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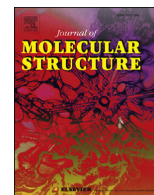

Binding of carbendazim to bovine serum albumin: Insights from experimental and molecular modeling studies

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ABSTRACT

Carbendazim (CBZ) is a widely used benzimidazole fungicide in agriculture to control a wide range of fruit and vegetable pathogens, which may lead to potential health hazards. To evaluate the potential toxicity of CBZ, the binding mechanism of bovine serum albumin (BSA) with CBZ was investigated by the fluorescence quenching technology, UV absorbance spectra, circular dichroism (CD), and molecular modeling. The fluorescence titration and UV absorbance spectra revealed that the fluorescence quenching mechanism of BSA by CBZ was a combined quenching process. In addition, the studies of CD spectra suggested that the binding of CBZ to BSA changed the secondary structure of protein. Furthermore, the thermodynamic functions of enthalpy change (ΔH^0) and entropy change (ΔS^0) for the reaction were calculated to be 24.87 kJ mol^{-1} and 162.95 J mol^{-1} K^{-1} according to Van't Hoff equation. These data suggested that hydrophobic interaction play a major role in the binding of CBZ to BSA, which was in good agreement with the result of molecular modeling study.

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1. Introduction

Carbendazim (CBZ, structure shown in Fig. 1) is a systemic fungicide of the benzimidazole (BZ) family, which has been widely used in agriculture for pre-and post-harvest treatment for the control of a wide range of fruit and vegetable pathogens in China and other countries in the world [1–4]. Generally, some benzimidazole fungicide rapidly degrade to carbendazim in water solution, such as benomyl and thiophanate [5,6]. It has been reported that carbendazim could disrupt various aspects of reproductive system, cause germ cell apoptosis, embryo toxicity, or teratogenesis in rats or human [7,8].

Serum albumins, including Bovine serum albumin (BSA) and Human serum albumin (HSA), are the main constituent of the blood plasma. It plays an important role in the binding and transport of various ligands to the target sites. The ligands are always fatty acids, dyes, drugs and pesticide residues [9–12]. It has been shown that the distribution, free concentration and the metabolism of the ligands can be influenced by their binding to protein in the bloodstream. Consequently, binding of small molecules to protein is imperative importance to many biological processes. While most of these reports focused on the interactions between SA and many drugs [13–16], the binding of benzimidazole pesticides to proteins has been seldom reported previously. The binding of pesticides to serum albumin changes not only the effectiveness and action of pesticides but also the activity of serum albumins, they may produce toxic effects to the protein which will finally affect the biological function of protein. BSA and HSA display approximately 76% sequence homology which have usually been used as model proteins to investigate the interaction between bioactive component and protein. Therefore BSA was usually selected as our protein model because of its medical importance, low cost and ready availability [17,18]. BSA consists of three linearly arranged domains (I–III): I (residues1-195), II (residues 196–383), III (residues 384–585), and each containing two subdomains (A and B). There are two tryptophans (Trp 134 and Trp 213) in BSA: Trp 134 is located on the surface of the molecule and Trp 213 resides in the hydrophobic pocket of sub-domain IIA [19,20]. The binding sites of BSA for ligands are often located in hydrophobic cavities of subdomains IIA and IIIA which are called sites I and II, respectively [21].

Since the widespread use of CBZ in agriculture may lead to potential health hazards (because of their toxicity or carcinogenicity), it is necessary to investigate the interaction of CBZ with protein. Pesticides-protein interaction experiments have a great

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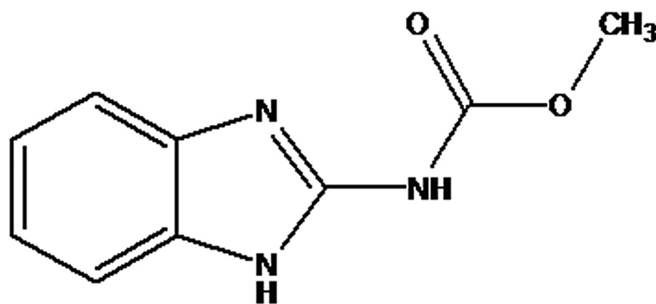

Fig. 1. The chemical structure of CBZ.

significance in discovering the transportation and distribution of pesticides in vivo.

In this paper, the interaction between CBZ and BSA has been studied using fluorescence, UV–vis absorption, circular dichroism (CD) spectra and molecular modeling methods. Binding parameters, such as the binding constant, number of binding sites, and binding force were obtained from the fluorescence data. These are the first spectroscopic results on CBZ-BSA interaction. In addition, the effect of CBZ on the structure of BSA was also examined.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA) was obtained from Sigma Chemical Company. All BSA solutions were prepared in pH 7.40 buffer solution, and BSA stock solution was kept in the dark at 275 K. Carbendazim (CBZ) (analytical grade) was purchased from Aladin Ltd. (Shanghai China). NaCl (analytical grade, 1.0 mol L^{-1}) solution was used to maintain the ion strength at 0.1. The buffer (pH 7.40) consists of tris (0.2 mol L^{-1}) and HCl (0.1 mol L^{-1}). The pH was checked with a suitably standardized pH meter. Distilled water was used in the experiments.

