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Interaction of prometryn to human serum albumin: Insights from spectroscopic and molecular docking studies

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ABSTRACT

Prometryn possesses much potential hazard to environment because of its chemical stability and biological toxicity. Here, the binding properties of prometryn with human serum albumin (HSA) and the protein structural changes were determined under simulative physiological conditions (pH 7.4) by multispectroscopic methods including fluorescence, UV–vis absorption, Fourier transform infrared (FT-IR) and circular dichroism (CD) spectroscopy, coupled with molecular modeling technique. The result of fluorescence titration suggested that the fluorescence quenching of HSA by prometryn was considered as a static quenching procedure. The negative enthalpy change (ΔH°) and positive entropy change (ΔS°) values indicated that the binding process was governed mainly by hydrophobic interactions and hydrogen bonds. The site marker displacement experiments suggested the location of prometryn binding to HSA was Sudlow's site I in subdomain IIA. Furthermore, molecular docking studies revealed prometryn can bind in the large hydrophobic activity of subdomain IIA. Analysis of UV–vis absorption, synchronous fluorescence, CD and FT-IR spectra demonstrated that the addition of prometryn resulted in rearrangement and conformational alteration of HSA with reduction in α-helix and increases in β-sheet, β-turn and random coil structures. This work provided reasonable model helping us further understand the transportation, distribution and toxicity effect of prometryn when it spreads into human blood serum.

1. Introduction

Pesticides, kinds of chemical reagents which are widely used in agriculture, have played an important role in minimizing weed management costs, killing insects and improving crop production. Many pesticides, including the herbicides, are highly toxic to terraneous and aquatic lives due to their great consumption in agricultural industry, and its uncontrolled or improper uses can result in adverse impact on food safety and human health [1,2]. Triazines herbicides have been extensively used as selective emergence herbicides in the control of weeds on agriculture in the last three decades, but triazines and their degradation products are very toxic and highly resistant which can survive many years in the soil, water and organisms. Therefore, they are considered as one of the most important classes of chemical pollutants [3]. It has been demonstrated that 1,3,5-triazines have mutagenic and sometimes pathogenic effects on living organisms [4].

Prometryn [2-(methylthio)-4,6-bis(isopropylamino)-s-triazine] (Fig. 1A), which belongs to the 1,3,5-triazine family, is registered for use in cotton, celery, pigeon pea, and recommended for control of broad-leaf weeds and grasses in vegetable crops [5,6]. However, prometryn possesses much potential hazard to environment because of its chemical stability, biological toxicity and being easy to gather in organisms, it may induce acute and chronic toxicity to human body through food chain. The "positive list system" was carried out in Japan since 2006, which sets strict maximum residue limit standards of prometryn in more than 100 kinds of food, and the value of maximum residue limit for most of the food is 0.05 mg/kg. Therefore, it is necessary to pay attention to the potential toxicity of prometryn.

Human serum albumin (HSA), the most abundant protein in human plasma, plays important roles in the transport and distribution of a large number of endogenous and exogenous ligands, including metabolites, fatty acids, amino acids and drugs [7,8]. HSA is a heart-shaped tridomain protein consists of a single poly-peptide chain with 585 amino acid residues, organized into three domains with each domain consisting of two identical subdomains A and B stabilized by seventeen disulfide bridges [9,10]. It has two well-known ligand binding sites, site I and site II, which are corresponded to the subdomains IIA and IIIA, respectively [11]. Most of the ligands are known to combine with HSA to form a complex at site I, and only a few at site II [12]. The study on the interaction mode of small molecules with serum albumin from qualitative and quantitative point of view is quite important because the distribution and metabolism of all kinds of small molecules in organisms are correlated with their binding affinities toward serum albumin in blood [13]. Binding of pesticides to

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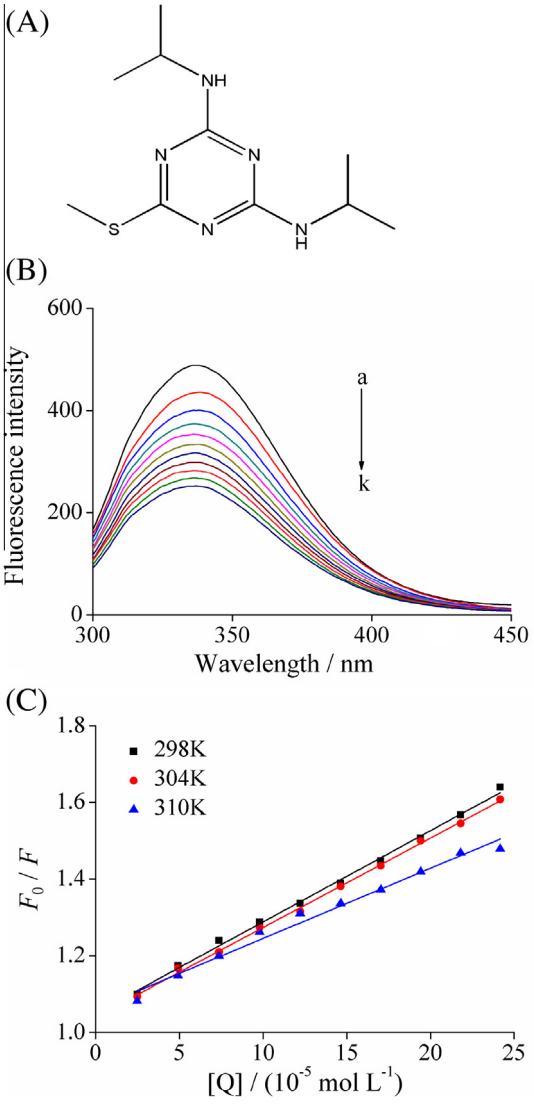

