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Experimental and theoretical study on the binding of 2-mercaptothiazoline to bovine serum albumin

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ABSTRACT

2-Mercaptothiazoline (MTZ) is widely utilized as a brightening and stabilization agent, corrosion inhibitor and antifungal reagent. The residue of MTZ in the environment is potentially hazardous to human health. In this study, the binding mode of MTZ with bovine serum albumin (BSA) was investigated using spectroscopic and molecular docking methods under physiological conditions. MTZ could spontaneously bind with BSA through hydrogen bond and van der Waals interactions with one binding site. The site marker displacement experiments and the molecular docking revealed that MTZ bound into site II (subdomain IIIA) of BSA, which further resulted in some backbone structures and microenvironmental changes of BSA. This work is helpful for understanding the transportation, distribution and toxicity effects of MTZ in blood.

1. Introduction

Serum albumins are the most abundant carrier proteins in the circulatory system and play an important role in the transportation and disposition of various endogenous and exogenous ligands, such as fatty acids, amino acids, metal ions, drugs and toxic substances [1–7]. For toxic substances, the distribution, free concentration, duration and intensity of their side effects can be significantly influenced by ligands–protein interactions in blood [8,9]. Therefore, the investigation of chemicals with the protein is of imperative and fundamental importance. Bovine serum albumin (BSA), a single chain of 583 amino acids, consists of three structurally homologous domains (I–III), and each domain contains two subdomains (A and B) [10,11]. The main binding sites of ligands on BSA are often located in two hydrophobic pockets in subdomains IIA and IIIA, namely sites I and II [12]. BSA has been one of the most extensively studied protein models in the related research [13,14].

2-Mercaptothiazoline (MTZ) is composed of an exocyclic mercapto group and a five-member heterocyclic ring containing S, N and C atoms [15]. MTZ has diverse pivotal roles in industrial processes. MTZ is commonly used as a brightening and stabilization agent in the electroplating solution of printed wiring board industry, and a corrosion inhibitor on the surface of the metal [16,17]. It is also used as an antifungal reagent in medical application [18]. The chemically modified MTZ was used for the purpose of the selective adsorption of the heavy metal ions in aqueous solutions [19]. Although the usability of MTZ is indisputable, MTZ is harmful according to its Material Safety Data Sheet and shows persistence to microbial degradation [20]. Previous studies reported that MTZ was frequently detected in wastewater effluents as well as river water [21,22]. Thus, MTZ as an aromatic pollutant exposes in the environment, leading to an increase in the possibility of its exposure to organisms.

Some publications have reported the toxic effects of MTZ in the past. The toxicity of MTZ on rats revealed that MTZ strongly reduced thyroid hormone levels and inhibited thyroid peroxidase [23,24]. Based on the characteristics of MTZ, it was used as an antithyroid drug [25]. MTZ was also found to inhibit both horseradish peroxidase and lactoperoxidase [24]. However, little work has been focused on the effect of MTZ on the carrier protein BSA. The purpose of this work was to explore the binding mechanism of MTZ with BSA by spectroscopic and molecular docking methods. The binding characteristics of MTZ–BSA were explored according to the fluorescence data. The precise binding site of MTZ on BSA was investigated in detail using displacement experiments and molecular docking. The UV–vis absorption, synchronous fluorescence and circular dichroism measurements were employed to investigate the conformational changes of BSA caused by MTZ. These results are expected to clarify the binding mechanism of MTZ with BSA and provide useful information for understanding the toxicological actions of MTZ at molecular level.

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2. Experimental methods

2.1. Reagents

Bovine serum albumin (BSA, CAS no.: 9048-46-8) and 2-mercaptothiazoline (MTZ, CAS no.: 96-53-7) were obtained from Sinopharm Chemical Reagent Co., Ltd. Phenylbutazone (PB, CAS no.: 50-33-9) and flufenamic acid (FA, CAS no.: 530-78-9) from Tokyo Chemical Industry Co. Ltd. and digitoxin (Dig, CAS no.: 71-63-6) from Dr. Ehrenstorfer GmbH were dissolved in ethanol to form a 1.0×10^{-3} mol L^{-1} solution, which were used to determine the binding sites of MTZ on BSA. A 0.2 mol L^{-1} mixture of phosphate buffer (mixture of $NaH_2PO_4\cdot2H_2O$ and $Na_2HPO_4\cdot12H_2O$, pH=7.4) was used to control the pH. All other reagents were of analytical grade and purchased from standard reagent suppliers. Ultrapure water (18.25 MΩ) was used throughout the experiments.

2.2. Apparatus and measurements

2.2.1. Fluorescence measurements.

All fluorescence spectra were recorded on an RF-5301PC fluorescence spectrophotometer (Shimadzu Japan) with a 1 cm cell. The excitation wavelength was 278 nm. The excitation and emission slit widths were set at 5 nm.

2.2.2. Displacement experiments.

The displacement experiments were performed using different site markers, namely, PB, FA and Dig for sites I, II and III, respectively, by keeping the concentrations of BSA and the markers constant at 3.0×10^{-7} mol L^{-1}, and then gradually adding MTZ (to give a final concentration of 5.0×10^{-5} mol L^{-1}). Fluorescence quenching spectra were measured at 291 K over a range of 290–500 nm. The binding constants of MTZ–BSA system in the presence of above site markers were calculated by the fluorescence data.

