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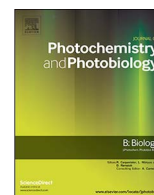

Elucidation of intermolecular interaction of bovine serum albumin with Fenhexamid: A biophysical prospect

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A B S T R A C T

Fenhexamid, as a hydroxyanilide, is widely applied to control Botrytis cinerea for protecting crops and fruits. But it could adversely affect human and animals health due to accumulation of residues in food production. Here, the affinity characteristics of fenhexamid on bovine serum albumin (BSA) was studied via a series of spectroscopic methods such as steady-state fluorescence spectroscopy, ultraviolet spectroscopy (UV), synchronous fluorescence spectroscopy (SFS), 3D fluorescence spectroscopy, and fourier transform infrared spectroscopy (FT-IR). The experimental results illustrated that the fluorescence quenching mechanism of BSA induced by fenhexamid was a static quenching. The binding constant (K_b) of fenhexamid with BSA was $2.399 \times 10^4\,M^{-1}$ at 298 K and the combination ratio was about 1:1. The competitive experiment demonstrated that fenhexamid was binding on the BSA at site II (subdomain IIIA), which was confirmed by the molecular docking studies. The negative values of thermodynamic parameter (ΔH^0, ΔS^0 and ΔG^0) revealed that the reaction of fenhexamid with BSA could proceed spontaneously, the van der Waals force and hydrogen bonding interaction conducted the main effect, and the binding process was enthalpy-driven. What's more, the 8-Anilino-1-naphthalenesulfonate (ANS) and sucrose binding studies were also performed and further verified the binding force between BSA and fenhexamid.

1. Introduction

Currently, study on the binding interactions of proteins with acute or chronic pesticides gradually becomes a topic interest among researchers [1–3]. These studies conducted a dominant effect on elucidating the binding mechanisms of pesticide with proteins at a molecular level, clarifying the toxic effect of pesticide to body, developing new compounds on an in-depth understanding their environmental compatibility and structural features [4,5]. Moreover, serum albumin is an ideal model for exploring the interaction on small molecules and protein in vitro.

Serum albumin, the most abundant soluble globular protein in the vertebrate blood, performs critical biological and physiological functions, and plays a dominant role in maintaining plasma oncotic pressure and adjusting the fluid distribution between body compartments [6,7]. Meanwhile, serum albumins have the ability to bind reversibly with multifarious endogeneous and exogeneous compounds such as fatty acids, hormones, nutrients and a variety of pharmaceuticals even toxic substances. Also, serum albumin represents the main determinant of drug disposition and efficacy as it increases the obvious solubility of hydrophobic drugs in the plasma [4,8,9]. It is generally recognized that serum albumins includes three domains (I - III), each domain has A and B sub-domains. The three domains were homologous but have different ligand-binding functions. Within each domain the first two loops, loops 1–2, 4–5, and 7–8, are grouped together as sub domains IA, IIA, and IIIA, respectively, and loops 3, 6, and 9 are called sub domains IB, IIB, and IIIB [10]. Among serum albumin, human serum albumin (HSA) and bovine serum albumin (BSA) are commonly studied as model albumin for assessing the intermolecular interaction of ligand with serum albumin. Both of them exhibit about 75.6% sequence homology and 76% tertiary structures similarity [4,11]. Fig. 1(a) shows the BSA structure clarifying three domains (I - III). BSA comprises 583 amino acids with 2 Trp and 20 Tyr residues and also contains the two most active binding sites, which are named as sites I and site II, respectively. The former positioned at sub-domain IIA and the latter is located at sub-domain IIIA. Here, BSA is used as a subject for our work due to its structural similarity with HSA, availability and affordability [12].

Fenhexamid (Fig. 1(b)), a hydroxyanilide, is used widely to control Botrytis cinerea (a common plant pathogenic fungus) for protecting some crops, many fruits and ornamental plants [13]. However, there is evidence that fenhexamid has been detected in fruits, vegetables and wine, and residue of fenhexamid is due to its stability and resistance to

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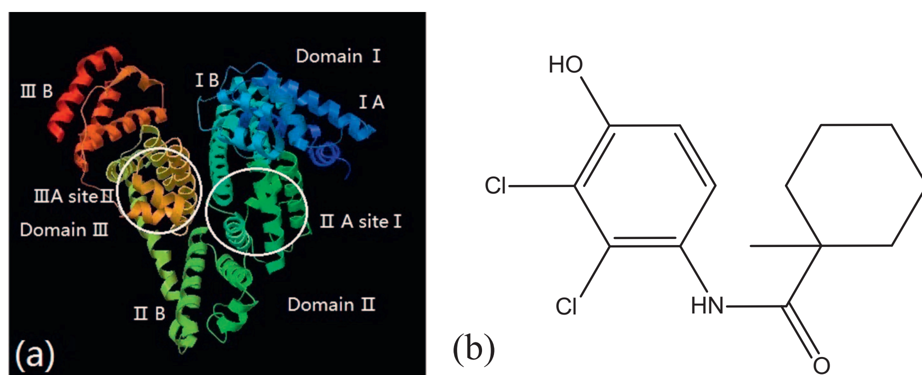

Fig. 1. (a) The structures of BSA (PDBID: 3V03) obtained from the Protein Data Bank (http://www.pdb.org/pdb/home/home.do). **(b)** The chemical structure of fenhexamid.

hydrolysis [14]. Fortunately, its acute hazard did not occur under normal uses [15]. While previous study present that fenhexamid have chronic toxicity for dogs (increase the morbidit of intracytoplasmic vacuoles in the adrenal cortex and decrease hemoglobin and hematocrit) [16]. In addition, fenhexamid could inhibit sterol biosynthesis in membranes by targeting 3-keto reductase in C4-demethylation [17]. Interestingly, 3-keto reductase is encoded by erg27 in fungus, which have homologs in HSD17B7 of humans [18]. Hence, it could adversely affect human and animals health due to accumulation of residues in food production. Currently, pesticides interaction with BSA is raising a considerable attention and arousing a wide interest in researchers due to its residues and toxic effect [19–21]. For all we know, there was no investigation on fenhexamid binding interaction with BSA.

