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Research Article

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Effects of coexistent drugs and metal ions on the interaction between topiramate amyl cephalosporin cefotaxime and bovine serum albumin

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ABSTRACT

In human physical conditions, the interaction of topiramate amyl cephalosporin cefotaxime (TACC) with bovine serum albumin (BSA) and the effects of coexistent drugs and metal ions were investigated by fluorescence spectroscopy. Results showed that the effect between TACC and BSA was static fluorescence quenching process, and the electrostatic attraction plays an important role in the conjugation reaction of BSA and TACC. Coexistent contents such as ampicillin, gentamicin, streptomycin, neomycin, kanamycin, thiamphenicol, erythromycin, acetyl spiramycin, sulfamethoxazole, penicillin G sodium salt, cefotaxime sodium, cefoperazone sodium, as well as Zn^{2+}、Co^{2+}、Ni^{2+}、Fe^{3+} *and* Mg^{2+} *have no effects on the binding of BSA-TACC system, but a bit of* Cu^{2+}*, moxifloxacin, ciprofloxacin, norfloxacin, chloramphenicol could disturb the stability of BSA-TACC system, making the fluorescence intensity changed a lot.*

Key words: bovine serum albumin; topiramate amyl cephalosporin cefotaxime; conjugation reaction; coexistent drugs; metal ions

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INTRODUCTION

Serum albumins, the most abundant plasma proteins, act as a transporter for a range of insoluble endogenous and exogenous compounds [1-2]. As the major soluble protein constituents of the circulatory system, they have many physiological functions. Many drugs and other bioactive small molecules bind reversibly to albumin [3-5] , they could bind with serum albumin mostly through the formation of a stable complexes reversibly. The drug-protein complex may be considered as a form of drug in the biology temporary storage, it can effectively avoid drug was eliminate from metabolism so quickly that it can maintain the total concentration and effective concentrations of blood medicine in plasma. Therefore, interaction of a drug with, and competition for, the binding sites on plasma proteins might strongly affect its distribution, elimination, as well as its pharmacodynamics and toxic properties [6]. Studies on the binding of drug with protein will facilitate interpretation of the metabolism and transporting process of drug, and will help to explain the relationship between structures and functions of protein.

Topiramate amyl cephalosporin cefotaxime (TACC), a member of the latest class of broad-spectrum cephalosporins, is characterized by a high degree of stability against hydrolytic bacterial enzymes. It has been classified as a fourth generation cephalosporin and is considered to be highly active against most of the Gram-negative bacteria. This

antibiotic does not harm anaerobic bacteria, hence spare the intestinal flora, unlike other antibiotics [7]. At present, the molecular interactions between BSA and many drugs have been investigated successfully in bio-medical domain [8-14]. However, the interaction between TACC and BSA has not been investigated, especially the effects of common ions and coexistent drugs on the binding of drugs to BSA. In this study, the interaction of TACC with BSA is investigated by fluorescence spectroscopy as well as the effects of common ions and coexistent drugs. This study is expected to provide important insight into the essence, potential toxicity between drugs and protein in real terms, and can also provide a useful clinical reference for future combination therapy.

EXPERIMENTAL SECTION

Apparatus

All fluorescence spectra were recorded with a Shimadzu RF-540 spectrofluorophotometer and a Hitachi F-4500 spectrofluorophotometer. Absorption was measured with an UV-vis 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).

Materials

Topiramate amyl cephalosporin cefotaxime (TACC) was obtained from Monitor of Chinese Veterinary Medicine (no less than 99.9% pure). Bovine serum albumin (BSA, no less than 99% pure) was purchased from Sigma Company. Warfarin (WF), ibuprofen (IB) and digitoxin (DG) were all obtained from Chinese Institute of Drug and Biological Products. Stock solutions of BSA (1.0×10^{-4} mol L^{-1}) and TACC (2.0×10^{-3} mol L^{-1}) were prepared. And all the stock solutions were further diluted as working solutions prior to use. All the common antibiotics were diluted to 1.0×10^{-3} mol L^{-1} and all the metal Ions were diluted to 1.0×10^{-4} mol L^{-1}. Tris-HCl buffer solution containing NaCl (0.15 mol L^{-1}) was used to keep the pH of the solution at 7.40. NaCl solution was used to maintain the ionic strength of the solution. All other reagents were of analytical grade and all aqueous solutions were prepared with newly double-distilled water and stored at 277K.

