about 10^{-8} s. K_{sv} is the Stern-Volmer quenching constant. K_q is the quenching rate constant of bimolecular and [L] is the concentration of the ligand. Based on the linear fit plot of F_0/F *versus* [L], the K_q values can be obtained. The calculated results were shown in Table-1. In Table-1, the values of K_{sv} decreased with the rising temperature in all systems, which indicated that the probable quenching mechanism of the interaction between bovine serum albumin and cefixime was initiated by complex formation rather than by dynamic collision[7]. In addition, K_q were much greater than 2×10^{10} M^{-1} s^{-1}, this also implied that the quenching of bovine serum albumin by cefixime may be a static process[8].

For static quenching process, the relationship between the fluorescence intensity and the concentration of quencher can be usually described by eqn. 2[9] to obtain the binding constant (K_a) and the number of binging sites (n) in most paper:

$$\log\{(F_0\text{-}F)/F\} = \log K_a + n\log[L] \quad (2)$$

where, K_a can be determined from the plot of log $[(F_0\text{-}F)/F]$ *versus* log [L]. Thus we can obtain K_a and *n* of cefixime with bovine serum albumin from the eqn. 2 and the calculated result

TABLE-1
QUENCHING REACTIVE PARAMETERS OF BOVINE SERUM ALBUMIN AND CEFIXIME AT DIFFERENT TEMPERATURES

T (K)	K_q (M^{-1} s^{-1})	K_{sv} (M^{-1})	r_1	K_a (M^{-1})	n	r_2
293	3.91×10^{12}	3.91×10^4	0.997 9	4.72×10^4	0.97	0.993 1
303	3.73×10^{12}	3.73×10^4	0.991 8	4.20×10^4	0.93	0.992 7
310	3.67×10^{12}	3.67×10^4	0.998 1	3.66×10^4	0.96	0.990 8

K_q is the quenching rate constant; K_a is the binding constant; n is the number of binding site. r_1 is the linear relative coefficient of $F_0/F \sim [L]$; r_2 is the linear relative coefficient of log $(F_0 - F)/F \sim$ log [L]

was shown in Table-1. As shown in Table-1, the fact that the values of *n* were all approximately to 1 implied that just one binding site for cefixime existed in bovine serum albumin. Meanwhile, the K_a decreased with the rising temperature, further suggested that the quenching was a static process[10].

Elastic scattering fluorescence spectroscopy studies: According to the experiment as discussed section elastic scattering fluorescence spectroscopy measurements, the interaction between bovine serum albumin and cefixime with cefixime as the detection object was investigated. The scattering fluorescence spectra of CFX-BSA system was shown in Fig. 2. As shown in Fig. 2, with gradual addition of bovine serum albumin to cefixime solution, the scattering intensity of the peak at 325 nm decreased. The results indicated that the interaction between bovine serum albumin and cefixime led to the formation of a complex between drug and protein[11]. According to the eqns. 1, 2, the calculated results were shown in Table-2. From Table-2, it can be seen that the number of binging sites (*n*) were all approximately to 1. Meanwhile, K_{a1} and K_{sv1} all were reduced with the rising temperatures, further suggested that the quenching was a static process, which were consistent with the results of classical fluorescence spectroscopy. This indicated that taking protein or drug as the object of detection, although detection methods were different, it can get the same interaction mechanism between drugs and protein. Besides, the K_{a1} values of elastic scattering fluorescence spectroscopy with drug as detection object are much greater than the K_a values of classical fluorescence spectroscopy with protein as detection object at the same temperature, which shows that in addition to Trp-212 of bovine serum albumin peptides, the others also interacts with cefixime. In addition to the "point to point" interaction between cefixime and Trp-212, the "point to side" interaction between cefixime and the other peptides in bovine serum albumin hydrophobic sub-domain also exists[12]. This shows that compared to classical fluorescence spectroscopy with protein as detection object, treating drugs as detection object can give more complete and more accurate expression of the interaction information of protein and drugs.

