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Fluorescence and circular dichroism studies of conjugates between metsulfuron-methyl and human serum albumin

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ABSTRACT

Metsulfuron-methyl is a sulfonylurea herbicide widely used for broad-leaved weed control in cereals. The binding interaction between metsulfuron-methyl and human serum albumin was elucidated by fluorescence, circular dichroism and molecular modeling. The results showed that the alterations of albumin secondary structure in the presence of herbicide induced the slight unfolding of the polypeptide chain of albumin. Fluorescence data revealed that the fluorescence quenching of albumin by herbicide was the result of the formation of the albumin–herbicide complex and hydrophobic and hydrogen bonds interactions were the dominant intermolecular force in stabilizing the complex. Fluorescence probes studies implied that the binding of herbicide to albumin primarily took place in subdomain IIA (Sudlow's site I), and this also corroborates with molecular modeling simulations. This study highlights for the first time the binding mechanism, specific binding site and binding region of herbicide on albumin at the first time. Therefore, this investigation enriches our information of the interaction of sulfonylurea herbicide to the physiologically protein albumin.

1. Introduction

Protein–ligand interactions play a crucial role in the distribution and transport of small molecules in biological system and process [1,2]. This interaction can take place with a variety of ingredients in blood plasma, including the most abundant plasma protein, human serum albumin (HSA). HSA has a molar mass of 66.5 kDa, and accounts for about 60% of the total protein in blood [3]. The first crystallographic analyses of HSA [3] revealed that HSA is a globular protein, consists of a single polypeptide chain of 585 amino acid residues and contains three homologous α-helical domains (I, II and III), each of which is composed of two subdomains (A and B) and is stabilized by 17 disulfide bonds and 1 free thiol at Cys-34. HSA is known to contain two major binding regions for ligand [4], namely Sudlow's site I and site II, which are located within hydrophobic cavities in subdomains IIA and IIIA, respectively. Site I is known as warfarin–azapropazone site and is formed as a pocket in subdomain IIA, which contains a single tryptophan residue in position 214 (Trp-214) [3]. Site II corresponds to the pocket of subdomain IIIA and is known as the indole-benzodiazepine site, which presents two important amino acid residues (Arg-410 and Tyr-411) [5]. HSA's most outstanding property is the ability to bind and transport many endogenous and exogenous compounds, such as bilirubin, fatty acids, steroids, drugs and dyes [6–8]. The multiple binding sites underlie the exceptional ability of HSA to interact with many ligands and make this protein an important regulator of intercellular fluid and toxicologic behavior of many ligands. It is also widely accepted that the degree of affinity between HSA and ligand can govern its distribution into tissues, affect its elimination and consequently affect its therapeutic or toxic effects [9]. Thus, the study on the interaction of ligand with HSA is of imperative and fundamental importance. This kind of studies may provide significant information on realizing the transport and metabolism process of ligand, the relationship between structure and function of HSA, and the chemical essence of the interaction between biomacromolecule and ligand.

Sulfonylurea herbicides have high herbicidal activity at low application rates (2–75 g of active ingredient per hectare). Among sulfonylurea products, metsulfuron-methyl (methyl 2-(4-methoxy-6-methyl-1,3,5-triazine-2-ylcarbamoylsulfamoyl) benzoate, MTS, structure shown in Fig. 1) is widely used due to its selectivity against a wide range of broad-leaved weeds in cereal,

Abbreviations: HSA, human serum albumin; Cys, cysteine; Trp, tryptophan; Arg, arginine; Tyr, tyrosine; MTS, metsulfuron-methyl; ALS, acetolactate synthase; ANS, 8-anilino-1-naphthalenesulfonic acid; Phe, phenylalanine; His, histidine; Leu, leucine; Lys, lysine; Ala, alanine; Val, valine; CD, circular dichroism; S.D., standard deviation.

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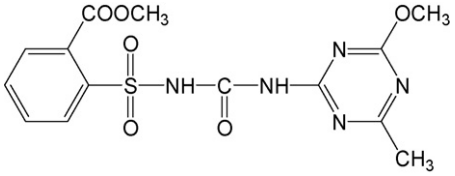

Fig. 1. Molecular structure of MTS.

pasture, plantation crops and non-crop situations after it was discovered in the mid-70s by Dr. George Levitt at DuPont [10]. It inhibits the acetolactate synthase (ALS), which is a key enzyme in the biosynthesis of branched chain amino acids, viz. valine, leucine and isoleucine, and thus inhibiting cell division [11]. MTS has widespread use in the fields around the world, e.g. USA, Europe and China. MTS will permeate into soil and can run off from cropland into rivers after application, causing surface water contamination. This herbicide may also persist in the environment for many months. In Europe, a limit value of 0.1 $\mu g\,L^{-1}$ was set for pesticides in drinking water by the European Community [12]. Recently, the Environmental Protection Agency (EPA) has indicated 50 $\mu g\,L^{-1}$ as the maximum concentration for sulfonylurea herbicides destined to human consumption [13]. In the past 20 years, several analytical methods have been proposed for the determination of MTS, for example, high performance liquid chromatography, gas chromatography, capillary electrophoresis, ELISA and mass spectrography [12]. However, there is no literature about the binding of MTS to HSA. Since MTS is used for many different ways and has been widely spread around the world, it is necessary to investigate the interaction between MTS and HSA, as it can provide salient insight into the interactions of the physiologically important protein HSA with sulfonylurea herbicides.

