Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 124 (2014) 84–90

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Investigation of interaction of antibacterial drug sulfamethoxazole with human serum albumin by molecular modeling and multi-spectroscopic method

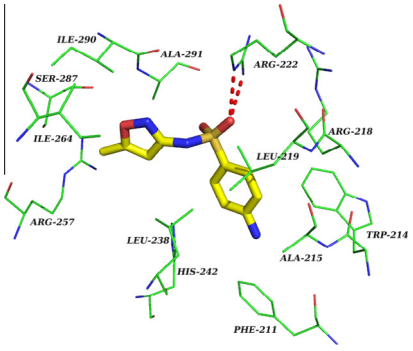
Qin Wang *, Sheng-Rui Zhang, Xiaohui Ji

School of Chemistry and Environment Science, Shaanxi University of Technology, Hanzhong, Shaanxi 723000, China

HIGHLIGHTS

- The interaction of SMX and HSA was firstly predicted through molecular modeling.
- The binding parameters were performed through the fluorescence quenching spectra.
- The thermodynamic parameters were calculated by the Van't Hoff equations.
- The conformational changes of HSA were confirmed by multi-spectroscopic methods.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 August 2013
Received in revised form 20 December 2013
Accepted 23 December 2013
Available online 7 January 2014

Keywords:
Sulfamethoxazole
Human serum albumin
Molecular interaction
Molecular modeling
Multi-spectroscopy

ABSTRACT

Interaction of sulfamethoxazole (SMX) with human serum albumin (HSA) was investigated by molecular modeling and multi-spectroscopic methods under physiological conditions. The interaction mechanism was firstly predicted through molecular modeling that confirmed the interaction between SMX and HSA. The binding parameters and the thermodynamic parameters at different temperatures for the reaction had been calculated according to the Stern–Volmer, Hill, Scatchard and the Van't Hoff equations, respectively. One independent class of binding site existed during the interaction between HSA and SMX. The binding constants decreased with the increasing temperatures, which meant that the quenching mechanism was a static quenching. The thermodynamic parameters of the reaction, namely standard enthalpy ΔH^0 and entropy ΔS^0, had been calculated to be -16.40 kJ mol^{-1} and 32.33 J mol^{-1} K^{-1}, respectively, which suggested that the binding process was exothermic, enthalpy driven and spontaneous. SMX bound to HSA was mainly based on electrostatic interaction, but hydrophobic interactions and hydrogen bonds could not be excluded from the binding. The conformational changes of HSA in the presence of SMX were confirmed by the three-dimensional fluorescence spectroscopy, UV–vis absorption spectroscopy and circular dichroism (CD) spectroscopy. CD data suggested that the protein conformation was altered with the reduction of α-helices from 55.37% to 41.97% at molar ratio of SMX/HSA of 4:1.

Introduction

Sulfonamides that possess a p-aminobenzenesulfonamide framework are a common class of antibiotics and are the first chemotherapeutic agents effective for the treatment of bacterial and protozoan infections in veterinary and human medicine practices [1–3]. In animal husbandry, sulfonamides are often added to the feed of swine, birds, and cattle for the prevention and treatment of infections or for growth promotion due to the advantages of broad antibacterial spectrum, high efficacy, and low prices [4,5]. Abuse of sulfonamides or insufficient withdrawal time can lead to

* Corresponding author. Tel.: +86 916 2642766; fax: +86 916 2641574.
E-mail addresses: qincaimuxu009@sina.cn (Q. Wang), Zhangshr09@lzu.edu.cn (S.-R. Zhang).

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http://dx.doi.org/10.1016/j.saa.2013.12.100

accumulation of these drugs in animal tissues, which may cause allergy, carcinogenesis, and formation of resistant bacteria in the human body [6–8]. As a member of the sulfonamide family, sulfamethoxazole (SMX) (structure shown in Fig. 1a) is a now widely used pharmaceutical product in treating urinary tract and lower respiratory tract infections [9–11]. However, SMX could be accumulated in human body and has potential toxicity to human health because of its carcinogenic potency and possible antibiotic resistance [12–14]. This problem has received increasing public attention, and many authorities around the world have proposed maximum residue limits (MRL) of sulfonamides that can be allowed in foods [15,16].

Human serum albumin (HSA) (crystal structure shown in Fig. 1b) is the most abundant protein in plasma and has been the most widely used model protein in evaluating the drug-protein system because it has been extensively characterized [17–19]. HSA contains three homologous domains (I–III): I (residues 1–195), II (196–383) and III (384–585); each domain can be divided into two subdomains (A and B) [20,21], and there is a large hydrophobic cavity in subdomain IIA where many small molecules can bind [22]. Crystal structure analysis has revealed that amino acid sequence of HSA contains a total of 17 disulfide bridges, one free thiol (Cys-34) and a single tryptophan (Try-214), and according to Peters, the tryptophan residue (Trp214) of HSA is in subdomain IIA [23]. As the major soluble protein constituent of the circulatory system, HSA has many physiological and pharmacological functions which are participating in absorption, distribution, metabolism and excretion of drugs [24–26]. Most drugs travel in plasma and reach the target tissues by binding to HSA, which is an important factor in determining drug pharmacokinetics including distribution and elimination [27–29]. Therefore, studies on the interaction between sulfonamides and HSA are necessary to understand the potential toxicity of sulfonamides to the human bodies.