UV-visible spectra studies: The binding constant K_b of protein and drug can be calculated on the following eqn. 3:

$$(A_0\text{-}A)^{-1} = A_0^{-1} + k_b^{-1}A_0^{-1}[L]^{-1} \quad (3)$$

where, A_0 and A are the absorption values in the absence and presence of ligand, respectively. And [L] is the concentration of the ligand. The UV-visible absorption spectra of cefixime in the absence and presence of bovine serum albumin were shown in Fig. 3. As shown in Fig. 3, with gradual addition of bovine serum albumin to cefixime solution, the intensity of the peak at 199 nm decreases with a significant red shift 12 nm, indicating that the interaction between bovine serum albumin and cefixime leads to the formation of a complex between drug and protein. Based on the linear regression plot of $(A_0\text{-}A)^{-1}$ *versus* $[L]^{-1}$, the K_b values can be obtained. The calculated results were shown in Table-3. As seen in Table-3, the binding constant K_b decreases with rising temperatures, which is consistent with the results of fluorescence methods. The K_b values are observed to be much larger than K_a of the classical fluorescence spectroscopy and close to K_{a1} obtained by the elastic scattering fluorescence spectroscopy. This phenomenon also shows that treating the drug as detection objects can give more complete and more accurate expression the interaction information of proteins and drugs. In this experiment, because of the calculation formula of reaction between drug and protein

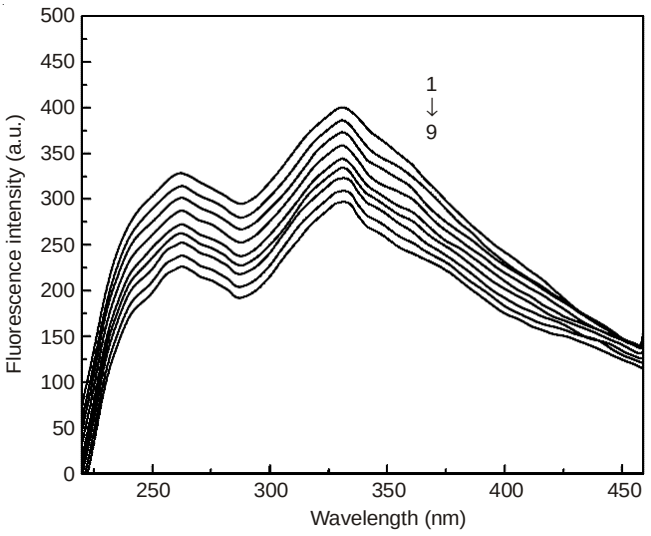

Fig. 2. Elastic scattering fluorescence spectra of BSA-CFX system (T = 310 K); $C_{CFX} = 2 \times 10^{-5}$ M, 1-9 C_{BSA} = (0, 2.0, 4.0, 5.0, 6.0, 8.0, 9.0, 10, 12) × 10^{-7} M

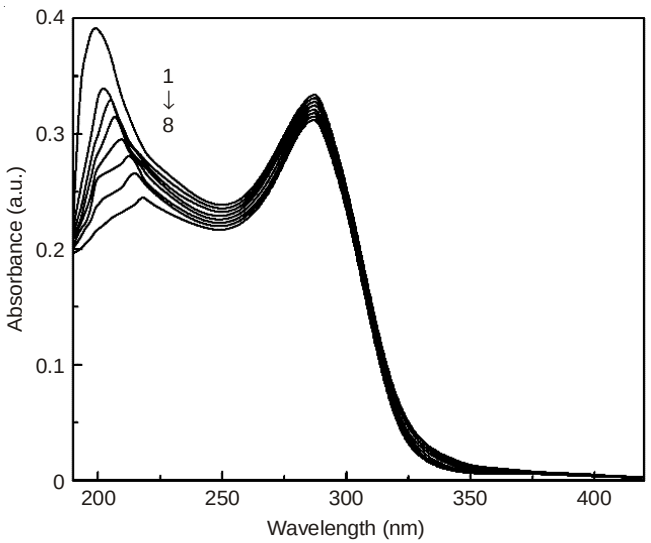

Fig. 3. Absorption spectra of CFX-BSA system (T = 310 K); $C_{CFX} = 4 \times 10^{-5}$ M, 1~8 C_{BSA} = (0, 0.4, 0.8, 1.2, 2.0, 2.5, 3.0, 3.5) × 10^{-6} M