Procedures

Fluorescence quenching experiments

In a typical fluorescence measurement, 1.0 mL of pH =7.40 Tris-HCl, 1.0 mL of 1.0×10^{-5} mol L^{-1} BSA solution and different concentrations of TACC were added into a 10 mL colorimetric tube successively. The samples were diluted to scaled volume with water, mixed thoroughly by shaking, and kept static for 20 min at different temperatures (298, 308 and 318 K). Excitation wavelength for BSA was 280 nm, excitation and emission slit widths set at 5 nm. The solution was subsequently scanned on the fluorophotometer and determined the fluorescent intensity at 343 nm. The fluorescence intensity of BSA without TACC was used as F_0. Meanwhile, the fluorescence intensity of compound was used as F.

Probe displace experiments

At 298 K, different concentrations of probe drug WF (or IB, DG) were added to the mixture of TACC-BSA systems. The molar ratio of TACC and BSA was kept at 5:1, and the of concentration of BSA is 1.0×10^{-6} mol L^{-1}. The mixtures were diluted to scaled volume with water, mixed thoroughly by shaking, and kept static for 20 min. The excitation wavelength for BSA was 280 nm, with the excitation and emission slit widths set at 5 nm. The fluorescence intensity of BSA only with TACC was used as F_0 to study the quenching effect of probe drugs to TACC-BSA system.

RESULTS AND DISCUSSION

Fluorescence quenching spectra of BSA-TACC system

Figure 1 shows the fluorescence spectra of BSA in the presence of different concentrations of TACC. It is shown that BSA has a strong fluorescence emission peak at 343 nm with excitation wavelength of 280 nm. The fluorescence intensity of BSA gradually decreased and there was almost no shift of the emission wavelength as increasing the concentration of TACC, indicating that there was the interaction between TACC and BSA. The quenching mechanism of the interaction between BSA and TACC was initiated by complex formation, and the microenvironment of BSA was changed during the binding reaction.

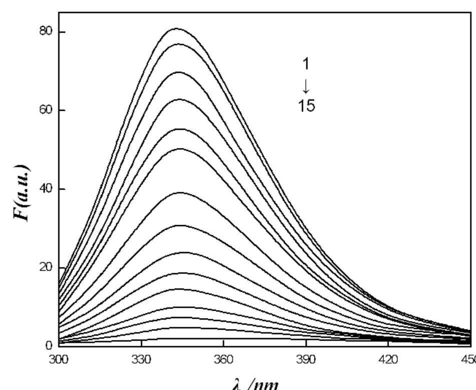

Fig. 1: Fluorescence spectra of BSA-TACC system（*T* =298K）

C_{BSA} =1.0×10^{-6} mol L^{-1}, 1~15 C_{TACC} =(0.0, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 12.0, 14.0, 16.0, 18.0)×10^{-5} mol L^{-1}; λ_{ex} =280 nm.

Fluorescence quenching mechanism of BSA-TACC system

Fluorescence quenching can occur by different mechanisms, it may be dynamic quenching, resulting from the collisional encounter between the drug and protein, or static quenching, resulting from the formation of a ground-state complex between the drug and protein. The Stern-Volmer equation is often applied to describe the fluorescence quenching and analyze the quenching mechanism [15] :

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

where F_0 and F are the fluorescence intensities in the absence and presence of ligand, respectively. τ_0 is the average lifetime of fluorescence without ligand, which is about 10^{-8} s. K_{sv} is the Stern-quenching constant. K_q is the quenching rate constant of biomolecule, 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. Higher temperature would result in faster diffusion and typically the dissociation of weekly bound complexes, leading to larger amount of dynamic quenching and smaller amounts of static quenching, respectively [7]. In this part, the values of K_{sv} decreased with the increase in temperature for all systems, which indicated that the probable quenching mechanism of the interaction between BSA and TACC was initiated by complex formation rather than by dynamic collision [16]. In addition, all the values of K_q were much greater than the maximum scatter collision quenching constant of various quenchers (2×10^{10} L mol^{-1} s^{-1}) [15] , this also suggested that the quenching mechanism was a static process [17].

