Journal of Photochemistry & Photobiology, B: Biology 173 (2017) 589–597

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

Characterizing the binding interaction of fungicide boscalid with bovine serum albumin (BSA): A spectroscopic study in combination with molecular docking approach

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ARTICLE INFO

Keywords:
Boscalid
Bovine serum album
Interaction
Spectroscopy
Molecular docking

ABSTRACT

Boscalid, a carboxamide fungicide, is used in the treatment of grey mould and powdery mildew, widely applied to a variety of crops and fruits such as rice, wheat, grapes and pears. It will become a potential risk for health due to its widely application and residue in crops and fruits. In this study, the binding interaction between boscalid and bovine serum albumin (BSA) was characterized using steady-state fluorescence spectroscopy, ultraviolet spectroscopy (UV), synchronous fluorescence spectroscopy, 3D fluorescence spectroscopy, Fourier transform infrared spectroscopy (FT-IR) and molecular docking to ascertain the store, transport and distribution of boscalid in vivo. The experimental results indicated that the fluorescence of BSA was quenched due to the forming the static boscalid–BSA complex with the binding constant of $4.57 \times 10^3\ M^{-1}$ at 298 K and boscalid bound on the subdomain III A (site II) of BSA through van der Waals force and hydrogen bonding interaction. The binding process of boscalid with BSA was spontaneous and enthalpy-driven process based on $\Delta G^0 < 0$ and $|\Delta H^0| > T|\Delta S^0|$ over the studied temperature range. Meanwhile, the obvious change in the conformation of boscalid was observed while the slight change in the conformation of BSA when binding boscalid to the BSA, implying that the flexibility of boscalid contributes to increasing the stability of the boscalid–BSA complex.

1. Introduction

Boscalid (Fig. 1A) belongs to the carboxamide fungicides, registered for use in the US in 2003 [1]. Its mode of action is inhibiting the succinate dehydrogenase [2]. Boscalid was used in the treatment of grey mould and powdery mildew and widely applied to a variety of crops and fruits such as rice, wheat, grapes and pears [3]. Thus, boscalid is relatively important for food protection to gain the suitable quality and quantity production of crops and provide a considerable economic return. However, some studies for boscalid indicate that it is present in surface water and has the marginal risk to leach to groundwater [4,5]. When it is widely sprayed on agricultural land, it will move from the fields after application to the air, soil and water environment, which will become a potential health risk not only for farm workers, but also for residents and children. Generally, acute and chronic pesticide poisoning often experience the storage and transportation of plasma in vivo. However, there is little information on evaluating the toxicity of boscalid rarely in vivo. To the best of our knowledge, up until now, the study of intermolecular interactions of SA with boscalid has not been reported. Therefore, the investigation of the binding interaction of serum albumin (SA) with boscalid can help us know transportation, distribution and elimination of boscalid in vivo and further understand the toxicological mechanisms of boscalid at a molecular level and design new chemicals with low toxicity functions.

SA is the most prominent protein in plasma, where it reaches a high concentration (ca. 0.6 mM). It can carry several endogenous and exogenous compounds to specific targets and serves as a transporter for drugs, metabolites, nutrients, and other molecules [6]. In addition, SA plays a leading role in maintaining colloid osmotic pressure. Among serum albumins, Human serum albumin (HSA) and bovine serum albumin (BSA) are widely used as model protein in life sciences, chemistry and clinical medicine. The similarity of BSA and HSA structure is very high, according to reference [7], its homology is up to 75.6%. They are both like heart-shaped, composed of three domains I, II and III. Each domain has A and B sub-domains, forming a hydrophobic cavity [8,9]. However, there are some differences between them. HSA contains a single tryptophan (Trp-214) [10], while BSA has two tryptophan residues (Trp-212, Trp-134). Trp-212 is buried in a hydrophobic pocket of the BSA (IIA sub-domain), and Trp-134 is located on the surface of the BSA (IB sub-domain) (Fig. 1B) [11]. Recently, BSA is often used as a

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http://dx.doi.org/10.1016/j.jphotobiol.2017.06.037
Received 20 January 2017; Received in revised form 14 June 2017; Accepted 24 June 2017
Available online 30 June 2017

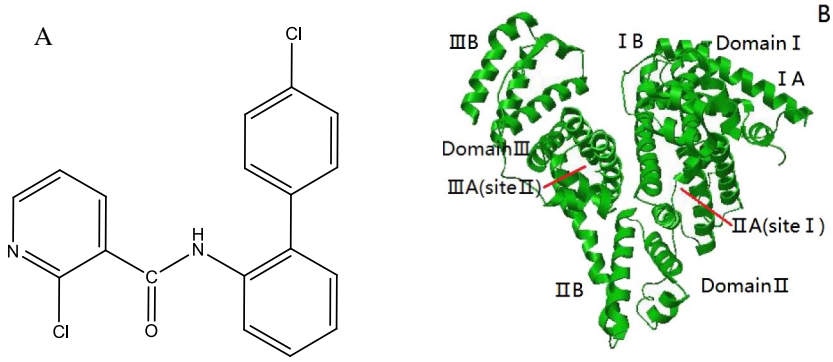

Fig. 1. (A) The chemical structure of boscalid. (B) The structures of BSA (PDBID: 3V03) obtained from the Protein Data Bank (http://www.pdb.org/pdb/home/home.do).

model molecule for evaluating the binding interaction of ligand with SA due to its structural similarity with HSA, low cost, ready availability and affordability [12,13].

