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Identification of pyrazosulfuron-ethyl binding affinity and binding site subdomain IIA in human serum albumin by spectroscopic methods

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ABSTRACT

Pyrazosulfuron-ethyl (PY) is a sulfonylurea herbicide developed by DuPont which has been widely used for weed control in cereals. The determination of PY binding affinity and binding site in human serum albumin (HSA) by spectroscopic methods is the subject of this work. From the fluorescence emission, circular dichroism and three-dimensional fluorescence results, the interaction of PY with HSA caused secondary structure changes in the protein. Fluorescence data demonstrated that the quenching of HSA fluorescence by PY was the result of the formation of HSA–PY complex at 1:1 molar ratio, a static mechanism was confirmed to lead to the fluorescence quenching. Hydrophobic probe 8-anilino-1-naphthalenesulfonic acid (ANS) displacement results show that hydrophobic patches are the major sites for PY binding on HSA. The thermodynamic parameters ΔH° and ΔS° were calculated to be $-36.32\ kJ\ mol^{-1}$ and $-35.91\ J\ mol^{-1}\ K^{-1}$, which illustrated van der Waals forces and hydrogen bonds interactions were the dominant intermolecular force in stabilizing the complex. Also, site marker competitive experiments showed that the binding of PY to HSA took place primarily in subdomain IIA (Sudlow's site I). What presented in this paper binding research enriches our knowledge of the interaction between sulfonylurea herbicides and the physiologically important protein HSA.

1. Introduction

Protein–ligand interactions play a critical role in the distribution, metabolism and transport of small molecules in biological systems and processes [1]. It is widely accepted that the degree of affinity between ligand and plasma protein can govern its distribution into target tissue, affect its elimination from the body, and influence its therapeutic or toxic effects [2,3]. Thus, the study on the interactions of small molecule with protein is of imperative and fundamental importance. One plasma protein which has been extensively investigated during such work is human serum albumin (HSA). HSA is the most abundant protein in blood, it constitutes up to 60% of the total plasma concentration [4] and contributes 80% of the colloid osmotic blood pressure [5]. He and Carter [6] have determined the three-dimensional structure of HSA and shown that it is a 65 kDa single chain, non-glycosylated polypeptide that folds into a heart-shaped structure containing approximately 67% α-helix [7]. HSA contains 585 amino acids, and its composed of three structurally homologous domains (I, II, and III), each of which contains two subdomains (A and B), and is stabilized by 17 disulfide bonds and 1 free thiol at Cys-34 [6]. HSA is known to contain two major binding sites for ligands, which located in hydrophobic cavities in subdomains IIA and IIIA (Sudlow's site I and site II) [8,9]. Site I is known as the warfarin-azapropazone site, formed as a pocket in subdomain IIA and it involves the sole tryptophan of HSA (Trp-214). Site II is located in subdomain IIIA and its known as the indole-benzodiazepine site, it is composed by hydrophobic amino acid residues and the pocket exterior which presented two important amino acids residues (Arg-410 and Tyr-411) [3,10]. In plasma, HSA is responsible for transporting and metabolizing many endogenous and exogenous ligands, such as fatty acids, metabolites, steroids, bilirubin, drugs and dyes [1,11,12]. The binding affinity of ligands to HSA can significantly alter their overall activity profile, including *in vivo* distribution, excretion and toxicity [3]. Therefore, it is important to understand the binding affinity and binding site of ligand with HSA. This kind of study may supply critical information on the therapeutic/toxicologic effectiveness of ligand, thus becoming a hot research field in chemistry, life sciences and clinical medicine.

Sulfonylurea herbicides were first introduced in 1982 by DuPont Agricultural Products and have high herbicidal activity at low application rates (<100 g of active ingredient per hectare) [13]. Among sulfonylurea products, pyrazosulfuron-ethyl

Abbreviations: PY, pyrazosulfuron-ethyl; HSA, human serum albumin; ANS, 8-anilino-1-naphthalenesulfonic acid; Cys, cysteine; Trp, tryptophan; Tyr, tyrosine; Arg, arginine; Phe, phenylalanine; ALS, acetolactate synthase; CD, circular dichroism; S.D., standard deviation.

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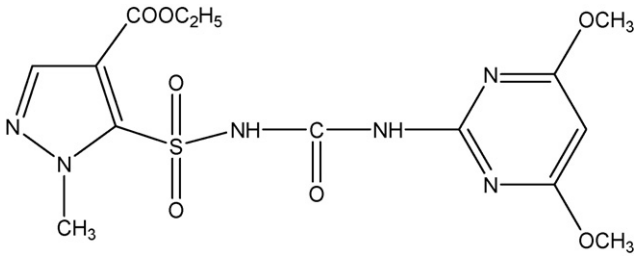

Fig. 1. Molecular structure of PY.