Table 1: Quenching reactive parameters of BSA and TACC at different temperatures

T / K	K_q / (L mol^{-1} s^{-1})	r_1	K_a / (L mol^{-1} s^{-1})	*n*	r_2
298	2.14×10^{12}	0.9958	2.65×10^{4}	1.17	0.9961
308	2.08×10^{12}	0.9969	2.44×10^{4}	1.11	0.9989
318	1.99×10^{12}	0.9978	2.31×10^{4}	1.06	0.9997

r_1 is the linear relative coefficient of F_0/F ~[C]; r_2 is the linear relative coefficient of $\log(F_0-F)/F$ ~$\log\{[D_t]-n[B_t](F_0-F)/F_0\}$. K_q is the quenching rate constant; K_a is the binding constant; n is the number of binding site.

Binding constant and number of binding site of BSA with TACC

For static quenching process, the relationship between the fluorescence intensity and the concentration of quencher can be usually described by derived Eq. (2) [18] to obtain the binding constant (K_a) and the number of binding sites (n) in most paper:

$$\log\left(\frac{F_0-F}{F}\right)=n\log K_a+n\log\left\{[D_t]-n\frac{F_0-F}{F_0}[B_t]\right\} \quad (2)$$

where $[D_t]$ and $[B_t]$ are the total concentrations of TACC and protein, respectively. On the assumption that n in the bracket is equal to 1, the curve of $\log(F_0\text{-}F)/F$ versus $\log\{[D_t]\text{-}[B_t](F_0\text{-}F)/F_0\}$ is drawn and fitted linearly, then the value of n can be obtained from the slope of the plot. If the n value obtained is not equal to 1, then it is substituted into the bracket and the curve of $\log(F_0\text{-}F)/F$ versus $\log\{[D_t]\text{-}n[B_t](F_0\text{-}F)/F_0\}$ is drawn again. The above process is repeated again and again till n obtained is only a single value or a circulating value. Based on the n obtained the binding constant K_a can be also obtained. In the work, a calculation program was developed. The calculation process can be finished with calculator based on the simple program and the calculating results can be obtained by inputting F, $[D_t]$ and $[B_t]$. The calculated results were shown in Table 1. As seen in Table 1, The order of magnitude of binding constants (K_a) was 10^4 indicated the existence of strong interaction between BSA and TACC. Meanwhile, the values of K_a decreased with the increasing temperature, further suggested that the quenching was a static process [19], hence it led to the reduced of the stability of binary systems.

Type of interaction force of the binary systems

In order to elucidate the interaction between drugs and BSA, the thermodynamic parameters were calculated from the Van't Hoff equation. Generally, the interaction forces between the small drug molecule and biological macromolecule include hydrogen bond, Van der Waals force, electrostatic interactions and hydrophobic force, etc [20]. Ross and Subramanian [21] have characterized the sign and magnitude of the thermodynamic parameter, enthalpy change (ΔH), free energy (ΔG) and entropy change (ΔS) of reaction, associated with various individual kinds of interaction. The reaction ΔH and ΔS can be regarded as constant if the temperature changes little. The thermodynamic parameters can be calculated on the basis of the following equation:

$$R\ln K=\Delta S-\Delta H/T \quad (3)$$

$$\Delta G=\Delta H-T\Delta S \quad (4)$$

Negative ΔH and positive ΔS indicate electrostatic interaction plays a major role in the binding reaction. Positive ΔH and ΔS are generally considered as the evidence for typical hydrophobic interactions. In addition, Van der Waals force and hydrogen bonding formation in low dielectric media are characterized by negative ΔH and ΔS [22].

