

# Study on the Binding Behavior of Lysozyme with Cephalosporin Analogues by Fluorescence Spectroscopy

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Received: 22 November 2008 / Accepted: 17 March 2009 / Published online: 3 April 2009  
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**Abstract** It was first found that the intrinsic fluorescence of lysozyme at 340 nm can be quenched by cephalosporin analogues through the static quenching and non-radiative energy transferring procedure. In the acetate buffer solution with pH 7.0 and 298 K, the quenching fluorescence intensity was in a good linearity over the concentration of drugs in the range of 1–100  $\mu\text{mol L}^{-1}$ , 0.1–100  $\mu\text{mol L}^{-1}$ , 0.5–100  $\mu\text{mol L}^{-1}$  and 0.05–100  $\mu\text{mol L}^{-1}$  for cefradine, cefuroxime, cefotaxime and ceftriaxone, respectively. The quenching ability or the binding ability of the studied drugs followed the pattern: ceftriaxone > cefotaxime > cefuroxime > cefradine, which was close to the order of their antibacterial ability. The binding parameters including the association constant and the number of binding potential point were calculated at different temperatures (288, 298 and 308 K), and thermodynamic parameters  $\Delta H^\circ$ ,  $\Delta S^\circ$  and  $\Delta G^\circ$  were given. The binding mode of lysozyme with cephalosporins showed that the hydrophobic effect might play a major role. The binding distance between cephalosporin and tryptophan residue in lysozyme was obtained. The results provided the quantitative information for the binding of cephalosporin to lysozyme, and it was suggested that the drugs probably bound to the active site near Trp62 in lysozyme.

**Keywords** Lysozyme · Cefradine · Cefuroxime · Cefotaxime · Ceftriaxone · Hydrophobic effect

## Introduction

Protein–drug interaction is the hot point in the fields of medicine, chemistry and biology [1, 2]. The studies on the interaction between serum albumin and drug were reported using optical methods, such as fluorescence spectroscopy [3, 4], circular dichroism (CD) [5], fourier transform infrared (FT-IR) [6] and chemiluminescence (CL) coupled ultrafiltration sampling [7, 8]. Lysozyme (14.4 kDa) is a small globular protein that contains structural elements commonly found in proteins. Lysozyme represented since its discovery a prototype molecule for understanding the complexity of the protein structure and function in physiology and disease [9], and because it has the physicochemical properties of the model protein. Thus, the study on the interaction of drugs with lysozyme has important meaning for providing useful information of the structure features to elucidate the therapeutic effectiveness of drugs [10]. It was reported the interaction of lysozyme with puerarin, alpinetin and cardamonin [11, 12] using FT-IR, CD, UV-Vis, and fluorescence spectroscopy. Recently, we have reported on the photochemical behavior of lysozyme-4-(2-pyridylazo)-resorcinol polymolecular complex [13] and lysozyme with bromophenol blue by fluorescence spectroscopy [14].

Cefradine, cefuroxime, cefotaxime and ceftriaxone (illustrated in Scheme 1), the first-, second- and third-generation agents of cephalosporin antibiotics, are semisynthetic antibiotic substances obtained from fungi, characterized by a broad antibacterial spectrum for a wide range of both Gram-positive and Gram-negative bacteria [15, 16]. Ceph-

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alosporin was grouped into “generations” based on the timing of introduction and antibacterial properties, and in general each newer generation of cephalosporin has significantly greater Gram-negative antimicrobial properties than the preceding generation [17]. In this work, the interaction of lysozyme with cephalosporin analogues including cefradine, cefuroxime, cefotaxime and ceftriaxone was studied in detail using fluorescence quenching method. It was found that the quenching ability or the binding ability of the studied drugs followed the pattern: ceftriaxone > cefotaxime > cefuroxime > cefradine, which was close to the order of their antibacterial ability. The binding parameters including the association constant and the number of binding potential point were calculated at different temperatures, and thermodynamic parameters  $\Delta H^\circ$ ,  $\Delta S^\circ$  and  $\Delta G^\circ$  were given. The binding mode of lysozyme with cephalosporins showed that the hydrophobic effect might play a major role. The distances between the drugs and the tryptophan residue in lysozyme were obtained according to the fluorescence resonance energy transferring theory.