Until now, the mechanisms of the interactions between sulfonamides and HSA have been investigated by several groups. Gao et al. investigated the interaction between HSA and sulfamethazine by capillary electrophoresis, fluorescence spectrometry, and circular dichroism (CD) [30]. Nandibewoor et al. performed the binding of SMX to bovine serum albumin by spectroscopic methods [31]. Zou et al. studied the binding of SMX to HSA by a technique of microdialysis with liquid chromatography [32]. However, the mechanism of the reaction, binding parameters, basic thermodynamic parameters and alteration in the protein secondary structure has not been investigated. In this paper, the mechanism of the reaction was firstly simulated using a SGI FUEL workstation. The binding constants, numbers of binding sites and basic thermodynamic parameters under different temperatures were calculated according to the, Modified Hill equation, Scatchard plots and Van't Hoff equation. The three dimensional fluorescence spectra were carried out to reveal the changes of HSA's tryptophan and tyrosine residues and the behavior of HSA's characteristic polypeptide backbone structure. Meanwhile, the alterations of the protein secondary structures induced by the addition of SMX were further investigated by UV–vis absorption spectroscopy and CD spectroscopy.

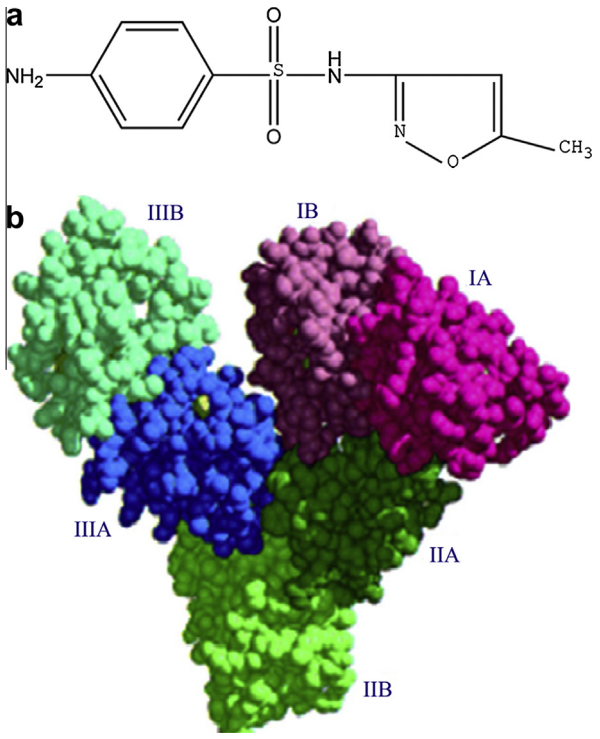

Fig. 1. (a) The structure of SMX and (b) crystal structure of HSA.

Materials and methods

Materials

SMX and HSA were purchased from Sigma Chemical Company. They were used without further purification. Tris(tris(hydroxymethyl)-amino-methane) was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). All solutions of HSA were prepared in pH 7.40 buffer solution, and stock solutions of HSA (3.0×10^{-5} mol L^{-1}) were kept in the dark at 4 °C. Tris (0.2 mol L^{-1})-HCl (0.1 mol L^{-1}) buffer solution containing NaCl (0.1 mol L^{-1}) was used to keep the pH of the solutions at 7.40. NaCl (1.0 mol L^{-1}) solution was used to maintain the ionic strength at 0.1. The stock solution (3.0×10^{-3} mol L^{-1}) of SMX was prepared by dissolving appropriate amounts of sulfonamides in anhydrous methanol and kept at 4 °C. All other reagents were of analytical reagent grade and doubly distilled water was used throughout the experiments.

Apparatus

All fluorescence spectra were recorded using a F97Pro spectrofluorophotometer (LengGuang Industrial Co., Ltd. of Shanghai, China). Fluorescence emission spectra were recorded from 270 to 450 nm (excitation wavelength 283 nm) using 10 nm/10 nm slit widths. An electronic thermo regulating water-bath (NTT-2100, EYELA, Japan) was used to control the temperature. UV–vis absorption spectra were recorded using a Tu-1901 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) equipped with a 1.0 cm quartz cell. CD measurements were carried out using an Olis DSM 1000 CD (American) in a cell of path length 1 mm at room temperature.

Methods

Molecular modeling study

The crystal structure of HSA in complex with R-warfarin was taken from the Brookhaven Protein Data Bank (entry codes 1h9z) [33]. The potential of the 3D structure of HSA was assigned based on the Amber 4.0 force field with Kollman−all-atom charges. The initial structure of SMX was generated by molecular modeling software Sybyl 6.9 [34]. The geometry of the molecule was subsequently optimized to minimal energy using the Tripos force field with Gasteiger–Marsili charges, and the FlexX program was