According to the binding constants K_a of TACC to BSA at different temperatures above (Table 1), the thermodynamic parameters were obtained conveniently. Therefore, the values of ΔH, ΔS and ΔG were -4.64 KJ mol^{-1}, 69.1 J mol^{-1} K^{-1}, -25.1 KJ mol^{-1} (T =298 K), respectively. The negative value of ΔG clarified an automatic reaction between TACC and BSA. The negative value of ΔH and positive value of ΔS showed that TACC mainly bound to BSA by the electrostatic attraction.

Identification of the binding sites of TACC on the BSA

BSA comprises three homologous domains that assemble to form a heart-shaped molecule. Each domain is a product of two sub-domains that possess common structural motifs. The principal regions of ligand binding to human serum albumin are located in hydrophobic cavities in sub-domains IIA and IIIA, which exhibit similar chemistry [23]. Site marker fluorescent probes are useful for rapid identification of drug binding sites [24]. In order to estimate the drug binding sites on BSA, Sudlow et al. has been proposed the percentage of probe replacement method using the following equation [25]:

$$probe\ displacement(\%)=F_2/F_1\times 100 \quad (5)$$

The molar ratio TACC:BSA was kept 5:1, while that different concentrations of various probe drugs each were added into it. In the study of the substitution reaction, F_1 and F_2 are the fluorescence intensities in the absence and presence probe drug. In this paper, we have considered WF, IB and DG as probe drugs which bind specifically to sites I, II and III, respectively. The processed results were shown in Figure 2 by Eq.(5).

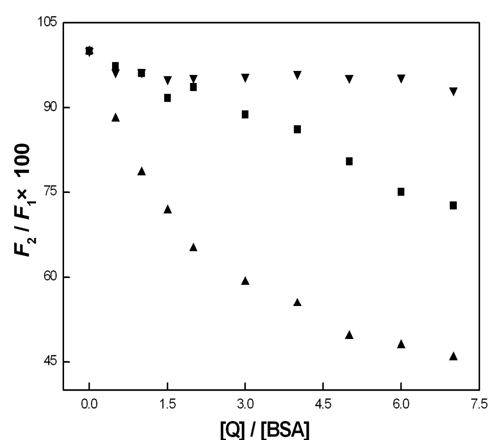

Fig. 2: Effect of site maker probe on the fluorescence intensities of BSA-TACC system. (T =298 K)
$C_{TACC} = C_{BSA} = 1.0\times10^{-6}$ mol L^{-1}; C_Q= (0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0)$\times10^{-6}$ mol L^{-1}
[Q]: ▲WF;■IB;▼DG;λ_{ex} =280 nm;

As seen in Figure 2, relative fluorescence intensities decreased obviously with increasing concentration of WF whereas it almost kept constant with the adding of IB and DG. It indicated that TACC was pronouncedly displaced by WF rather than IB or DG, hence it could be inferred that the binding site for TACC was site I which located in sub-domain IIA of BSA (as show in figure 3).

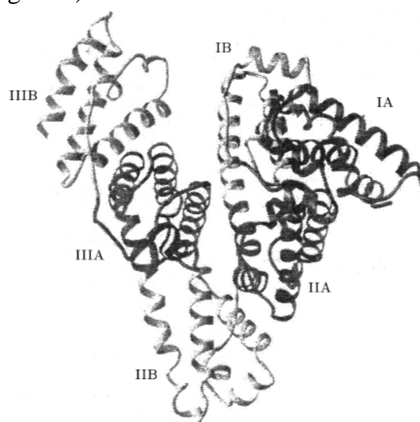

Fig. 3: Schematic drawing of the BSA molecule. Sub-domains are marked with IA, IB, IIA, IIB, IIIA and IIIB, respectively