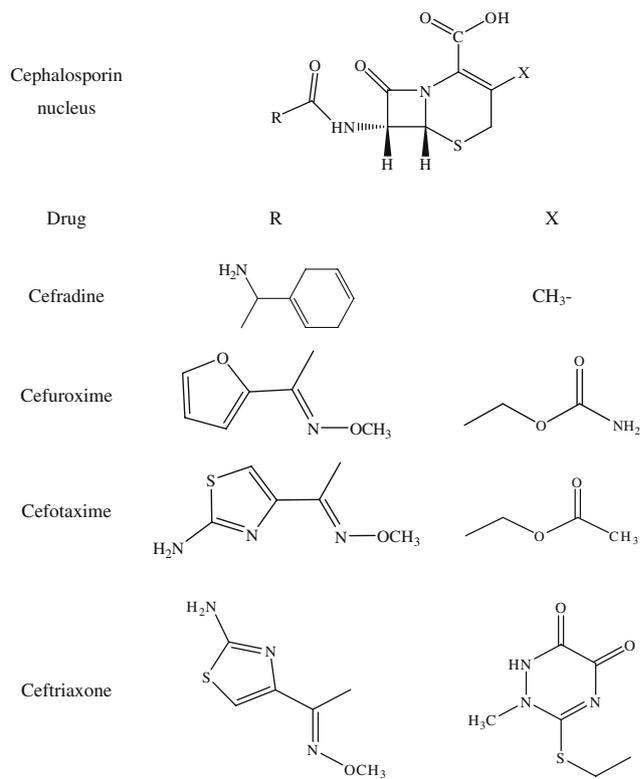
## Experimental section

### Apparatus

All fluorescence measurements were carried out on a Hitachi F-4500 fluorophotometer (Kyoto, Japan) equipped with a xenon lamp and a 1.0 cm cell, with the slit's width of Ex/Em 5.0/5.0 nm and the PMT voltage at 700 V. The UV spectra were obtained from a Lambda-40 spectrophotometer (Perkin Elmer, USA) coupled with a 1.0 cm quartz cell. All pH measurements were made with a pHs-3C digital pH meter (Shanghai Leici Device Works, China) with a combined glass-calomel electrode. The temperature was controlled by a water-bath and temperatures were kept in a certain range ( $T \pm 0.1$  °C) throughout the experiment.

### Reagents

In this work, all reagents used were of analytical-reagent grade unless specified and doubly deionized water was purified in a Milli-Q system (Millipore, Bedford, MA, USA) used for the preparation of solutions in the whole procedure. Lysozyme (Sigma) was purchased from local market without further purification was used to prepare a stock solution ( $50 \mu\text{mol L}^{-1}$ ) after diluted with doubly deionized water, and the stock solution was kept in a brown flask at 4 °C. Cefradine, cefuroxime, cefotaxime and ceftriaxone standard solutions were supplied by Xi'an Medicine Purchasing and Supply Station, China, to prepare a  $1.0 \times 10^{-2} \text{ mol L}^{-1}$  stock solution in a calibrated flask.



**Scheme 1** Molecular structure of cefradine, cefuroxime, cefotaxime and ceftriaxone

NaCl ( $5 \text{ mol L}^{-1}$ ) and acetate buffer (pH 7.0) solutions were used to adjust the ionic strength and the acidity of the system, respectively. Common metal ions ( $1 \text{ mmol L}^{-1}$ ) were prepared from  $\text{CoCl}_2$ ,  $\text{NiCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{Fe}(\text{NO}_3)_3$ ,  $\text{CuSO}_4$ ,  $\text{CrCl}_3$ ,  $\text{FeSO}_4$ , and  $\text{AlCl}_3$ , respectively, and their final concentration  $10 \mu\text{mol L}^{-1}$  was used in fluorescence titration [18]. All working strength solutions in the experiment were prepared freshly.

### General procedure

To a 10 mL comparison color tube, 0.5 mL acetate buffer, 0.1 mL lysozyme and appropriate volume standard solutions of drugs were added in order. The mixture was diluted using doubly deionized water to the mark and homogenized for determination. The fluorescence intensity was measured at  $\lambda_{\text{em}}$  340 nm in the wavelength range of 290–450 nm upon excitation at 280 nm.