Our work is aiming to explore the binding characteristics of fenhexamid on BSA including binding mechanism, binding affinity, and binding sites. We also attempt to determine the binding distance via FRET and further to explore the effect of fenhexamid on the secondary structure of BSA. To gain these information, we performs thoroughly and systematically an investigation on the fenhexamid–BSA interaction using fluorescence spectroscopy, SFS, 3D fluorescence spectroscopy, UV–vis absorption spectroscopy, FT-IR, and molecular modeling techniques.

2. Materials and Methods

2.1. Reagents and Solutions

Fenhexamid (≥98%) and sucrose (> 99%) were purchased from Shanghai Titan Scientific Co., Ltd. (Shanghai, China). Fatty acid-free BSA (≥98%) was purchased from Aladdin Industrial Corporation (Shanghai, China). Tris (hydroxymethyl) aminomethane (Tris) (≥99%) was purchased from Aladdin Industrial Corporation (Shanghai, China). Phenylbutazone (≥99.9%) was purchased from Hubei Hengshuo Chemical Co., Ltd. (Hubei, China). Ibuprofen (≥99.9%) was supplied from Zhejiang University of Technology (Zhejiang, China). Ammonium 8-Anilino-1-naphthalenesulfonate (ANS-NH4) (> 95.0%) was purchased from Tokyo Chemical Industry Co., Ltd. Ethanol (> 99.7%) was purchased from Anhui Ante Food Co., Ltd.

BSA stock solution with the concentration of 1.5 μM and 100 μM, respectively, was prepared in 0.050 M Tris–HCl buffer solution containing 0.15 M NaCl (pH 7.40). The sucrose (3 M) and ANS (3×10^{-3} M) solutions were prepared in water. The stock solutions of fenhexamid, phenylbutazone and ibuprofen were separately prepared in anhydrous ethanol with the concentration of 3×10^{-3} M. All stock solutions were stored in the dark at 4 °C.

2.2. UV Spectra Measurements

Absorption spectra measurements were performed on a UV-1601 spectrophotometer (Shimadzu, Japan) with 1 cm cell. All BSA solutions (1.5 μM) with and without fenhexamid (from 0 to 18 μM) were determined at 298 K with recording from 200 to 500 nm and the corresponding fenhexamid solutions were used for blank correction.

2.3. Fluorescence Spectra Measurements

2.3.1. Steady Fluorescence Spectra Measurements

All fluorescence spectra measurements were carried out on a F97pro Spectrofluorimeter with a 1 cm cell (Shanghai Leng Guang Industrial Co., Ltd., China).

For steady fluorescence studies, all BSA solutions (1.5 μM) with increment of fenhexamid (from 0 to 1.8 μM) were determined at three temperatures (298, 303 and 308 K) with 5/10 nm slit widths. The excitation wavelength was set at 285 nm, while the emission wavelength was set in the range from 300 to 500 nm. Here, the inner-filter effect (IFE) can be ignored, because the totality of absorbance of mixture solution of fenhexamid (1.8 μM) at the excitation and emission wavelength of BSA was < 0.01.

To illustrate the possible quenching mechanism between BSA and fenhexamid, the fluorescence quenching experiments were carried out at 298, 303 and 308 K and the quenching constant were obtained from Stern–Volmer equation [22]:

$$\frac{F_0}{F} = 1 + K_{SV}[Q] = 1 + k_q \cdot \tau_0 [Q] \tag{1}$$

where $[Q]$ means the concentration of fenhexamid. K_{SV} is the quenching constant. k_q is the quenching rate constant of protein. τ_0 is the average fluorescence lifetime of BSA without fenhexamid and its value is 6 ns [23]. F and F_0 denote the fluorescence intensities with or without fenhexamid, respectively.

The binding parameters for the BSA–fenhexamid complex were calculated at 298, 303 and 308 K from fluorescence quenching data using modified Stern–Volmer equation [22]:

$$log\frac{F_0 - F}{F} = log \ \ K_b + n \ \ log[Q] \tag{2}$$

where K_b is the binding constants and n is the binding site number. The values for K_b and n can be obtained from the intercept and slope.

Thermodynamic parameters such as enthalpy (ΔH^0), entropy (ΔS^0) and free energy change (ΔG^0) for the binding interaction of fenhexamid with BSA were obtained by conducting the fluorescence experiments at 298, 303 and 308 K. The ΔH^0 and ΔS^0 be obtained through from Eq. (3), and the ΔG^0 can be obtained through the eq. (4):

$$ln \ \ K_b = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \tag{3}$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{4}$$

where K_b is the binding constants and R is gas constant ($R = 8.314$).

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