Journal of Luminescence 130 (2010) 2013–2021

Contents lists available at ScienceDirect

Journal of Luminescence

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

Determining the binding affinity and binding site of bensulfuron-methyl to human serum albumin by quenching of the intrinsic tryptophan fluorescence

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ARTICLE INFO

Article history:
Received 26 June 2009
Received in revised form
13 May 2010
Accepted 18 May 2010
Available online 26 May 2010

Keywords:
Bensulfuron-methyl
Human serum albumin
Fluorescence spectroscopy
Circular dichroism
Molecular modeling
Ligand binding

ABSTRACT

Bensulfuron-methyl (BM) is a highly active sulfonylurea herbicide for use on paddy rice. Steady state fluorescence, UV/vis absorption, circular dichroism (CD), time-resolved fluorescence and molecular modeling methods have been exploited to determine the binding affinity and binding site of BM to human serum albumin (HSA). From the synchronous fluorescence, UV/vis, CD and three-dimensional fluorescence spectra, it was evident that the interaction between BM and HSA induced a conformational change in the protein. Steady state and time-resolved fluorescence data illustrates that the fluorescence quenching of HSA by BM was the formation of HSA–BM complex at 1:1 molar ratio. Site marker competitive experiments demonstrated that the binding of BM to HSA primarily took place in subdomain IIIA (Sudlow's site II), this corroborates the hydrophobic probe ANS displacement and molecular modeling results. Thermodynamic analysis displays hydrophobic, electrostatic and hydrogen bonds interactions are the major acting forces in stabilizing the HSA–BM complex.

1. Introduction

Sulfonylurea herbicides were first introduced in 1982 by DuPont agricultural products and have been used successfully for weed control in crops. Among sulfonylurea herbicides, bensulfuron-methyl (2-(4,6-dimethoxypyrimidin-2-carbamoyl-sulfamoyl)-*o*-toluic acid methyl ester, structure shown in Fig. 1 is widely utilized to control weeds in paddy soils in USA, Europe, China, Japan and other regions of the world [1]. It is a broad spectrum product for pre-emergence or early post-emergence control of most broad-leaved grasses and sedges in transplanted or direct seeding paddy rice. Due to its high herbicidal activity and good crop selectivity, bensulfuron-methyl (BM) is typically applied at rates $< 100\ g\ ha^{-1}$ [1]. The BM inhibits acetolactate synthase, which catalyzes the first common reaction in the biosynthesis of branched chain amino acids viz. valine, leucine and isoleucine, and thus inhibiting cell division [2]. However, the widespread application of BM has led to concerns over contamination of surface and groundwater because it can enter ecosystems by sprays, drift, surface runoff, accidental spills or irrigation with contaminated water [3]. In addition, the low volatility and photodegradation of BM in natural condition result in the long persistence of this herbicide in the environment (over 100 days). Recently, a limit value of 0.05 ppm was set for sulfonylurea herbicides in drinking water and fruits by the Environmental Protection Agency (EPA) [4]. Also, the European Union indicates $0.1\ \mu g\ L^{-1}$ as the maximum concentration for a single pesticide in water for human consumption [5]. Many articles have appeared in the literature about the determination of BM, such as chromatography, mass spectrometry and immunoassay [6,7]. But there have no reports on the binding affinity and binding site of BM to plasma protein (especially serum albumin), as it can supply salient insight into the mechanism of interaction between sulfonylurea herbicides and physiologically important protein.

It is generally accepted that the distribution, metabolism, excretion and toxicity of ligands are correlated with their affinities towards protein, especially serum albumin. To clearly understand the characterization of the binding process by estimating the binding affinity and binding site is of imperative and fundamental importance in the biological system [8]. One plasma protein that has been extensively studied during such work is human serum albumin (HSA). HSA is the most abundant protein in blood plasma, it constitutes up to 60% of the total protein and contributes 80% of the colloid osmotic blood pressure [9]. In blood plasma, the exceptional ability of HSA is its capacity to bind a diverse variety of ligands, such as fatty acids, metabolites, bilirubin, steroids, drugs and dyes [10], due to the presence of two principal binding sites (i.e. Sudlow's site I and site II) as well as some minor binding sites (e.g. tamoxifen and digitoxin sites). The affinity of binding between ligand and HSA

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doi:10.1016/j.jlumin.2010.05.019

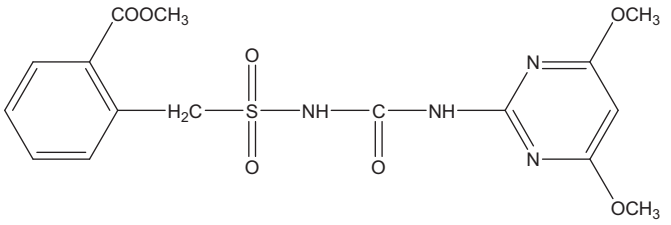

Fig. 1. Molecular structure of BM.

can dominate its distribution into target tissues, affect its elimination from the body, and influence its therapeutic/toxicologic effects; it is then important to determine the binding affinity and binding site in order to comprehend the molecular basis of these interactions and provide basic information on the pharmacological/toxicological action, distribution and transportation of ligands [11].