## Results and discussion

### Optimization of the experimental conditions

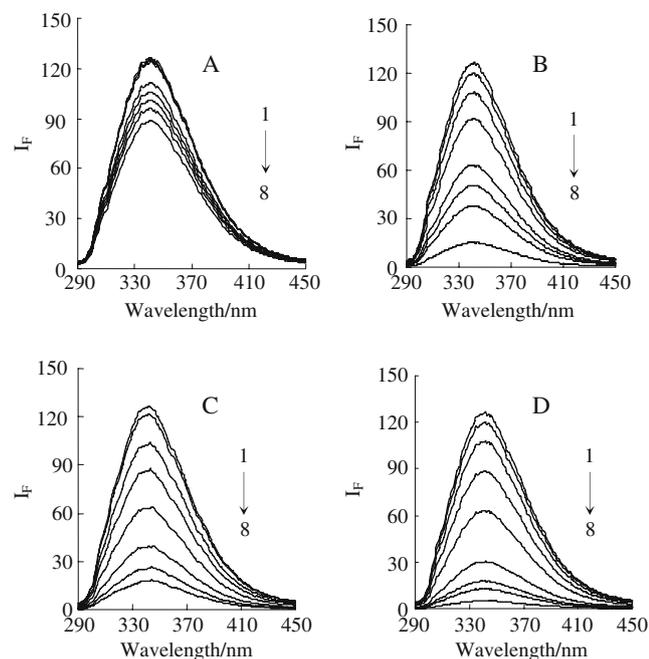
The experimental parameters including temperature, pH and the ionic strength were tested using cefotaxime as the

representative before examining the interaction of lysozyme and drugs.

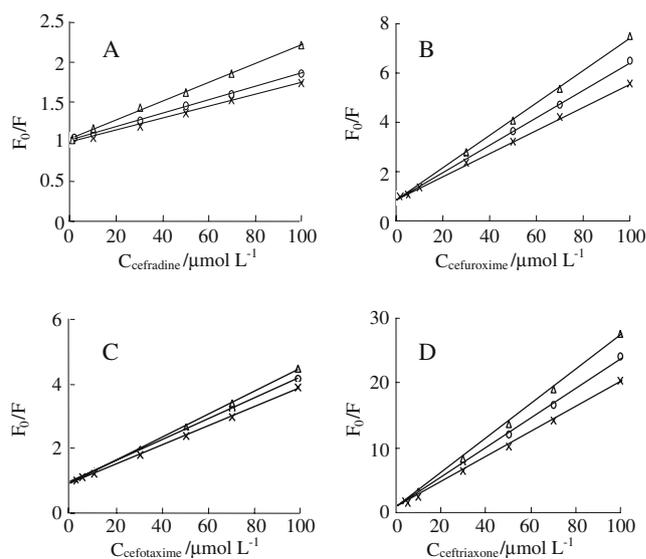
The binding constant of lysozyme and cefotaxime was obtained at different temperatures (288, 298, 308, and 318 K). It was observed that the binding constant was greatest at 288 K and the slowest interaction rate was obtained. Less time was needed for the interaction at 298 K than 308 and 318 K, and the sensitivity at 298 K was higher than that of 308 and 318 K. Thus the most appropriate temperature for the measurement was at 298 K.

The influence of pH was examined in the range of pH 5.0–9.0. The stable fluorescence intensity for the complex of lysozyme–cefotaxime could be achieved with pH 6.5–7.5. Therefore, acetate buffer solution at pH 7.0 was used in this work.

To understand the role of the electrostatic interactions in the binding process, the ionic strength dependence of the binding of cefotaxime to lysozyme was studied. It was performed in the presence of 0.1, 0.5 and 1.0 mol L<sup>-1</sup> NaCl at pH 7.0 and 298 K. It was found that the value of the binding constant of lysozyme–cefotaxime remained almost the same within the standard deviation as ionic strength increased, which indicated that the electrostatic attraction did not show a significant contribution in the binding process. So the procedure was carried out without addition to NaCl solution for adjusting ionic strength.



**Fig. 1** Fluorescence spectra of lysozyme with drugs **a** cefradine (1–100  $\mu\text{mol L}^{-1}$ ), **b** cefuroxime (0.1–100  $\mu\text{mol L}^{-1}$ ), **c** cefotaxime (0.5–100  $\mu\text{mol L}^{-1}$ ), **d** ceftriaxone (0.05–100  $\mu\text{mol L}^{-1}$ ). Concentration: lysozyme, 0.5  $\mu\text{mol L}^{-1}$ ; drug (1) 0  $\mu\text{mol L}^{-1}$ , (8) 100  $\mu\text{mol L}^{-1}$



