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Complex of nicosulfuron with human serum albumin: A biophysical study

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ABSTRACT

Nicosulfuron is a sulfonylurea herbicide developed by DuPont that has been used successfully for weed control in maize. The binding mechanism and binding site identified in human serum albumin (HSA) with the use of fluorescence, circular dichroism (CD) and molecular modeling is the subject of this paper. From the CD, synchronous and three-dimensional fluorescence results, it was apparent that the interaction of nicosulfuron with HSA caused secondary structure changes in the protein. Fluorescence data revealed that the nicosulfuron induced the fluorescence quenching of HSA through a static quenching procedure. Thermodynamic analysis results implied the role of hydrophobic and hydrogen bonds interactions in stabilizing the nicosulfuron–HSA complex. Site marker competitive experiments showed the binding of nicosulfuron to HSA primarily took place in subdomain IIA (Sudlow's site I), this corroborates the guanidine hydrochloride (GuHCl) induced denaturation of HSA, hydrophobic probe ANS displacement and molecular modeling results. In this work, the presented binding research extends our knowledge of the binding properties of sulfonylurea herbicides to the important plasma protein HSA.

1. Introduction

Sulfonylurea herbicides were first introduced in 1982 by DuPont Agricultural Products. Due to its high herbicidal activity and low toxicity for mammals, they have widespread control over broad-leaved weeds and some grasses in cereals and are widely used for a variety of crops [1]. Among sulfonylurea products, nicosulfuron (2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-*N*,*N*-dimethyl-3-pyridinecarboxamide) is very effective even applied at low rates (<100 g ha^{-1}) and is widely used for selective post-emergence control of annual and perennial grasses in maize [2]. It inhibits the enzyme acetolactate synthase (ALS), which is a key enzyme in the biosynthesis of branched chain amino acids viz. valine, leucine and isoleucine, and hence inhibiting cell division [3]. The highest use of nicosulfuron is on maize and approximately 200,000 lb annually [4], after the foliar application, the herbicide permeates into soil and can subsequently run off from cropland into rivers, ponds and lakes, causing surface and groundwater contamination. Since European Union indicates 0.1 μg L^{-1} as the maximum limit value for a single pesticide in drinking water destined to human consumption, several analytical methods have been proposed for the determination of nicosulfuron, such as chromatography, mass spectrometry, immunoassay, capillary electrophoresis [1,3]. Sulfonylurea (e.g. nicosulfuron) has been proved to induce hypoglycemia in humans [5,6]. Recently, Simpson and colleagues [7] have clearly demonstrated that sulfonylureas increase the risk of cardiovascular disease; moreover, their result adds endorsement to a casual connection by identifying a dose-related effect on the risk of death. It is worth noting that the free concentration available for the toxic action can be obviously reduced for pesticides with high affinity to proteins, for the reason that proteins can dominate the extent and duration of toxicological effect in the body, although bindings of pesticide to proteins are often lower than that to the target enzyme [8]. However, no reports in the literature have appeared regarding the binding mechanism and binding site of nicosulfuron on protein at molecular level. Owing to the widespread use of nicosulfuron in fields around the world, which implied the potential toxicological risk to humankind, the investigation on the interaction of nicosulfuron with protein is of great importance.

Human serum albumin (HSA) is the most abundant protein in blood plasma, which constitutes about 60% of the total plasma protein, and providing about 80% of the blood osmotic pressure [9]. The exceptional feature of HSA is its ability to bind and transport of many endogenous and exogenous substances, for example, fatty acids, metabolites, drugs, dyes and pesticides [10,11]. Also, it is widely accepted that the degree of affinity between ligand and HSA can dominate its distribution into target tissue, affect its elimination from the body, and finally influence its therapeutic

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or toxic effects, biotransformation and biodistribution etc. of ligands [12]. Besides, there is testimony of conformational changes of protein caused by its interaction of ligands, and these changes seem to affect the secondary and tertiary structure of protein [12]. Thus, it is significant to study the interaction of nicosulfuron with HSA, this sort of studies may supply pivotal information on the structural characteristics that determine the toxic effect of nicosulfuron, since it has been proved that the distribution and free concentration of various ligands can be drastically altered as a result of their binding to HSA, and therefore become a focal research area in chemistry, life science and clinical medicine.

