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Study on the interaction characteristics of cefamandole with bovine serum albumin by spectroscopic technique

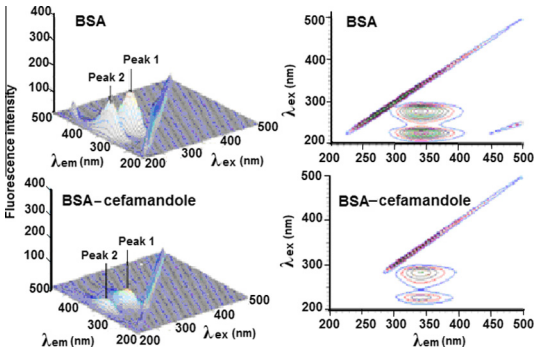
Qian Wang, Xuyang Liu, Ming Su, Zhihong Shi, Hanwen Sun *

College of Chemistry and Environmental Science, Hebei University, Key Laboratory of Analytical Science and Technology of Hebei Province, Baoding 071002, PR China

HIGHLIGHTS

- Quenching mechanism of BSA fluorescence by cefamandole was discussed.
- The fluorescence quenching is due to static quenching and energy transfer.
- The van der Waals interaction and hydrogen bonding play major roles in the binding.
- Primary binding of cefamandole located at site III in sub-domain III_A of BSA.
- Conformational change of BSA in the presence of cefamandole was observed.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction of cefamandole with bovine serum albumin (BSA) was studied by fluorescence quenching in combination with UV–Vis spectroscopic method under near physiological conditions. The fluorescence quenching rate constants and binding constants for BSA–cefamandole system were determined at different temperatures. The fluorescence quenching of BSA by cefamandole is due to static quenching and energy transfer. The results of thermodynamic parameters, ΔH (-268.0 kJ mol^{-1}), ΔS (-810.0 J mol^{-1} K^{-1}) and ΔG (-26.62 to -8.52 kJ mol^{-1}), indicated that van der Waals interaction and hydrogen bonding played a major role for cefamandole–BSA association. The competitive experiments demonstrated that the primary binding site of cefamandole on BSA was located at site III in sub-domain III_A of BSA. The distance between cefamandole and a tryptophane unit was estimated to be 1.18 nm based on the Förster resonance energy transfer theory. The binding constant (K_A) of BSA–cefamandole at 298 K was 2.239×10^4 L mol^{-1}. Circular dichroism spectra, synchronous fluorescence and three-dimensional fluorescence studies showed that the presence of cefamandole could change the conformation of BSA during the binding process.

Introduction

Cefamandole is a cephalosporin antibiotic used to treat a host of infections and is usually delivered by injection into the vein or muscle. It is very effective against Gram-negative coccus and hemophilus, and was mainly used to treat bacterial infections such as respiratory tract infections, biliary tract infections, and urinary tract infections. The clinically used form of cefamandole is cefamandole nafate, which is administered parenterally and decomposed rapidly to cefamandole in the body [1–3]. Serum albumin is one of the most abundant proteins in circulatory system of a wide variety of organisms and one of the most extensively studied proteins at all. The albumins make a significant contribution to

* Corresponding author. Tel.: +86 312 5079719; fax: +86 312 5079739.
E-mail address: hanwen@hbu.cn (H. Sun).

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colloid osmotic blood pressure and aid in the transport, distribution and metabolism of many endogenous and exogenous ligands. Protein–drug binding greatly influences absorption, distribution, metabolism and excretion properties of typical drugs [4,5]. Protein binding has long been considered one of the most important physicochemical characteristics of drugs, playing a potential role in distribution, excretion and therapeutic effectiveness. Thereby, it is important and necessary to study the interaction of drug with serum albumins at molecular level.

There were a few of reports for studying the interactions of cephalosporin and protein. The binding of some cephalosporins of pharmacological interest to serum albumin was studied using ultrafiltration method [6,7] and differential scanning calorimetry method [8], but no information regarding interaction forces and conformation change of BSA. Fluorescence spectroscopy is an effective tool for the investigation of conformational changes of protein under physiological conditions because of its accuracy, sensitivity, rapidity and convenience [9]. It can reveal the accessibility of drugs to albumin's fluorophores, which can help us to understand the binding mechanisms of albumin–drug and to provide information on the structural features for determining the therapeutic effectiveness of the drug. However, one of the major problems associated with measurement of fluorescent organic matter in natural samples is the inner-filter effect (IFE), sometimes referred to as self-absorption [10]. This is caused by the absorption of the exciting as well as the fluorescent light (primary and secondary IFE, respectively) by the fluorophore itself, alternatively by another component of the sample. The intrinsic fluorescence of lysozyme at 340 nm can be quenched by cephalosporin analogues through the static quenching and non-radiative energy transferring procedure [11]. The interaction of cephalosporin-medicine with bovine serum albumin (BSA) was investigated by fluorescence methods [12,13], but not correction of the inner-filter effect. There is no report for studying the interactions of cefamandole and serum albumin protein.