**Fig. 2** The Stern-Volmer plots of lysozyme–drugs **a** cefradine, **b** cefuroxime, **c** cefotaxime, **d** ceftriaxone *open triangle* 288 K, *open circle* 298 K, *multiplication symbol* 308 K

#### Quenching effect of drugs on lysozyme fluorescence

The fluorescence quenching includes two procedures: the static quenching and the dynamic quenching. With the increasing of temperature, for the former it will reduce the stability of formative compound and the quenching constant, and for the latter it will increase the effective collision number of ions, enhance energy transfer and make the quenching constant augment. To get the quenching mode for the binding interaction of lysozyme with drugs, a series of experiments were carried out. The fluorescence spectra of lysozyme with cefradine, cefuroxime, cefotaxime and ceftriaxone were obtained, as shown in Fig. 1. It was observed that lysozyme had strong fluorescence emission intensity and the addition of the drugs caused a dramatic change in the fluorescence spectrum of lysozyme. The decreased fluorescence intensity was proportional to the concentration of the drugs in the range of 1–100  $\mu\text{mol L}^{-1}$ , 0.1–100  $\mu\text{mol L}^{-1}$ , 0.5–100  $\mu\text{mol L}^{-1}$  and 0.05–100  $\mu\text{mol L}^{-1}$  for cefradine, cefuroxime, cefotaxime and ceftriaxone, respectively. It was suggested the quenching ability of the studied drugs following the pattern: ceftriaxone > cefotaxime > cefuroxime > cefradine, which was close to the order of their antibacterial ability. With the same molar ratio for drug:lysozyme of 60:1, lysozyme fluorescence was quenched by 21.0, 60.4, 48.1 and 86.2% in the presence of cefradine, cefuroxime, cefotaxime and ceftriaxone, respectively. At higher concentration of the drug (molar ratio 200:1), lysozyme fluorescence decreased by only 46.4% in the presence of cefradine and was complete or almost complete quenched by other drugs. It was suggested

**Table 1** Dynamic quenching constant of lysozyme–drug

Drug	$T$	$K_q \times 10^{-12}$	$R^2$
	$K$	$L \text{ mol}^{-1} \text{ s}^{-1}$	
Cefradine	288	1.17±0.01	0.9985
	298	0.84±0.02	0.9990
	308	0.76±0.01	0.9991
Cefuroxime	288	6.56±0.01	0.9995
	298	5.58±0.01	0.9996
	308	4.6 9±0.02	0.9995
Cefotaxime	288	3.53±0.01	0.9990
	298	3.23±0.01	0.9991
	308	2.96±0.02	0.9989
Ceftriaxone	288	26.45±0.01	0.9996
	298	22.80±0.02	0.9995
	308	19.38±0.01	0.9993

that a high affinity of binding site to lysozyme was close to tryptophan residues in its structure. A slight red shift of emission peak was observed for the binding of cefotaxime and ceftriaxone to lysozyme indicating that the microenvironment of lysozyme was changed.

The Stern-Volmer graphs of lysozyme–cephalosporins with drugs concentration in the range of 1–100  $\mu\text{mol L}^{-1}$  at different temperatures were shown in Fig. 2 in order to illustrate ulteriorly the quenching mechanism. It can be seen that the curves were in a good linear relationship and the slope was decreased with temperature increasing, indicating the static quenching played a main role in the binding process.

On the other hand, assumed the procedure to be the dynamic quenching. From Stern-Volmer quenching equation [19],

$$\frac{F_0}{F} = 1 + K_q \tau_0 C_Q = 1 + K_{sv} C_Q \quad (1)$$

$F_0$  and  $F$  denote the steady-state fluorescence intensity in the absence and the presence of quencher (cephalosporins);  $K_q$ ,  $\tau_0$ ,  $K_{sv}$  and  $C_Q$  are the quenching rate constant of the biomolecule, the average lifetime of the molecule without quencher, the dynamic quenching constant and the concentration of quencher, respectively.  $K_{sv} = K_q \tau_0$  and  $K_q = K_{sv} / \tau_0$ , the fluorescence lifetime of the biopolymer ( $\tau_0$ ) is  $10^{-8}$  s [20], and quenching rate constant ( $K_q$ ,  $L \text{ mol}^{-1} \text{ s}^{-1}$ ) can be obtained from the slope of the curve. The values of quenching rate constant  $K_q$  for lysozyme–cephalosporins were summarized in Table 1. It was obvious that the quenching constants were at  $10^{12}$  level and by far greater than the maximum scatter collision-quenching constant of various quenchers with biopolymers at  $2.0 \times 10^{10} L \text{ mol}^{-1} \text{ s}^{-1}$  [21]. It was deduced that the quenching interaction of

