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Complexation of insecticide chlorantraniliprole with human serum albumin: Biophysical aspects

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ABSTRACT

Chlorantraniliprole is a novel insecticide belonging to the diamide class of selective ryanodine receptor agonists. A biophysical study on the binding interaction of a novel diamide insecticide, chlorantraniliprole, with staple *in vivo* transporter, human serum albumin (HSA) has been investigated utilizing a combination of steady-state and time-resolved fluorescence, circular dichroism (CD), and molecular modeling methods. The interaction of chlorantraniliprole with HSA gives rise to fluorescence quenching through static mechanism, this corroborates the fluorescence lifetime outcomes that the ground state complex formation and the predominant forces in the HSA–chlorantraniliprole conjugate are van der Waals forces and hydrogen bonds, as derived from thermodynamic analysis. The definite binding site of chlorantraniliprole in HSA has been identified from the denaturation of protein, competitive ligand binding, and molecular modeling, subdomain IIIA (Sudlow's site II) was designated to possess high-affinity binding site for chlorantraniliprole. Moreover, using synchronous fluorescence, CD, and three-dimensional fluorescence we testified some degree of HSA structure unfolding upon chlorantraniliprole binding.

1. Introduction

It is well-known that the absorption, distribution, metabolism, and excretion (ADME) properties of various ligands are intensely affected by protein–ligand conjugation in the blood plasma [1]. Characterization of the binding process by appraising the binding parameter, i.e. binding constant, the number of binding site, binding energetic, binding location, and conformation changes of macromolecule, is essential to understand interactions in the biological systems and processes [2]. Within the plasma proteins, human serum albumin (HSA) is undoubtedly the most principal carrier protein for ligands. In human body, HSA concentrations range from 35 to 50 g L^{-1}, making it the most copious protein in the blood plasma, accounting for about 60% of the total protein content and providing about 80% of the blood osmotic pressure [3]. He and Carter [4] have determined the three-dimensional structure of HSA and have shown that it is a globular protein consisting of a single polypeptide chain of 585 amino acids, largely helical (~67%) and having a molecular weight of 66.5 kDa. HSA comprises of three homologous domains (I, II, and III), each of which contains two subdomains (A and B) that share common structural elements, and is stabilized by 17 disulfide bridges and 1 free thiol at Cys-34 [3]. Although still partly controversial, what the consensus exists today is that there are two primary binding sites for ligands on HSA are within two hydrophobic cavities in subdomains IIA and IIIA, namely Sudlow's sites I and II [5], as well as some minor sites (e.g. tamoxifen and digitoxin sites [6]). Due to these binding sites, this protein is responsible for distributing and metabolizing many endogenous and exogenous ligands such as fatty acids, bilirubin, pharmaceuticals, metal ions, dyes, and pesticides [2,7,8], to assist their transport and to enhance their solubility. It is also generally accepted today that the extent of binding between a ligand and HSA can dominate its distribution into tissues, influence its elimination from the body, and finally affect its therapeutic or toxic effects [2,9]. Thus, investigation on the interaction of ligand with HSA is of imperative and fundamental importance. The information on the association of HSA and ligand can make us better comprehend the distribution, accumulation patterns, and toxicity of ligand *in vivo*.

Chlorantraniliprole, 3-bromo-*N*-[4-chloro-2-methyl-6-[(methylamino)carbonyl]phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide (structure shown in Fig. 1), is a new insecticide in

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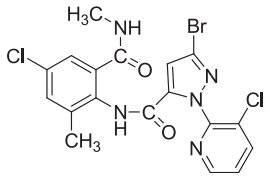

Fig. 1. Molecular structure of chlorantraniliprole.