In this work, cefamandole was selected as model drug. To provide important insight into the interaction of the physiologically important protein BSA with drugs, this study examined, for the first time, the interaction between BSA and cefamandole under near physiological conditions by the fluorescence quenching in combination with UV–Vis spectroscopic method.

Experimental

Drugs and reagents

Commercially available bovine serum albumin (BSA, catalog no. A-7030, purity: 98%, M: 68,000) was purchased from Sigma Chemical Company. BSA stock solution (1.0×10^{-4} M) was prepared by dissolving an appropriate amount of BSA with 0.1 M Tris–HCl (pH 7.4) buffer solution, and kept in the dark at 4 °C. BSA working solutions were prepared by diluting the stock solution with water. Cefamandole sodium (purity: 98.7%) was purchased from the North China Pharmaceutical Co. Ltd. (Shijiazhuang, China). A stock solution (1.0×10^{-3} M) of cefamandole was prepared in water, and stored in refrigerator at −4 °C. Tris–HCl buffer (pH 7.40) consists of Tris (0.1 M), HCl (0.1 M), and NaCl (0.5 M). NaCl solution was used to maintain the ion strength. All chemicals were of analytical reagent grade or better. Purified water was prepared by an XGJ-30 highly pure water machine (Yongcheng purification Science & Technology Co. Ltd., Beijing, China).

Equipment

All fluorescence measurements were performed on an F-7000 Fluorescence spectrophotometer (Hitachi, Japan) which was equipped with a 1 cm quartz cell and thermostat bath. The spectrum data points were collected from 280 to 500 nm. The widths of the excitation and the emission slit were both set at 5 nm. Fluorescence measurements were carried out at room temperatures.

Circular dichroism (CD) spectra were obtained on a MOS-450/SFM300 circular dichroism spectrometer (Bio-Logic Com. Germany). The absorption spectra were performed on a TU-1900 double light Spectrophotometer (Beijing TAYASAF Science & Technology Co., Ltd., China) using a 1 cm quartz cell in the wavelength range of 200–500 nm. All pH measurements were performed with a pHS-3C pH meter (Shanghai, China).

Determination of fluorescence intensity

Five 10-mL clean and dried test tubes were taken, and 2 mL of 0.5 M NaCl, 2.0 mL Tris–HCl buffer (pH 7.40), 0.25 mL of 4.0×10^{-5} M BSA, and different volumes (2.0–4.0 mL) of cefamandole standard solution of 1.0×10^{-4} M were added in each test tube, and diluted to 10 mL with water. The concentration of BSA was 1.0×10^{-6} M, and that of cefamandole was 0.4, 0.8, 1.2, 1.6, and 2.0×10^{-6} M. Sixth test-tube containing only BSA solution at pH 7.4 was marked as "control", and seventh test-tube containing only 1×10^{-5} M cefamandole was used for the comparison. After mixing the solutions, these were allowed to stand for 15 min for maximum binding of cefamandole to BSA. The fluorescence intensity after the correction of inner-filter effect was calculated by the equation [14]: $F_{cor} = F_{obs}\ \exp(\frac{1}{2}A_{ex} + \frac{1}{2}A_{em})$, where F_{obs} is fluorescence intensity measured before the correction of inner-filter effect, A_{ex} and A_{em} are absorbance of the test solution at excitation and emission wavelengths, respectively. The corrected fluorescence intensity was used for studying on the interaction of cefamandole and BSA. After corrected inner-filter effect, fluorescence intensity (F_0) in the absence of quencher cefamandole and the fluorescence intensity (F) in the presence of quencher cefamandole were measured at a wavelength of λ_{ex} 280 nm and λ_{em} 340 nm under temperature of 298, 303 and 308 K for estimating the interaction between cefamandole and BSA.

Results and discussion

Fluorescence quenching mechanism

Fluorescence quenching refers to any process that decreases the fluorescence intensity of a sample. A variety of molecular interactions can result in fluorescence quenching of excited state fluorophores. These include molecular rearrangements, energy transfer, ground state complex formation and collisional quenching. Fig. 1 shows the fluorescence spectra of BSA in the absence and presence of cefamandole after corrected inner-filter effect as well as absorption spectra.

No fluorescence of cefamandole was observed. The fluorescence spectra of BSA show a broad band with maximum at ~340 nm. It is observed that both fluorescence intensity and absorbance of BSA decrease with increasing concentration of cefamandole. A maximum fluorescence emission of BSA underwent spectral shift from 341.0 to 342.8 nm, and a maximum absorption of BSA underwent spectral shift from 283 to 270 nm. It is suggested that BSA and cefamandole formed a comp lex, and an energy transfer between cefamandole and BSA occurred.

The fluorescence quenching data are analyzed by the Stern–Volmer equation [15]:

$$F_0/F = 1 + k_q\tau_0[Q] = 1 + k_{sv}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensity in the absence and presence of quencher, respectively. k_q is the quenching rate constant, τ_0 is the fluorescence life time of biopolymer BSA