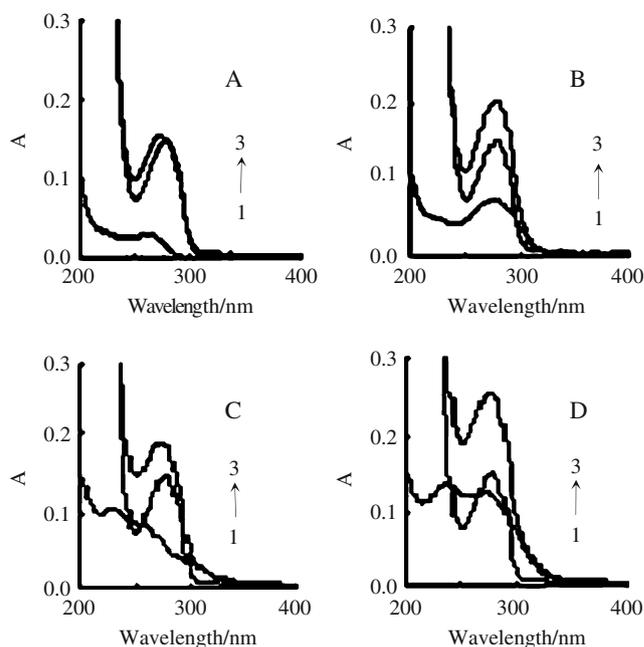
lysozyme and cephalosporins was not initiated by the dynamic collision but forming the ground-state compounds.

### Absorption spectra of lysozyme–drug

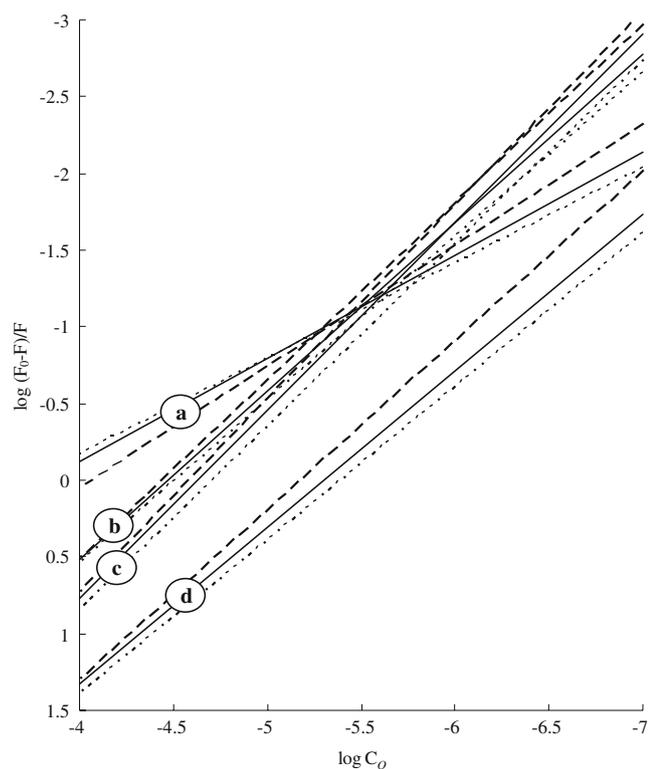
The absorption spectra of lysozyme with drugs are shown in Fig. 3. It can be observed that the addition of drugs caused the increase of the absorption at the wavelength of 280 nm (the characteristic absorption peak of lysozyme) and a little blueshift of the absorption peak exhibited upon addition of drug. It indicated that there were interactions between the drugs and the ground-state of lysozyme. Accordingly, they caused the changes in the absorption spectrum of lysozyme. It can be deduced that the binding ability of the drugs followed the pattern: ceftriaxone > cefotaxime > cefuroxime > cefradine, which consisted with that of their antibacterial capability [22]. It was reported the dynamic quenching can affect only the excitation state of the quenching molecule but not the absorption spectrum of the quenching substances [19]. So it was further proved that the quenching of cephalosporins on lysozyme fluorescence was owing to the formation of ground-state complex and belonged to the static quenching mode.