TABLE-2
QUENCHING REACTIVE PARAMETERS OF CEFIXIME AND BOVINE SERUM ALBUMIN AT DIFFERENT TEMPERATURES

T (K)	K_{q1} ($M^{-1}s^{-1}$)	K_{sv1} (M^{-1})	r_3	K_{a1} (M^{-1})	n	r_4
293	8.17×10^{14}	8.17×10^{6}	0.999 1	3.03×10^{6}	0.93	0.996 6
303	6.35×10^{14}	6.35×10^{6}	0.996 9	2.57×10^{6}	0.93	0.998 6
310	5.17×10^{14}	5.17×10^{6}	0.993 7	2.45×10^{6}	0.91	0.999 6

r_3 is the linear relative coefficient of $F_0/F \sim [L]$; r_4 is the linear relative coefficient of $\log \{(F_0 - F)/F\} \sim \log [L]$

TABLE- 3
BINDING CONSTANTS OF CEFIXIME-BOVINE SERUM ALBUMIN SYSTEM BY UV-VISIBLE AT DIFFERENT TEMPERATURES

T (K)	K_b (M^{-1})	Linear regression equation	r_5
293	1.67×10^6	$(A_0\text{-}A)^{-1} = 6.158 + 3.677 \times 10^{-6} [L]^{-1}$	0.9909
303	1.48×10^6	$(A_0\text{-}A)^{-1} = 5.134 + 3.477 \times 10^{-6} [L]^{-1}$	0.9972
310	1.27×10^6	$(A_0\text{-}A)^{-1} = 5.498 + 4.338 \times 10^{-6} [L]^{-1}$	0.9968

K_b is the binding constant; r_5 is the linear relative coefficient of $(A_0\text{-}A)^{-1} \sim [L]^{-1}$

protein was inferred by Scatchard model, the differences of binding constants may be caused by the differences between fluorescence method and UV-visible.

Type of interaction force of BSA-CFX systems: The type of interaction forces between proteins and drugs were hydrogen bonds, electrostatic forces, van der Waals forces and hydrophobic forces, respectively. If the temperature does not vary significantly, the ΔH could be regarded as a constant[14]. The thermodynamic parameters can be determined by the following van't Hoff equation[15]:

$$R \ln K = \Delta S - \Delta H/T \quad (4)$$

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

According to the relevant thermodynamic parameters of small molecule drugs and biological macromolecules, the type of interaction force can be simply judged. According to eqns. 4, 5, the values of ΔH, ΔS and ΔG were obtained: ΔH = -11.08 KJ mol^{-1}, ΔS = 51.61 J mol^{-1} K^{-1}, ΔG = -27.08 KJ mol^{-1} (CFS, 310 K); ΔH = -9.66 KJ mol^{-1}, ΔS = 91.12 J mol^{-1} K^{-1}, ΔG = -37.92 KJ mol^{-1} (elastic scattering fluorescence spectroscopy, 310 K); ΔH = -11.90 KJ mol^{-1}, ΔS = 78.46 J mol^{-1} K^{-1}, ΔG = -36.22 KJ mol^{-1} (UV-visible, 310 K). The reaction process of cefixime and bovine serum albumin was a spontaneous molecular interaction procedure in which entropy increased and Gibbs free energy decreased[16]. ΔH < 0 and ΔS > 0 showed that the electrostatic interaction play a major role in the binding process[17]. Comparing to ΔS, ΔG obtained by classical fluorescence spectroscopy and elastic scattering fluorescence spectroscopy, we could know that the reaction using cefixime as the focus was easier. The thermodynamic parameters obtained by elastic scattering fluorescence spectroscopy and cefixime are consistent, which indicates that the elastic scattering fluorescence spectroscopy with drug as detection object to study the interaction mechanism of cefixime with bovine serum albumin is correct.