The object of this work is to determine the binding affinity and binding site of BM to HSA and to evaluate the binding property between BM and HSA by quenching the intrinsic Trp fluorescence. The secondary structure alternations of HSA in the presence of BM and the HSA–BM interaction in the presence of site markers (phenylbutazone for subdomain IIA, flufenamic acid for subdomain IIIA, and 8-anilino-1-naphthalenesulfonic acid (ANS) for hydrophobic probe) are also analyzed.

2. Materials and methods

2.1. Materials

HSA (fatty acid free $<0.05\%$), BM, and ANS (Crystal, purity $>97\%$) were purchased from Sigma-Aldrich chemical company. All other reagents were of analytical reagent. Milli-Q ultrapure water was used throughout the experiments. The buffer used was Tris-HCl consisting of 0.2 M Tris/0.1 M HCl and 0.1 M NaCl at pH 7.4. The pH was checked with a suitably standardized Orion-868 pH meter (Orion, USA). Dilutions of the HSA stock solution (1.0×10^{-5} M) in Tris-HCl buffer solution were prepared immediately before use and the concentration of HSA was determined spectrophotometrically using $E^{1\%}_{1\,cm}=5.30$ [12]. The stock solution of BM was prepared in acetone.

2.2. Apparatus and methods

2.2.1. Fluorescence emission spectra

Fluorescence emission spectra were performed on a F-4500 spectrofluorimeter (Hitachi, Japan) equipped with 1.0 cm quartz cell and a thermostatic bath. The excitation and emission slits with a band pass of 5 nm were used for all the measurements. An excitation wavelength of 295 nm was chosen and very dilute solutions were used in the experiments (HSA 1.0×10^{-6} M, BM in the range of 0–22.5×10^{-6} M). The quenching effect of acetone was evaluated and no effect was observed on the HSA fluorescence emission spectra with the addition of acetone (data not shown), which suggested no change in HSA conformation.

2.2.2. UV/vis absorption spectra

UV/vis absorption spectra were registered on a Cary-300 (Varian, USA) spectrophotometer with a 1.0 cm quartz cuvette at 291 K.

2.2.3. CD spectra

CD spectra were recorded with a Jasco-810 spectropolarimeter (Jasco, Japan) using a 0.2 cm path length quartz cell. Measurements were taken at wavelengths between 200 and 260 nm with 0.1 nm step resolution and averaged over five scans recorded at a speed of 50 nm min^{-1}. All observed CD spectra were baseline subtracted for buffer and the results were expressed as MRE (mean residue ellipticity) in deg cm^2 $dmol^{-1}$, which is defined as

$$MRE=\frac{\theta_{obs}}{10\times n\times l\times C_p} \quad (1)$$

where θ_{obs} is the CD in millidegree, n is the number of amino acid residues (5 8 5), l is the path length of the cuvette, and C_p is the HSA molar concentration. α-helical content was calculated from the MRE values at 208 nm using the following equation described by Greenfield and Fasman [13]:

$$\%\alpha-helix=\frac{MRE_{208}-4000}{33{,}000-4000} \quad (2)$$

2.2.4. Three-dimensional fluorescence spectra

Three-dimensional fluorescence spectra were measured under the following conditions: the emission wavelength was recorded between 200 and 500 nm, the initial excitation wavelength was set to 200 nm with increment of 10 nm, the number of scanning curves was 16, and other scanning parameters were identical to those of the fluorescence emission spectra.

2.2.5. Time-resolved fluorescence spectra

Time-resolved fluorescence spectra were executed in a time-correlated single photon counting system from FL920P spectrometer (Edinburgh Instruments, UK) with $\lambda_{ex}=295$ nm. The data are fitted to biexponential functions after deconvolution of the instrumental response function by an iterative reconvolution approach by the DAS6 decay analysis software utilizing reduced χ and weighted residuals as parameters for goodness of fit. Average fluorescence lifetime (τ) for biexponential iterative fittings was calculated from the decay times and the relative amplitudes (A) using the following equation:

$$\tau=\tau_1A_1+\tau_2A_2. \quad (3)$$

2.2.6. Site marker experiments

Site marker competitive experiments: Binding location studies between HSA and BM in the presence of two site markers (phenylbutazone and flufenamic acid) were performed using fluorescence titration methods. The concentration of HSA and phenylbutazone/flufenamic acid was stabilized at 1.0×10^{-6} M, BM was then gradually added to the HSA-site markers mixtures. An excitation wavelength of 295 nm was selected and the fluorescence spectra were recorded in the range of 290–450 nm.

2.2.7. Hydrophobic probe ANS

ANS binding studies: In the first series of experiments, HSA concentration was kept fixed at 1.0×10^{-6} M and BM/ANS concentration was varied from 2.5 to 22.5×10^{-6} M, HSA fluorescence was recorded ($\lambda_{ex}=295$ nm, $\lambda_{em}=333$ nm). In the second series of experiments, BM was added to solutions of HSA and ANS held in equimolar concentration (1.0×10^{-6} M), BM concentration was also varied from 2.5 to 22.5×10^{-6} M and the fluorescence of ANS was measured ($\lambda_{ex}=370$ nm, $\lambda_{em}=465$ nm [14]).

2.2.8. Molecular modeling

Molecular modeling of the HSA–BM association reaction was performed on SGI Fuel Workstation. The crystal structure of HSA