Fig. 1. (A) Molecular structure of prometryn. (B) Effect of prometryn on the fluorescence spectra of HSA (pH 7.4, T = 298 K, λ_{ex} = 280 nm, λ_{em} = 338 nm). c(HSA) = 1.5 × 10^{-6} mol L^{-1}; c(prometryn) = 0, 2.47, 4.92, 7.37, 9.80, 12.22, 14.64, 17.04, 19.42, 21.80, and 24.17 × 10^{-5} mol L^{-1} for curves a → k, respectively. (C) The Stern–Volmer plots for the fluorescence quenching of HSA by prometryn at different temperatures.

plasma proteins has toxicological importance, since the degree and time of action in the body affect duration and intensity of their effects [14].

The aim of this work was to explore the binding properties including binding mechanism, binding constant and binding sites of prometryn binding to HSA and the effect of the complexation on the protein structure by fluorescence, UV–vis absorption, circular dichroism (CD), and Fourier transform infrared (FT-IR) spectroscopy along with molecular modeling. The present work is expected to provide some insights into the transporting, distribution and toxicity effect of prometryn in body.

2. Materials and methods

2.1. Materials

HSA (fatty-acid free) was purchased from Sigma Chemical Company (St. Louis, USA). The HSA stock solutions were prepared by dissolving its crystals of 33.3 mg in 0.05 mol L^{-1} Tris–HCl buffer of pH 7.4 containing 0.10 mol L^{-1} NaCl to form a 1.0 × 10^{-4} - mol L^{-1} stock solution and then diluted to the required concentrations with the buffer. Prometryn (purity ⩾ 99.2%) was obtained from Sigma–Aldrich Laborchemikalien GmbH, and its stock solution (1.06 × 10^{-2} mol L^{-1}) was prepared in anhydrous methanol. All other chemicals were of analytical reagent grade, and ultrapure water was used throughout the experiment. All stock solutions were stored at 0–4 °C.

2.2. Apparatus and methods

2.2.1. Fluorescence measurements

Fluorescence measurements were performed with a Hitachi spectrofluorimeter Model F-7000 (Hitachi, Japan) equipped with a 150 W xenon lamp and 1.0 cm quartz cuvette and a thermostat bath. A 3.0 mL solution containing 1.5 × 10^{-6} mol L^{-1} HSA was added to the quartz cuvette, and then titrated by successive addition of a 1.06 × 10^{-2} mol L^{-1} of prometryn stock solution using a micropipette (to give a final concentration of 2.417 × 10^{-4} mol L^{-1}). These well-mixed solutions were stood for 3 min to equilibrate, and then the fluorescence spectra were measured at three temperatures (298, 304, and 310 K) with the widths of both the excitation and emission slits set at 2.5 nm. The exciting wavelength was 280 nm, and fluorescence emission spectra were recorded in the range of 300–450 nm. In this work, all fluorescence intensities were corrected for the absorption of excited light and the re-absorption of emitted light. The following formula was used to correct the inner-filter effect [15]:

$$F_c = F_m e^{(A_1+A_2)/2} \quad (1)$$

where F_c and F_m are the corrected and measured fluorescence, respectively. A_1 and A_2 are the absorbance of prometryn at excitation and emission wavelengths, respectively. The intensity of fluorescence used in this paper was the corrected fluorescence intensity.

Synchronous fluorescence spectra of HSA in the absence and presence of the increasing amount of prometryn were recorded from 260 to 340 nm by setting the excitation and emission wavelength interval ($\Delta\lambda$) at 15 and 60 nm.

The competitive experiments were performed to identify the binding site of prometryn on HSA using the fluorescence titration method in the presence of different site markers viz, Eosin Y, ibuprofen and digitoxin for sites I, II and III, respectively. The concentrations of HSA and the site markers were kept at 1.5 × 10^{-6} mol L^{-1}, and prometryn was then gradually added to the Eosin Y–HSA, ibuprofen–HSA or digitoxin–HSA mixtures. The fluorescence spectra were recorded in the range of 300–450 nm at 298 K upon excitation at 280 nm.

2.2.2. UV–vis absorption measurements

UV–vis absorption spectra were recorded on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Japan) and a quartz cell of 1.0 cm was used for the measurements. The absorption spectra of HSA in the absence and presence of prometryn, and the absorption spectra of corresponding concentration of prometryn solution were measured over a wavelength range of 200–320 nm in pH 7.4 Tris–HCl buffer at room temperature.

2.2.3. CD measurements

CD measurements of HSA in the presence and absence of prometryn were made in the UV-region (200–250 nm) under constant nitrogen flush on a Bio-Logic MOS 450 CD spectrometer with a cell of 1.0 mm path length at room temperature. The concentration of HSA was kept at 2.0 × 10^{-6} mol L^{-1}, and molar ratios of prometryn