The present paper deals with the mechanism of binding of MTS as a ligand with HSA by fluorescence, circular dichroism (CD) and molecular modeling. Partial binding parameters of MTS to HSA have been calculated. The changes of HSA secondary structure were quantitatively analyzed by CD and three-dimensional fluorescence spectroscopy. In addition, a competitive study of HSA interacted with MTS and site markers (phenylbutazone and flufenamic acid) should help understand preferential binding at the molecular level. Besides, the binding region for MTS towards HSA was confirmed by the evidences from hydrophobic probe 8-anilino-1-naphthalenesulfonic acid (ANS) displacement experiments and molecular modeling simulations.

2. Experimental

2.1. Materials

HSA (fatty acid free < 0.05%) and MTS were purchased from Sigma–Aldrich Chemical Company. All other reagents were of analytical reagent grade. Milli-Q ultrapure water was used throughout the experiments. NaCl (1.0 M) solution was used to maintain the ionic strength at 0.1. Tris (0.2 M)–HCl (0.1 M) buffer solution containing NaCl (0.1 M) was used to keep the pH of the solution at 7.4. Dilutions of the HSA stock solution (1.0×10^{-5} M) in Tris–HCl buffer were prepared immediately before use, and the concentration of HSA was determined spectrophotometrically using $E_{1cm}^{1\%}$ of 5.30 at 280 nm [14]. The stock solution of MTS was prepared in acetone.

2.2. Apparatus and methods

Steady state fluorescence spectra were performed on a F-4500 fluorophotometer (Hitachi, Japan) equipped with a 1.0 cm quartz cell and a thermostat bath. The excitation and emission slits with a band pass of 5.0 nm were used for all the measurements. The excitation wavelength was set at 295 nm to selectively excite the Trp residue, and the emission spectra were recorded in the wavelength range from 290 to 450 nm at a scanning speed of 240 nm min^{-1}.

CD spectra were carried out with a Jasco-810 spectropolarimeter (Jasco, Japan) using a 1.0 cm path length quartz cuvette. Calibration of the instrument was adjusted using (+)-10-camphorsulfonic acid. Measurements were taken at wavelength between 200 and 260 nm with 0.1 nm step resolution and averaged over five scans recorded as 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

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

where θ_{obs} is the CD in millidegree, n is the number of amino acid residues (585), 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 [15]:

$$\%\alpha\text{-helix} = \frac{\mathrm{MRE}_{208} - 4000}{33,000 - 4000} \quad (2)$$

Three-dimensional fluorescence spectra were performed under the following conditions: the emission wavelength was recorded between 200 and 450 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 steady state fluorescence spectra.

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

ANS displacement experiments: In the first series of experiments, interaction of MTS and ANS with HSA was studied under identical conditions. HSA concentration was kept fixed at 1.0×10^{-6} M, and MTS/ANS concentration was varied from 1.0 to 9.0×10^{-6} M, then fluorescence emission spectra of HSA was recorded (λ_{ex} = 295 nm, λ_{em} = 335 nm). In the second series of experiments, MTS was added to solutions of HSA and ANS held in equimolar concentrations (1.0×10^{-6} M), the concentration of MTS was also varied from 1.0 to 9.0×10^{-6} M and the fluorescence of ANS was recorded (λ_{ex} = 370 nm, λ_{em} = 470 nm).

Molecular modeling of the HSA–MTS association reaction was performed on SGI Fuel Workstation. The crystal structure of HSA was downloaded from Brookhaven Protein Data Bank (entry codes 1H4K, resolution 2.4 Å, http://www.rcsb.org/pdb). The two-dimensional structure of MTS was downloaded from PubChem (http://pubchem.ncbi.nlm.nih.gov). The potential of the three-dimensional structure of HSA was assigned according to the AMBER force field with Kollman all-atom charges. The initial structure of MTS was generated by molecular modeling software Sybyl 7.3. The geometry of the molecule was subsequently optimized to minimal energy using the Tripos force field with Gasteiger-Hückel charges, and the Surflex docking program was applied to calculate the possible conformation of the ligand that binds to the protein [16].

Statistical analysis: All experiments were performed in triplicate, the mean values, standard deviations, and statistical differences were estimated using analysis of variance with 95% confidence limits ($p < 0.05$); the mean values were compared using Student's