2.2.3. Molecule docking investigation

Docking calculations were carried out using AutoDock 4.2 (developed by The Scripps Research Institute, USA) [26]. Docking calculations were carried out on a BSA protein model (PDB code 4F5S) [27,28]. The initial structure of MTZ was taken from the ZINC database [29]. With the aid of AutoDock, the ligand root of MTZ was detected and rotatable bonds were defined. All hydrogen atoms and compute gasteiger charges were added into the BSA protein model. To recognize the binding sites in BSA, blind docking was carried out and grid maps of $126\times126\times126$ Å grid points and 0.375 Å spacing were generated. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) search method. Each run of the docking experiment was set to terminate after a maximum of 250,000 energy evaluations and the population size was set to 150. The conformation with the lowest binding free energy was used for further analysis.

2.2.4. UV–visible absorption measurements

The absorption spectra were recorded on a double beam UV-6100 spectrophotometer (Mapada, China) equipped with 1.0 cm quartz cells. Slit width was set at 2.0 nm. The wavelength range was 205–245 nm.

2.2.5. Synchronous fluorescence measurements

Synchronous fluorescence spectra of BSA in the absence and presence of MTZ were measured ($\Delta\lambda=15$ nm, $\lambda_{em}=280$–340 nm and $\Delta\lambda=60$ nm, $\lambda_{em}=310$–380 nm, respectively) by an RF-5301PC fluorescence spectrophotometer (Shimadzu, Japan). The excitation and emission slit widths were set at 5 nm.

2.2.6. Circular dichroism (CD) measurements

CD spectra were made on a MOS-450/AF-CD spectropolarimeter (Bio-Logic, France) in a 1.0-cm cell at room temperature. Bandwidth was 4 nm and scanning speed was 1 nm/2 s.

3. Results and discussion

3.1. Fluorescence analysis of BSA–MTZ binding characteristics

3.1.1. Fluorescence quenching

The interaction of MTZ with BSA was monitored by measuring the fluorescence quenching of BSA with increasing concentrations of MTZ. Fig. 1 shows the fluorescence quenching spectra of BSA induced by different concentrations of MTZ in phosphate buffer with pH 7.4 at the excitation wavelength of 278 nm. It is apparent that the fluorescence of BSA regularly decreased with the increasing concentration of MTZ, indicating that MTZ could interact with BSA and quench its intrinsic fluorescence.

Fluorescence quenching mainly takes place by two different mechanisms (dynamic and static quenching). In order to verify the mechanism, we plotted F_0/F against the concentration of MTZ as shown in Fig. 2. The fluorescence data was analyzed according to the Stern–Volmer equation [30,31]

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

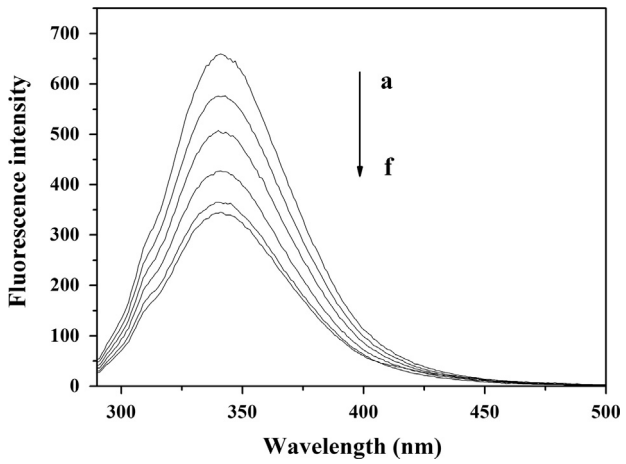

Fig. 1. Effect of MTZ on BSA fluorescence. Conditions: BSA: 3.0×10^{-7} mol L^{-1}; MTZ/($\times10^{-5}$ mol L^{-1}): (a) 0, (b) 1, (c) 2, (d) 3, (e) 4, (f) 5; pH 7.4; T=291 K.

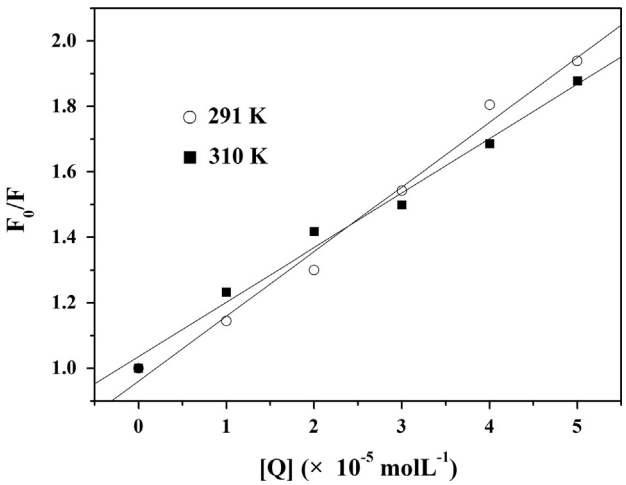

Fig. 2. Stern–Volmer plots for the quenching of BSA by MTZ at 291 and 310 K.