Effects of common ions and coexistent drugs on the binding of TACC and BSA

According to this method about the percentage of probe replacement method described above, we have taken assays to study the effects of some common ions and antibiotics on the binding of BSA-TACC system. The molar ratio TACC:BSA was kept 5:1, while the concentration of BSA was 1×10^{-6} M. F_1 and F_2 are the fluorescence intensities in the absence and presence antibiotice. It supposed that there was no effect on the binding of BSA-TACC system when the value of $(F_2-F_1)/F_1$ was less than ±5% by Eq. (5), that was, this antibiotice could coexist with TACC. The calculated percentage of coexist drugs replacement F_2/F_1 were shown in Table 2. The results showed that 100 times of ampicillin, gentamicin, streptomycin, acetyl spiramycin, sulfamethoxazole, 10 times of penicillin G sodium salt, Zn^{2+}, Co^{2+}, Ni^{2+}, 8 times of Fe^{3+}, 5 times of Mg^{2+}, cefotaxime sodium, cefoperazone sodium could coexist with TACC, but 1 time of Cu^{2+}, moxifloxacin, ciprofloxacin, norfloxacin, chloramphenicol could disturb the stability of BSA-TACC system, making the fluorescence intensity changed a lot. It might result that quinolone and chloromycetin drugs have strong ability to bind to BSA, hence, there were certain competition among them for the protein.

Table 2: The percentage of coexist drugs replacement on the binding of BSA-TACC system

Coexist drug	C_d /mol L^{-1}	F_2/F_1(%)	Coexist drug	C_d /mol L^{-1}	F_2/F_1(%)
penicillin G sodium salt	1.0×10^{-5}	97.18	cefoperazone sodium	5.0×10^{-6}	95.74
ampicillin	1.0×10^{-4}	98.59		5.0×10^{-5}	46.83
gentamicin	1.0×10^{-4}	97.82	norfloxacin	1.0×10^{-6}	99.46
streptomycin	1.0×10^{-4}	96.92		2.0×10^{-5}	54.50
neomycin	1.0×10^{-4}	103.21	moxifloxacin	1.0×10^{-6}	95.50
kanamycin	1.0×10^{-4}	98.97		1.0×10^{-5}	50.92
acetyl spiramycin	1.0×10^{-4}	99.69	ciprofloxacin	1.0×10^{-6}	88.96
erythromycin	1.0×10^{-4}	97.18		2.0×10^{-5}	48.81
sulfamethoxazole	1.0×10^{-4}	97.18	Mg^{2+}	5.0×10^{-6}	98.04
thiamphenicol	1.0×10^{-4}	101.92	Fe^{3+}	8.0×10^{-6}	95.69
chloramphenicol	1.0×10^{-6}	96.51	Zn^{2+}	1.0×10^{-5}	100.26
	5.0×10^{-5}	47.35	Co^{2+}	1.0×10^{-5}	95.29
cefotaxime sodium	5.0×10^{-6}	99.60	Ni^{2+}	1.0×10^{-5}	96.86
	8.0×10^{-5}	50.39	Cu^{2+}	1.0×10^{-6}	101.05
				4.0×10^{-5}	62.09

C_d is the concentration of coexist drug; F_1 and F_2 are the fluorescence intensities in the absence and presence coexist drug.

The competition would lead to binding constant K_a changed spontaneously. The fluorescence intensity of BSA reduced to 50% when there were 80 times of cefotaxime sodium, 50 times of cefoperazone sodium, chloramphenicol, 20 times of ciprofloxacin, norfloxacin and 10 times of moxifloxacin in this system, which indicated that these coexistent drugs have displaced the combination of TACC and BSA. The competition could also affect the stayed-time from the blood, as well as plasma concentration and effective concentration, having effects on the efficacy of drugs when these antibiotics coexist with TACC on the binding reaction of BSA. However, aminoglycosides, macrolides, sulfonamides and penicillins antibiotics have no effects on the binary system, that is, those antibiotics will not affect the transportation of TACC though BSA when they coexist with TACC, and also have no effects on the efficacy of TACC.