### Association constant and binding site of lysozyme–drug

When small molecules bind independently to a set of equivalent sites on biomacromolecules, the equilibrium



**Fig. 3** The absorption spectra of lysozyme–drugs **a** cefradine, **b** cefuroxime, **c** cefotaxime, **d** ceftriaxone **1** drug, **2** lysozyme, **3** lysozyme–drug. Concentration: lysozyme,  $2.0 \mu\text{mol L}^{-1}$ ; drug,  $2.0 \mu\text{mol L}^{-1}$



**Fig. 4** The relationship between  $\log(F_0-F)/F$  and  $\log C_Q$  under different temperatures **a** cefradine, **b** cefuroxime, **c** cefotaxime, **d** ceftriaxone. Dotted line 288 K, solid line 298 K, broken line 308 K. Concentration: lysozyme,  $1.0 \mu\text{mol L}^{-1}$ ; drugs,  $0.1\text{--}100 \mu\text{mol L}^{-1}$

between the free and the bound small molecules can be expressed by

$$\log \frac{F_0 - F}{F} = \log K + n \log C_Q \quad (2)$$

where  $K$  is the binding constant to a site and  $n$  is the number of binding potential points, that is the amount of small molecules can be bound by a macromolecule, respectively. According to the Eq. (2), the relationships between  $\log(F_0-F)/F$  and  $\log C_Q$  for lysozyme–cephalosporins were plotted in Fig. 4. The values of  $K$  and  $n$  can be obtained from the relationships of  $\log(F_0-F)/F$  and  $\log C_Q$  were listed in Table 2.  $K$  values of the four drugs except cefradine were at  $10^5$  level suggesting that there existed a high binding affinity of drugs to lysozyme, and it was mainly increased with the “generation” of cephalosporin varying from the 1st to the 3rd one. And  $n$  value was ranging from 0.62 to 1.26 approximately equal to 1.0 indicating that there was one class of binding site to cephalosporin analogues in lysozyme.

It was reported that 80% of fluorescence of lysozyme was due to Trp62 and Trp108 residues, and the oxidation of either Trp62 or Trp108 was accompanied by a drastic decrease in fluorescence intensity [23]. Trp62 has been suggested to be involved in van der Waals or hydrophobic

**Table 2** Binding parameters of lysozyme–drug at different temperatures

Drug	$T$	$K$	$n$	$R_2$
	K	$\text{L mol}^{-1}$		
Cefradine	288	$2.02 \pm 0.01 \times 10^2$	0.62	0.9967
	298	$3.72 \pm 0.01 \times 10^2$	0.67	0.9979
	308	$1.62 \pm 0.01 \times 10^3$	0.79	0.9984
Cefuroxime	288	$3.87 \pm 0.02 \times 10^5$	1.19	0.9981
	298	$4.73 \pm 0.01 \times 10^5$	1.23	0.9991
	308	$5.83 \pm 0.02 \times 10^5$	1.26	0.9983
Cefotaxime	288	$5.98 \pm 0.01 \times 10^4$	1.06	0.9987
	298	$8.06 \pm 0.01 \times 10^4$	1.10	0.9992
	308	$1.32 \pm 0.02 \times 10^5$	1.16	0.9986
Ceftriaxone	288	$1.50 \pm 0.01 \times 10^5$	0.96	0.9962
	298	$2.56 \pm 0.02 \times 10^5$	1.02	0.9990
	308	$7.66 \pm 0.02 \times 10^5$	1.14	0.9976

effect with substrate and inhibitors [24, 25], and the indole ring of Trp62 was changed in the interaction of Trp62 with the inhibitor tri-N-acetylglucosamine [26]. Combined the above information with  $n$  value, it was suggested that cephalosporins tested most likely bound to the active site near Trp62 in lysozyme.

**Thermodynamic parameters and nature of the binding force for lysozyme–drug**

In order to estimate the interaction mechanism of lysozyme with cephalosporins, the thermodynamic parameters were calculated using the van’t Hoff equation. The acting forces between a pharmaceutical and a biomolecule include hydrogen bond, van der Waals forces, hydrophobic effect and electrostatic attraction. If the temperature ( $T$ ) changes