2.2. Apparatus and methods

All fluorescence spectra were recorded on a RF-5301PC Spectrofluoro-photometer (Shimadzu, Japan). The excitation and emission slit widths were both 5 nm. The excitation wavelength was 280 nm, and the emission wavelengths were red at 300–495 nm.

Fluorescence titration experiments: 2.0 ml solution containing appropriate concentration of BSA was titrated manually by successive addition of a 1.0×10^{-3} mol L^{-1} ethanol stock solution of CBZ (to give a final concentration of 7.4, 14.7, 22.0, 29.1, 36.1, 43.0, 50.0, 56.6 and 63.0×10^{-6} mol L^{-1}) with trace syringes, and the fluorescence intensity was measured (excitation at 280 nm and emission at 338 nm). All experiments were measured at different temperature (296, 303 and 310 K). The temperature of sample was kept by recycled water throughout the experiment.

The UV absorbance spectra were recorded using a TU-1901 UV–vis Spectrophotometer with a 1 cm quartz cell (Beijing Purkinje General Instrument Co., Ltd., Beijing, China).

Circular dichroism was made on a Jasco-20 automatic recording spectropolarimeter (Japan), using a 2 mm cell at 296 K. The spectra were recorded in the range of 200–300 nm. The results are expressed as molar ellipticity ([θ]) in deg cm^2 $dmol^{-1}$. The α-helical content of HSA was calculated from the [θ] value at 208 nm using the equation: $\alpha\%\text{helix} = \{(-[\theta]_{208}-4000)/(33000-4000)\} \times 100$ as described by Lu et al. [22].

Molecular modeling was investigated through SGI FUEL WORKSTATION. The crystal structure of BSA was taken from the Brookhaven Protein Data Bank (entry code 3V03). The potential of the 3-D structure of BSA was assigned according to the Amber 4.2 force field with Kollman-all-atom charges. The initial structures of CBZ were generated by molecular modeling soft-ware SYBYL 6.9 [23]. The geometries of these compounds were subsequently optimized using the Tripos force field with Gasteiger-Marsili charges. FlexX program was applied to calculate the possible conformation of the ligands that bind to the protein.

3. Results and discussion

3.1. The mechanism of fluorescence quenching

Fluorescence quenching technique was applied to investigate whether CBZ interact with BSA. It is a powerful method to study interactions of several substances with protein which can reveal the accessibility of quenchers to albumin's fluorophore groups [24–26]. Fig. 2 shows the fluorescence emission spectra of BSA with varying concentration of CBZ. BSA has a strong fluorescence emission with a peak at 340 nm at λ_{ex} 280 nm, while CBZ was almost non-fluorescent at λ_{ex} 280 nm. It can be seen that the fluorescence intensity of BSA decreased regularly with increasing concentration of CBZ. In addition, the reduction of the fluorescence intensity was calculated to be 62.7% at the highest CBZ concentration. Furthermore, there was a blue shift (from 340 to 331 nm) of emission with the addition of CBZ. These results suggest that the binding of CBZ to BSA quenches the intrinsic fluorescence of BSA and affects the conformation of protein.

Fluorescence quenching can be classified as static quenching and dynamic quenching which can be distinguished by their differing dependence on temperature.

For dynamic quenching, the quenching constants increase with increasing temperature, while the quenching constants decrease with increasing temperature for static quenching [25]. The Stern-Volmer Equation (1) is often applied to recognize the quenching mechanism:

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

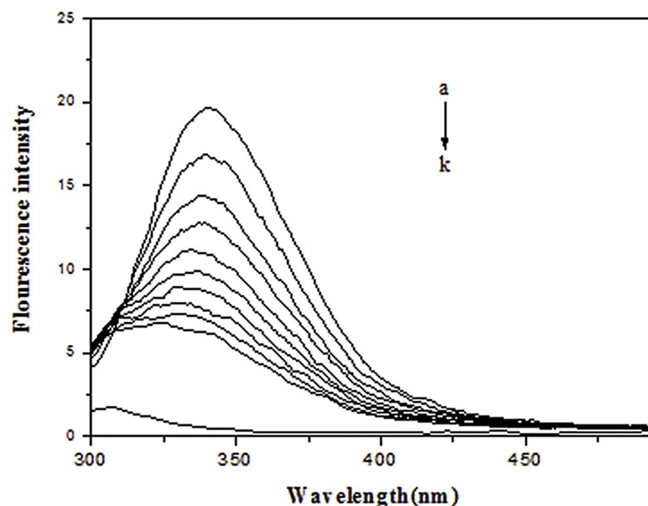

Fig. 2. The fluorescence emission spectra of CBZ-BSA system at excited 280 nm: (a) 3.0×10^{-6} mol L^{-1} BSA; (b–j) 3.0×10^{-6} mol L^{-1} BSA in the presence of 7.4, 14.7, 22.0, 29.1, 36.1, 43.0, 50.0, 56.6 and 63.0×10^{-6} mol L^{-1} CBZ, respectively; (k) 7.4×10^{-6} mol L^{-1} CBZ. T = 296 K, pH = 7.4.

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