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The interaction between 4-aminoantipyrine and bovine serum albumin: Multiple spectroscopic and molecular docking investigations

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ABSTRACT

4-Aminoantipyrine (AAP) is widely used in the pharmaceutical industry, in biochemical experiments and in environmental monitoring. AAP as an aromatic pollutant in the environment poses a great threat to human health. To evaluate the toxicity of AAP at the protein level, the effects of AAP on bovine serum albumin (BSA) were investigated by multiple spectroscopic techniques and molecular modeling. After the inner filter effect was eliminated, the experimental results showed that AAP effectively quenched the intrinsic fluorescence of BSA via static quenching. The number of binding sites, the binding constant, the thermodynamic parameters and binding subdomain were measured, and indicated that AAP could spontaneously bind with BSA on subdomain IIIA through electrostatic forces. Molecular docking results revealed that AAP interacted with the Glu 488 and Glu 502 residues of BSA. Furthermore, the conformation of BSA was demonstrably changed in the presence of AAP. The skeletal structure of BSA loosened, exposing internal hydrophobic aromatic ring amino acids and peptide strands to the solution.

1. Introduction

Serum albumins are the major soluble proteins of the circulatory system (accounting for 50–60% of the total plasma protein) [1]. Serum albumins have many physiological functions [2]. The most important property of the abundant serum protein is that they serve as a transport vehicle for a variety of endogenous and exogenous compounds [3]. It has been shown that the distribution, free concentration and the metabolism of many biologically active compounds, such as metabolites, drugs and even some toxins, are correlated with their binding to serum albumin [4]. Furthermore, it has also been demonstrated that conformational changes of serum albumin can be caused by interactions with small molecule ligands, which may influence serum albumin's biological function as a carrier protein [5,6]. The interactions of serum albumin with ligands have attracted a great deal of interest for many years due to their application in a great variety of biological [7], pharmaceutical [8], toxicological [9] and cosmetic [10] systems. As the sequences of human serum albumin (HSA) and BSA are 76% similar, BSA is commonly substituted for HSA in experiments due to its availability and lower cost [11,12].

4-Aminoantipyrine (AAP, structure in the inset of Fig. 1) is a metabolite of aminophenazone and is an aromatic substance with analgesic, antipyretic and anti-inflammatory properties [13]. However, AAP usually produces side effects such as the risk of agranulocytosis [14]. Although AAP is scarcely ever administered as an analgesic because of side effects, as a raw material, it is mostly used to produce 4-aminoantipyrine derivatives, which have better biological activities [15,16]. In addition, it is used as a reagent for biochemical reactions producing peroxides or phenols [17,18] and can also be used to detect phenols in the environment [19]. Since AAP is widely used in the pharmaceutical industry, biochemical research and environmental monitoring, AAP has become an environmental pollutant.

The toxic effect of 4-aminoantipyrine on experimental animals has been reported [20]. AAP can reduce blood flow [21] and 13,14-dihydro-15-keto prostaglandin F2 alpha concentration [22] after it is infused into the blood. AAP can form stable complexes with heme [23]. The interaction of AAP with bovine hemoglobin has been studied in our laboratory and AAP has an obvious denaturing effect on bovine hemoglobin [24]. Considering the important physiological functions of serum albumin in circulatory system, it would be interesting to observe the effect of AAP on serum albumin. In the present work, we investigated the toxic effects of AAP on BSA under simulative physiological conditions by spectroscopic and molecular modeling methods. After considering the inner filter effect, we estimated the association constants, number of binding

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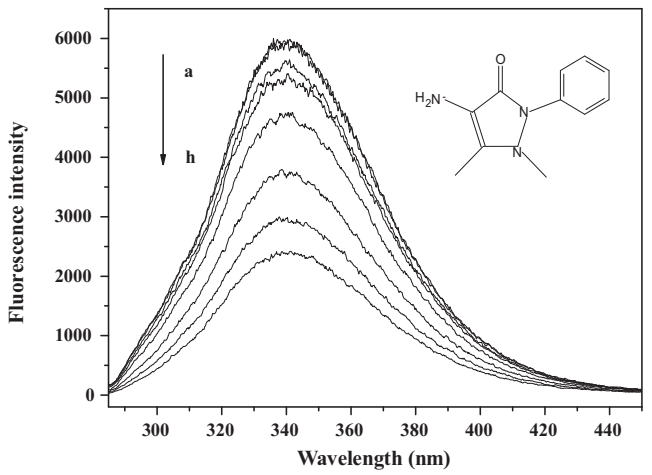

Fig. 1. The influence of AAP on the fluorescence emission spectra of BSA. *Conditions*: BSA – 1.0×10^{-6} mol L^{-1}; AAP – (a) 0 mol L^{-1}, (b) 1×10^{-6} mol L^{-1}, (c) 5×10^{-6} mol L^{-1}, (d) 1×10^{-5} mol L^{-1}, (e) 2×10^{-5} mol L^{-1}, (f) 4×10^{-5} mol L^{-1}, (g) 6×10^{-5} mol L^{-1}, (h) 8×10^{-5} mol L^{-1}; pH 7.4; T = 294 K.

sites, thermodynamic parameters, and binding force for the interaction of AAP with BSA. The specific binding site of AAP on BSA was investigated in detail. The effect of AAP on the microenvironment and conformation of BSA was also investigated. This study provides basic data for clarifying the binding mechanisms of AAP with serum albumin and is helpful for understanding its effect on protein function during its transportation in the blood and its toxicity in vivo.