**Table 3** Thermodynamic parameters of lysozyme–drug

Drug	$T$	$\Delta H^\circ$	$\Delta S^\circ$	$\Delta G^\circ$
	K	$\text{kJ mol}^{-1}$	$\text{J mol}^{-1}\text{K}^{-1}$	$\text{kJ mol}^{-1}$
Cefradine	288	$77.54 \pm 0.01$	$311.98 \pm 0.01$	$-12.71 \pm 0.01$
	298			$-14.66 \pm 0.01$
	308			$-18.92 \pm 0.02$
Cefuroxime	288	$15.13 \pm 0.02$	$159.47 \pm 0.02$	$-30.81 \pm 0.02$
	298			$-32.37 \pm 0.01$
	308			$-33.99 \pm 0.02$
Cefotaxime	288	$29.38 \pm 0.01$	$193.14 \pm 0.01$	$-26.34 \pm 0.01$
	298			$-27.99 \pm 0.01$
	308			$-30.19 \pm 0.02$
Ceftriaxone	288	$60.63 \pm 0.02$	$308.71 \pm 0.02$	$-28.54 \pm 0.01$
	298			$-30.85 \pm 0.02$
	308			$-34.69 \pm 0.02$

**Table 4** Effect of common metal ions on the binding constant of lysozyme–cefotaxime

Metal ion	$K' \times 10^{-4}$ L mol <sup>-1</sup>	$R^2$	$K'/K$
Without	8.06	0.9992	–
Co <sup>2+</sup>	12.40	0.9971	1.538
Ni <sup>2+</sup>	9.51	0.9959	1.180
Mg <sup>2+</sup>	2.35	0.9951	0.292
Fe <sup>3+</sup>	1.25	0.9965	0.155
Cu <sup>2+</sup>	5.38	0.9974	0.667
Cr <sup>3+</sup>	2.01	0.9962	0.249
Fe <sup>2+</sup>	2.47	0.9961	0.306
Al <sup>3+</sup>	4.12	0.9972	0.511

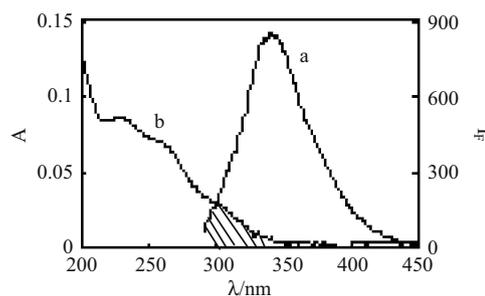
little the enthalpy change ( $\Delta H^\circ$ ) does not vary significantly and can be regarded as a constant. In the equations,

$$\ln K = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \Delta G^\circ = -RT \ln K = \Delta H^\circ - T \Delta S^\circ \quad (3)$$

$K$  is the binding constant;  $R$  is the gas constant,  $\Delta H^\circ$ ,  $\Delta S^\circ$  and  $\Delta G^\circ$  are the enthalpy, entropy and free energy change, respectively. The values of  $\Delta H^\circ$ ,  $\Delta S^\circ$  and  $\Delta G^\circ$  at different temperatures obtained were summarized in Table 3. It can be seen that  $\Delta G^\circ < 0$ ,  $\Delta H^\circ > 0$  and  $\Delta S^\circ > 0$ . The negative sign for  $\Delta G^\circ$  meant that the binding process was spontaneous and the formation of lysozyme–drug was an endothermic reaction with positive enthalpy change. According to the sign and the magnitude of the thermodynamic parameters in protein–ligand association process characterized in literature [27], it was deduced that the binding force was mainly on the hydrophobic effect.

Effect of common metal ions on the binding of drug to lysozyme

The influence of the metal ions on the binding constant of lysozyme–cephalosporins was examined at 298 K. For the lysozyme–cefotaxime complex, the results were listed in Table 4. It can be seen that the binding constant value was decreased in the presence of metal ions except Co<sup>2+</sup> and Ni<sup>2+</sup>, implying weaker binding between lysozyme and cefotaxime. The reduction of binding constant value obtained with metal ions might be resulted from the binding of metal ions with cefotaxime which reduced the binding of lysozyme–cefotaxime. It was indicated that the binding of cefotaxime to protein might be inhibited in blood, and more cefotaxime dosage should be implemented for exerting sufficiently the pharmacodynamic cefotaxime. The similar results were obtained for cefradine, cefuroxime and ceftriaxone.