Conclusion

In this paper, the interaction of cefixime with bovine serum albumin was studied at different temperatures with the methods of classical fluorescence spectroscopy and elastic scattering fluorescence spectroscopy and verified by UV-visible. Compared binding constants of the three methods, shows that the K_a values of classical fluorescence spectroscopy was smaller than elastic scattering fluorescence spectroscopy and UV-visible and the later two values are close to each other. That means taking drugs as detection object for elastic scattering fluorescence spectroscopy can be more comprehensive and more accurate when expressing the interaction information between protein and drug in terms of fluorescence. In addition to Trp of bovine serum albumin peptides, the others also interact with cefixime. Linear phase relations of linear regression equation of the elastic scattering fluorescence spectroscopy method are above 0.99. Although the values for the elastic scattering fluorescence spectroscopy and UV-visible of binding constant are slightly different, the difference is minimal. It also suggests that the elastic scattering fluorescence spectroscopy method is reasonable. elastic scattering fluorescence spectroscopy is a challenge for classical fluorescence spectroscopy and it provides a new way to study the interaction more accurately between drugs and proteins, which will further improve the study of the reaction mechanism between drugs and proteins.

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REFERENCES

1. A. Varlan and M. Hillebrand, *Rev. Roum. Chim*., **55**, 69 (2010).
2. X.R. Pan, R.T. Liu, P.F. Qin, L. Wang and X.C. Zhao, *J. Lumin*., **130**, 611 (2010).
3. C.F. Bohren and D.R. Hufillann, Absorption and Scattering Light by Smal1 Particles, John Wiley & Sons, New York (1983).
4. T. Tan, R. Huang and Z.N. Xia, *Chin. J. Anal. Chem.*, **35**, 1415 (2007).
5. S.F. Yu, *World Notes on Antibiotics*, **16**, 339 (1995).
6. I. Matei and M. Hillebrand, *J. Pharm. Biomed. Anal*., **51**, 768 (2010).
7. Y.J. Hu, Y.O. Yang, A.M. Bai, W. Li and Y. Liu, *J. Solution Chem*., **39**, 709 (2010).
8. N. Barbero, E. Barni, C. Barolo, P. Quagliotto, G. Viscardi, L. Napione, S. Pavan and F. Bussolino, *Dyes Pigments*, **80**, 307 (2009).
9. Y.L. Wei, J.Q. Li, C. Dong, S.M. Shuang, D.S. Liu and C.W. Huie, *Talanta*, **70**, 377 (2006).
10. S.A. Markarian and M.G. Aznauryan, *Mol. Biol. Rep*., **39**, 7559 (2012).
11. C.Z. Zheng, H.P. Wang, W. Xu, C.Y. Xu, J.G. Liang and H.Y. Han, *Spectrochim. Acta A*, **118**, 897 (2014).
12. F. Wang, W. Huang, B. Tang and X.J. Liu, *Chin. J. Anal. Chem*., **34**, S239 (2006).
13. R. Huang, Z.N. Xia and P. Gong, *Spectrosc. Spect. Anal*, **28**, 161 (2008).
14. Y.M. Song, Z. Liu, K.J. Wang and N.N. Luan, *Acta Chim. Sin*., **68**, 2191 (2010).
15. P. Ju, H. Fan, T. Liu, L. Cui, S.Y. Ai and X.N. Wu, *Biol. Trace Elem. Res*., **144**, 1405 (2011).
16. J. Mariam, P.M. Dongre and D.C. Kothari, *J. Fluoresc*., **21**, 2193 (2011).
17. P. Sen, S. Fatima, B. Ahmad and R.H. Khan, *Spectrochim. Acta A*, **74**, 94 (2009).