CONCLUSION

The interaction through electrostatic force between TACC and BSA was studied by using fluorescence spectroscopy at different temperatures. The experiment also studied the effects of common ions and coexistent drugs on the binary system of BSA-TACC. This study is expected to provide important insight into the essence, potential toxicity between drugs and protein in real terms, and can also provide a useful clinical reference for future combination therapy.

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REFERENCES

[1] QL Guo; R Li; FL Jiang; JC Tu; LW Li; Y Liu, *Acta. Phys.-Chim.Sin.*, **2009**, 25(10), 2147-2154.
[2] Y Bhatt; D Shah, *J. Chem. Pharm. Res.*, **2012**, 4(3), 1708-1715.
[3] YJ Hu; Y Liu; XS Shen; XY Fang; SS Qu, *J. Mol. Struct.*, **2005**, 738(1-3), 143-147.
[4] YJ Hu; Y Liu; JB Wang; XH Xiao; SS Qu, *J. Pharm. Biomed. Anal.*, **2004**, 36(4), 915-919.
[5] J Guharay; B Sengupta; PK Sengupta, *Proteins*, **2001**, 43(2), 75-81.
[6] C Bertucci; E Domenici, *Curr. Med. Chem.*, **2002**, 9(15), 1463-1481.
[7] A Tamta; M Chaudhary; R Sehgal, *Int. J. Pharm.*, **2010**, 6(2), 111-116.
[8] SY Bi; YT Sun; CY Qiao; HQ Zhang; CM Liu, *J. Lumin.*, **2009**, 129(5), 541-547.
[9] A Rogozea; M Iulia, *J. Phys. Chem. B.*, **2012**, 116(49), 14245-14253.
[10] B Kaboudin; K Moradi; MR Faghihi, *J. Lumin.,* **2013**, 139, 104-112.
[11] BR Thorat; R Jadhav; M Mustapha; S Lele; D Khandekar; P Kamat; S Sawant; D Arakh; RG Atram; R Yamgar, *J. Chem. Pharm. Res.*, **2012**, 4(1), 18-26.
[12] PN Naik; SA Chimatadar, *Spectrochim. Acta, Part A.*, **2009**, 73(5), 841-845.
[13] N Zhou; YZ Liang; P Wang, *J. Mol. Struct.,* **2008**, 872(2-3), 190-196.
[14] D Ran; X Wu; J Zheng, *J. Fluoresc.*, **2007**, 17(6), 721-726.
[15] RE Maurice; AG Camillo, *Anal Biochem*, **1981**, 114(2), 199-212.
[16] Y Zhang; J Zhang; F Li, *Mol Biol Rep*, **2011**, 38(4), 2445-2453.
[17] F Ding; N Li; BY Han; F Liu; L Zhang; Y Sun, *Dyes and Pigments*, **2009**, 83(2), 249-257.
[18] SY Bi; CY Qiao; DQ Song; Y Tian; DJ Gao; Y Sun; HQ Zhang, *Sens. Actuators. B.*, **2006**, 119(1), 199-208.
[19] N Shahabadi; M Maghsudi, *J. Mol. Struct.*, **2009**, 929(1-3), 193-199.

[20] N Wang; L Ye; F Yan; R Xu, *Int. J. Pharm.*, **2008**, 351(1-2), 55-60.
[21] PD Ross; S Subramanian, *Biochemistry*, **1981**, 20(11), 3096-3102.
[22] M Gharagozlou; DM Boghaei, *Spectrochim. Acta, Part A.*, **2008**, 71(4), 1617-1622.
[23] XM He; DC Carter, *Nature*, **1992**, 358(16), 209 -215.
[24] G Sudlow; DJ Birkett; DN Wade, *Mol. Pharmacol.*, **1976**, 12(6), 1052 -1061.
[25] K Yamasaki; T Maruyama; U Kragh-Hansen; M Otagiri, *Biochim. Biophys. Acta.*, **1996**, 1295(2), 147-157.