**Fig. 5** Overlap of the fluorescence spectrum of lysozyme (a) and the absorption spectrum of cefotaxime (b). Concentration: lysozyme, 2.0 μmol L<sup>-1</sup>; cefotaxime, 2.0 μmol L<sup>-1</sup>

Energy transfer from lysozyme to drug

The overlap of the UV absorption spectrum of cefotaxime and the fluorescence emission spectrum of lysozyme was shown in Fig. 5, and others with the similar phenomenon were omitted. According to Förster dipole–dipole non-radiative energy transfer theory [28], energy transfer will happen under these conditions: (1) the donor can produce fluorescence emission; (2) the fluorescence emission spectrum of donor and the UV absorption spectrum of acceptor have enough overlap; (3) the distance between donor and acceptor is approached and lower than 7 nm. The energy transfer effect is related not only to the distance between donor and acceptor, but to the critical energy transfer distance, as

$$E = \frac{R_0^6}{R_0^6 + r^6} \quad (4)$$

where  $r$  is the distance between the donor (lysozyme) and the acceptor (cefotaxime),  $R_0$  is the critical distance when the transfer efficiency is 50%, which can be calculated by

$$R_0^6 = 8.8 \times 10^{-25} A^2 N^{-4} \Phi J \quad (5)$$

where  $A^2$  is the spatial orientation factor of the dipole,  $N$  is the refractive index of the medium,  $\Phi$  is the fluorescence quantum yield of the donor,  $J$  is the overlap integral of the

**Table 5** The spectral overlap integral, energy-transfer efficiency and action distance of lysozyme–drug

Drug	$J$ cm <sup>3</sup> L mol <sup>-1</sup>	$E$ %	$R_0$ nm	$r$ nm
Cefradine	$1.92 \times 10^{-14}$	8.89	2.81	4.13
Cefuroxime	$3.36 \times 10^{-14}$	12.91	3.09	4.24
Cefotaxime	$4.32 \times 10^{-14}$	20.19	3.22	4.05
Ceftriaxone	$5.60 \times 10^{-14}$	38.55	3.36	3.63

fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor. Therefore,

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4\Delta\lambda}{\sum F(\lambda)\Delta\lambda} \quad (6)$$

where  $F(\lambda)$  is the fluorescence intensity of the donor at wavelength  $\lambda$ ,  $\varepsilon(\lambda)$  is the molar absorptivity of the acceptor at wavelength  $\lambda$ . The energy transfer efficiency is given by

$$E = 1 - \frac{F}{F_0} \quad (7)$$

$J$  can be evaluated by integrating the spectra with shadow in Fig. 3 for  $\lambda=290\text{--}450$  nm. And in the present case,  $A^2=2/3$ ,  $N=1.336$  and  $\Phi=0.14$  [29], according to the Eqs. (4–7), the average distance between the binding site and the tryptophan in lysozyme  $r$  and the critical distance  $R_0$  can be calculated. The results were illustrated in Table 5. Obviously, the distance was lower than 7 nm, which showed that there existed non-radiative energy transfer between lysozyme and drugs [30].

## Conclusions

The fluorescence, UV-Vis spectrometry and fluorescence resonance energy transferring methods were provided for the study of intermolecular interactions between lysozyme and cephalosporin analogues. It was indicated that the static quenching played a main role in the binding process and there existed non-radiative energy transfer between lysozyme and drugs. The hydrophobic effect was the major binding force in the reaction of lysozyme with cephalosporins. In the acetate buffer solution with pH 7.0 and 298 K, the quenching fluorescence intensity was in a good linearity over the concentration of drugs in the range of  $1\text{--}100 \mu\text{mol L}^{-1}$ ,  $0.1\text{--}100 \mu\text{mol L}^{-1}$ ,  $0.5\text{--}100 \mu\text{mol L}^{-1}$  and  $0.05\text{--}100 \mu\text{mol L}^{-1}$  for cefradine, cefuroxime, cefotaxime and ceftriaxone, respectively. It was also interesting to compare the quenching effect of cefradine, cefuroxime, cefotaxime and ceftriaxone on lysozyme fluorescence and their binding ability for the formation of lysozyme–drug complexes with the antibacterial ability of the drugs. The association constant, binding potential point and binding site between lysozyme and the drugs was discussed and the action distance was obtained. The study is expected to provide important insight into the interactions of the physiologically important protein lysozyme with cephalosporin analogues. The measures are of particular interest in clinical and pharmaceutical assessment.

**Acknowledgement** The authors gratefully acknowledge the financial support from Shaanxi Province Nature Science Foundation, the Foundation of Ministry of Education and the NWU Graduate Innovation and Creativity Funds, China, Grant No. 2006B05, No. 07JK395 and No. 08YZ42, and Shaanxi Key Laboratory of Analytical Chemistry.

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