Numerous methods have been utilized to study the ligand–protein interactions, including ultrafiltration, ultracentrifugation, equilibrium dialysis, fluorescence, UV/vis, capillary electrophoresis, NMR, surface plasmon resonance, chromatography, etc. [10,11,13,14]. Among them, fluorescence spectroscopy has been widely used due to its inimitable sensitivity, rapidity, and simplicity [15]. At the same time, rigorous literature survey divulges that attempts have not yet been made to investigate the binding mechanism and binding site of interaction of nicosulfuron with HSA. The intent of the study was to determine the binding mechanism and binding site of nicosulfuron with HSA by fluorescence, circular dichroism (CD) and molecular modeling methods. Great essays were made to investigate the binding properties pertain to the secondary structure changes of HSA, quenching mechanism, thermodynamic functions, specific binding site and binding patches. This work should give more understanding on realizing the transport and metabolism process of nicosulfuron, the relationship of structure and function of HSA, and the chemical essence of the interaction between biomacromolecule and ligand.

2. Materials and methods

2.1. Materials

HSA (fatty acid free <0.05%) and nicosulfuron were obtained from Sigma–Aldrich Chemical Company, and used without further purification. All other reagents were of analytical reagent. Milli-Q ultrapure water was applied throughout the experiments. NaCl (1.0 M) solution was employed 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. 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 were prepared immediately before use, and the concentration of HSA was determined spectrophotometrically using $E_{1\ \mathrm{cm}}^{1\%}$ of 5.30 at 280 nm [16]. The stock solution of nicosulfuron was prepared in Tris–HCl buffer.

2.2. Apparatus and methods

2.2.1. 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 as a speed of 20 nm min^{-1}. All observed CD spectra were baseline subtracted for buffer solution and the results were taken as Mean Residue Ellipticity (MRE) in deg cm^2 dmol^{-1} which is defined as

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

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 [17]:

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

2.2.2. Fluorescence spectra

Steady state fluorescence spectra were performed on a F-4500 spectrofluorometer (Hitachi, Japan) equipped with 1.0 cm quartz cuvette and a thermostat bath. Fluorescence quenching spectra were recorded at 291, 297, 303 and 309 K in the range of 250–450 nm. The width of the excitation and emission slits was set to 5.0 nm for all the measurements. An excitation wavelength of 295 nm was chosen and very dilute solution was applied in the experiment (HSA 1.0×10^{-6} M, nicosulfuron in the range of 0–9.0×10^{-6} M). Fluorescence spectra were the average of five scans with the baseline corrected by Tris–HCl buffer as the control.

2.2.3. Three-dimensional fluorescence spectra

Three-dimensional fluorescence spectra were measured under the following conditions: the emission wavelength was recorded between 200 and 500 nm, and 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.

2.2.4. Site-specific probe

Site marker competitive experiments: binding location studies between nicosulfuron and HSA in the presence of four site markers (phenylbutazone, ibuprofen, digitoxin and hemin) were measured using the fluorescence titration method. The concentrations of HSA and site markers were held in equimolar (1.0×10^{-6} M), then nicosulfuron was added to the HSA-site markers mixtures. An excitation wavelength of 295 nm was chosen and the fluorescence emission wavelength was registered from 250 to 450 nm.

2.2.5. Hydrophobic probe ANS

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 nicosulfuron/ANS concentration was varied from 1.0 to 9.0×10^{-6} M, fluorescence emission spectra of HSA was recorded (λ_{ex} = 295 nm, λ_{em} = 334 nm). In the second series of experiments, the nicosulfuron was added to solutions of HSA and ANS held in equimolar concentrations (1.0×10^{-6} M), the concentration of nicosulfuron was also varied from 1.0 to 9.0×10^{-6} M and ANS fluorescence was measured (λ_{ex} = 370 nm, λ_{em} = 465 nm).

2.2.6. Molecular modeling

Molecular modeling of the HSA–nicosulfuron interaction was performed on SGI Fuel Workstation. The crystal structure of HSA was downloaded from Brookhaven Protein Data Bank (entry codes 1H4 K, resolution 2.4 Å, http://www.rcsb.org/pdb). The two-dimensional structure of nicosulfuron 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 nicosulfuron 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 [18].