(ethyl-5-(*N*-(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl)-1-methyl-1*H*-pyrazole-4-carboxylate, structure shown in Fig. 1), is a sulfonylurea herbicide widely used for selective post-emergence control of annual and perennial grasses and broad-leaved weeds in cereals because of its high herbicidal activity and low toxicity for mammals [14,15]. 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 therefore cell division was hindered [16]. However, the widespread use of pyrazosulfuron-ethyl (PY) is a potential water pollutant and presents environmental risk, especially for human beings. Due to PY fairly high water solubility result in its high mobility, it has been detected in surface and groundwater. Recently, a limited value of 0.05 ppm was set for sulfonylurea herbicides in drinking water by the Environmental Protection Agency (EPA) [17]. The European Union also indicates 0.1 $\mu g\,L^{-1}$ as the maximum concentration for a single pesticide in water destined to human consumption [13]. Some articles have appeared in this literature regarding the degradation and determination of the sulfonylurea herbicides [15,18–20], but critical literature survey reveals that attempts have not been made so far to identify the binding affinity and binding site of interaction of PY with HSA.

The aim of this study was to identify the binding affinity of PY in HSA, special emphasis was put on which binding site (Sudlow's site I or site II) is the major site for PY binding on HSA molecule. Another object of the work was to investigate the alterations of HSA secondary structure in the presence of PY. Finally, this report should give more understanding on realizing the transport and metabolism process of PY, the relationship between the structure and function of HSA, and the chemical essence of the interaction between biomacromolecule and small molecule.

2. Materials and methods

2.1. Materials

HSA (fatty acid free < 0.05%) and PY were purchased from Sigma–Aldrich Chemical Company. All other reagents were of analytical reagent. Milli-Q ultrapure water was used throughout the experiments. NaCl (1.0 M) solution was applied to maintain the ionic strength at 0.1. Tris (0.2 M)–HCl (0.1 M) buffer solution containing NaCl (0.1 M) was employed 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 solution were prepared immediately before use, and the concentration of HSA was determined spectrophotometrically using $E_{1\,cm}$ 1% of 5.30 at 280 nm [21]. The stock solution of PY was dissolved in acetone.

2.2. Apparatus and methods

Circular dichroism (CD) spectra were performed on 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° $cm^2\,dmol^{-1}$ which is defined as

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

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 [22]:

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

Steady state fluorescence spectra were performed on a F-4500 spectrofluorometer (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, PY in the range of 0–4.5×10^{-7} 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 show), which suggested no change in HSA conformation.

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 at 200 nm with increment of 10 nm, the number of scanning curves was 16, and other scanning parameters were just the same as those of the fluorescence emission spectra.

Hydrophobic probe 8-anilino-1-naphthalenesulfonic acid (ANS) displacement experiments: in the first series of experiments, HSA concentration was kept fixed at 1.0×10^{-6} M, and PY/ANS concentration was varied from 0.5 to 4.5×10^{-7} M, HSA fluorescence emission spectra were recorded (λ_{ex} = 295 nm, λ_{em} = 335 nm). In the second series of experiments, PY was added to solutions of HSA and ANS held in equimolar concentrations (1.0×10^{-6} M), the concentration of PY was also varied from 0.5 to 4.5×10^{-7} M, and the fluorescence of ANS was performed (λ_{ex} = 370 nm, λ_{em} = 470 nm).

Site marker competitive experiments: binding location studies between HSA and PY in the presence of two site markers (phenylbutazone and flufenamic acid) were measured using the fluorescence titration method. PY was added to solutions of HSA and site markers held in equimolar concentrations (1.0×10^{-6} M), PY concentration was varied from 0.5 to 4.5×10^{-7} M. An excitation wavelength of 295 nm was selected and the fluorescence of HSA was recorded.

2.3. Statistical analysis

All experiments were performed in triplicate, and the mean values were calculated. All statistic data were processed using the OriginPro Software (OriginLab Corporation, USA).

3. Results and discussion

3.1. Changes of the HSA secondary structure

The conformational changes of HSA was evaluated by measurement of the intrinsic fluorescence of HSA Trp residue before and after the addition of PY, because the intramolecular forces, which were involved to maintain the secondary structure, could be altered, thus resulting in the conformational changes of HSA [23]. Fig. 2 shows the fluorescence emission spectra of HSA with vari-