2. Experimental

2.1. Reagents

Bovine serum albumin (BSA) was obtained from Sinopharm Chemical Reagent Co., Ltd. 4-Aminoantipyrine (AAP) was obtained from Tianjin Chemical Reagent Co. Ltd. AAP was dissolved with ultrapure water as a stock solution, 1.0×10^{-3} mol L^{-1}. Phenylbutazone (PB), flufenamic acid (FA), and digitoxin (Dig) were obtained from Tokyo Chemical Industry Co. Ltd. and were dissolved in ethanol to form a 1.0×10^{-3} mol L^{-1} solution, which was used to determine the binding sites of AAP on BSA. A 0.2 mol L^{-1} mixture of phosphate buffer (mixture of $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 12H_2O$, pH 7.4) was used to control the pH. 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 F-4600 fluorescence spectrophotometer (Hitachi, Japan) with a 1 cm cell. The excitation wavelength was 278 nm. The excitation and emission slit widths were set at 5 nm. Scan speed was 1200 nm/min. PMT (Photo Multiplier Tube) voltage was 680 V.

Synchronous fluorescence spectra of BSA in the absence and presence of 4-aminoantipyrine were measured ($\Delta\lambda$ = 15 nm, λ_{ex} = 265–320 nm and $\Delta\lambda$ = 60 nm, λ_{ex} = 250–310 nm, respectively). The excitation and emission slit widths were set at 5 nm. Scan speed was 1200 nm/min. PMT voltage was fixed at 680 V.

2.2.2. UV–vis absorption measurements

The absorption spectra were recorded on a double beam UV-2450 spectrophotometer (Shimadzu, Japan) equipped with 1.0 cm quartz cells. Slit width was set at 2.0 nm. The wavelength range was 310–200 nm.

2.2.3. Circular dichroism (CD) measurements

CD spectra were made on a J-810 Spectropolarimeter (Jasco, Tokyo, Japan) in a 1-cm cell at room temperature. Bandwidth was 1 nm and scanning speed was 200 nm/min.

2.2.4. Molecule docking investigation

Docking calculations were carried out using AutoDock 4.2. The structure of AAP was generated by Materials Studio 4.4. With the aid of AutoDock, the ligand root of AAP was detected and rotatable bonds were defined.

As the crystal structure of BSA is unavailable in Protein Data Bank [25], a homologous model structure was used for the docking studies with AAP. A BLAST search in the PDB with a BSA sequence [Swissprot sequence ALBU_BOVIN (P02769)] revealed 75% identity with HSA. The SAM_T06 server was used to obtain the model structure, which has been used for previous docking studies [26–28]. AutoDock used the local search to search for the optimum binding site of small molecules to the protein. To recognize the binding sites in BSA, blind docking was carried out, with the grid size set to 110, 110 and 110 along the *X*-, *Y*- and *Z*-axes with 0.375 Å grid spacing. The center of the grid was set to 35.604, 4.724 and −24.211 Å. Maximum number of iterations was 300 and maximum number of successes/failures in a row before changing rho was 4. The conformation with the lowest binding free energy was used for further analysis.

3. Results and discussion

3.1. Fluorescence measurements

Fluorescence has been widely used to investigate the interaction between ligands and proteins and can give some information about the quenching mechanism, binding constants and binding sites.

The inner filter effect (IFE) would affect fluorescence measurements [29]. To determine whether an IFE induced by the absorption of excitation and emission radiation is significant in this system, we checked the sum of the absorbance at 278 nm (excitation wavelength) and 340.8 nm (fluorescence peak) under different conditions. The differences in the sums could cause no more than 5% percentage error for each sample, so IFE was ignored here.

Changes of emission spectra can provide information about their structure and dynamics [30]. Fluorescence emission spectra of BSA with different AAP concentrations were recorded at room temperature (Fig. 1). It can be seen from Fig. 1 that the fluorescence intensity decreased with the increasing concentration of AAP. The fluorescence quenching of BSA by AAP indicated that AAP can bind to BSA and alter the structure of BSA.

3.2. The fluorescence quenching mechanism

Since the intrinsic fluorescence of BSA can be quenched by AAP, we further explored the quenching mechanism. Quenching mechanisms are usually divided into dynamic quenching and static quenching. Since higher temperature results in larger diffusion coefficients, the dynamic quenching constants will increase with increasing temperature. In contrast, increased temperature is likely to result in decreased stability of complexes, and thus lower the value of the static quenching constants [31]. The quenching mode was primarily described as dynamic. In order to test this view, we used the well-known Stern–Volmer equation [32]:

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