In order to elaborate the binding interaction between boscalid and BSA, we employed the fluorescence spectroscopic titration, ultraviolet spectroscopy (UV), synchronous fluorescence spectroscopy, 3D fluorescence spectroscopy, FT-IR and molecular docking for obtaining the important information of binding constant (K_b) of boscalid-BSA complex, the specific binding site of boscalid on BSA, and effect of boscalid binding to BSA on their conformation, among others. It can be expected that this study will provide basic data for further elucidating the binding mechanisms of boscalid with SA at a molecular level, clarifying the toxicity of boscalid to body, and thus reinforcing the supervision of food safety.

2. Materials and Methods

2.1. Reagents and Solutions

Fatty acid-free BSA was purchased from Shanghai Shenhang Biotechnology Co., Ltd. (Shanghai, China), which was not purified before used. Boscalid (≥ 98%) was purchased from Shanghai Titan Scientific Co., Ltd. (Shanghai china). Tris(hydroxymethyl) aminomethane (Tris) (≥ 99%) was purchased from Bobo Biotechnology Co., Ltd. (Shanghai, China). HCl (≥ 36%) was purchased from Juhua Reagent Co., Ltd. (Quzhou, China). Sodium chloride (NaCl) was purchased from Zhongxing Chemical Reagent Co., Ltd. (Zhejiang, China). Phenylbutazone (≥ 99.9%) was purchased from Hubei Hengshuo Chemical Co., Ltd. (Hubei, China). Diazepam (≥ 99.9%) was supplied from Zhejiang University of Technology (Zhejiang, China).

The Tris (0.050 M) was adjusted to pH = 7.40 by 36% HCl. BSA (1.5 μM) and NaCl (0.15 M) were dissolved in Tris–HCl buffer solution (pH = 7.40) to prepare BSA stock solution. The stock solution of boscalid (3×10^{-3} M), phenylbutazone (3×10^{-3} M) and diazepam (3×10^{-3} M) were prepared in anhydrous ethanol, respectively. All stock solutions were kept in the dark at 4 °C. And, all the fresh working solutions were prepared daily from the stock solutions by proper dilution.

All other chemicals and solvents were of analytical reagent grade. The double-distilled water was used throughout the experiments.

2.2. Fluorescence Spectra Measurements

Fluorescence spectra of all BSA solutions in the absence and presence of boscalid under simulated physiological conditions (pH = 7.4) were recorded on a F97pro Spectrofluorimeter with a 1 cm quartz cell (Shanghai Leng Guang Industrial Co., Ltd., Shanghai, China) from 200 to 500 nm with 5/10 nm slit widths at λ_{ex} = 285 nm at three temperatures (298, 303 and 308 K). Accurately transferred 3.0 mL of BSA solution to a quartz cell and then titrated using the stock solutions of boscalid with incremental 1.0 μL. Because the accumulative volume of the stock solution of boscalid was < 6.0 μL, the effect on the net volume increase could be ignored.

The inner-filter effect (IFE) caused by the absorption of the exciting (primary) as well as the fluorescent light (secondary) by the fluorophore itself, or by another component of the sample, which will reduce the fluorescence intensity of fluorophore. However, the sum of the absorbance of boscalid (< 2.0 μM) and BSA (1.5 μM) at 343 nm was < 0.002. The inner filter effect can be neglected according to the correction equation [14].

2.3. UV Spectra Measurements

UV spectra of all BSA solutions in the absence and presence of boscalid were recorded on a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) with 1 cm quartz cells from 190 to 500 nm at room temperature. The corresponding boscalid solution was used for blank correction.

2.4. Synchronous Fluorescence Measurements

The synchronous fluorescence measurements of all BSA solutions in the absence and presence of boscalid were carried out on a F97Pro Spectrofluorimeter with 10/10 nm slit widths with a 1 cm quartz cell (Shanghai LengGuang Industrial Co., Ltd., Shanghai, China) by scanning two intervals of $\Delta\lambda$ = 15 nm and $\Delta\lambda$ = 60 nm ($\Delta\lambda = \lambda_{em} - \lambda_{ex}$) at 298 K, which characterizing the properties of Tyr and Trp residues, respectively. The excitation wavelength range was set from 240 to 340 nm.

2.5. Three-dimensional Fluorescence Measurements

The three-dimensional fluorescence measurements of all BSA solutions were performed using F97 Pro Spectrofluorimeter with a 1 cm quartz cell (Shanghai LengGuang Industrial Co., Ltd., Shanghai, China) with 10/10 nm slit widths at room temperature. The number of scanning and the increment were set at 76 and 2 nm per scanning, respectively. The excitation wavelength range was set from 200 to 350 nm and the emission wavelength range was recorded from 200 to 600 nm.

2.6. FT-IR Measurements

FT-IR spectra of all BSA solutions in the absence and presence of boscalid were measured on Nicolet 5700 FT-IR spectrophotometer (Thermo Nicolet, Waltham, America) equipped with a Ge/KBr beamsplitter and a DTGS detector. The sample solutions were placed between ZnS windows. All spectra were estimated from 4000 to 1000 cm^{-1} with 100 scans at a resolution of 4 cm^{-1}. The FT-IR spectra of free BSA and its boscalid complex were analyzed using the self-deconvolution with second derivative resolution enhancement implemented in nicolet omnic software to characterize each peak position for overlap peak. Finally, the data files were imported and treated using a digital curve-