the anthranilic diamide developed by DuPont Crop Protection, and it has proven to be efficacious against *Lepidoptera* pests, as well as against selected species in the orders *Coleoptera*, *Diptera*, and *Hemiptera* [10]. Chlorantraniliprole has also shown high efficacy against various turfgrass pests including the white grub and can be applied as early as early spring for preventive white grub control due to its long residual activity [11]. Chlorantraniliprole acts as allosteric activator on the ryanodine receptors in insects and affects calcium homeostasis by unregulated release and depletion of internal calcium stores in muscles, thus causing feeding cessation, lethargy, muscle paralysis, and ultimately death of the insect [12]. However, the insecticide will lead to concerns over contamination of surface and groundwater as well as agricultural products after application, as it can enter ecosystems by sprays, drifts, surface runoff, accidental spills, or irrigation with contaminated water. A limit value of 0.1 ppm was set for chlorantraniliprole in vegetables and fruits by the Environmental Protection Agency (EPA) [13]. Also, Health Canada's Pest Management Regulatory Agency (PMRA) prescribes 0.01 ppm as the maximum residue limit of chlorantraniliprole in meat for human consumption [14]. Recently, Caboni et al. [15] have used liquid chromatography–tandem mass spectrometric ion-switching determination of chlorantraniliprole in fruits and vegetables. However, critical literature survey declares that attempts have not been made so far to investigate the association between chlorantraniliprole and HSA by spectroscopic and computational methods, as they can supply salient insight into the mechanism of interaction between chlorantraniliprole and physiologically important protein.

Till date, many techniques have been exploited for the protein–ligand binding study, including equilibrium dialysis, ultrafiltration, ultracentrifugation, fluorescence, capillary electrophoresis, surface plasmon resonance, calorimetry, surface tension, chromatography, crystallology, and so on [3,4,16–19]. Among them, fluorescence spectroscopy has been confirmed to provide the most comprehensively qualitative and quantitative information on the protein–ligand interactions [20,21]. The current work explored the potential non-covalent binding of chlorantraniliprole to HSA, the major protein in the human intra- and extra-vascular space. Exhaustive analysis of the steady-state and time-resolved fluorescence and circular dichroism (CD) of the HSA–chlorantraniliprole coordinated complex and molecular modeling simulations offered essential data on the observed association reaction. These types of analyses could be utilized for future investigations on plasma protein–insecticide interactions *in vivo*, since free concentration available for the toxic effect can be effectively reduced for insecticide with high-affinity to proteins [22,23], although affinity of insecticides to proteins is often lower than to the enzyme targets.

2. Materials and methods

2.1. Materials

HSA (essential globulin free, fatty acid free $<0.05\%$) and chlorantraniliprole were received from Sigma-Aldrich (USA) and used without further purification. Milli-Q ultrapure water (Millipore, USA) was used throughout the experiments. All the experiments were performed in Tris (0.2 M)–HCl (0.1 M) buffer solution of pH=7.4, with an ionic strength 0.1 in the presence of NaCl, except where specified, and the pH was checked with a suitably standardized Orion-868pH 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 $E^{1\%}_{1\,cm}$=5.3 or by the method of Lowry et al. [24,25]. All other reagents employed were of analytical grade and obtained from Sigma-Aldrich.

2.2. Methods

2.2.1. Steady-state fluorescence

Steady-state fluorescence were acquired on a F-4500 spectrofluorimeter (Hitachi, Japan) equipped with 1.0 cm quartz cell and a thermostatic bath. The excitation and emission slits were set at 5.0 nm each, intrinsic fluorescence was measured by exciting the stirred protein solution at 295 nm to selectively excite the tryptophan (Trp) residue, and the emission spectra were recorded in the wavelength range 300–500 nm at a scanning speed of 240 nm min^{-1}. Tris–HCl buffer solution of chlorantraniliprole in corresponding concentrations was subtracted from all measurements.

2.2.2. Time-resolved fluorescence

Time-resolved fluorescence was collected with a FL920P spectrometer (Edinburgh, UK), using the time-correlated single photon counting system with a hydrogen flash lamp excitation source, in air equilibrated solution at an ambient temperature. The excitation wavelength was 295 nm and the number of counts performed in the channel of maximum intensity was 1000. Fluorescence lifetime decay curves were analyzed with a nonlinear least-squares iterative method using the accompanying software from Edinburgh, or the Edinburgh FAST software, and deconvolution of the instrumental response time from the experimental data. The resolution limit after deconvolution was 0.2 ns. Goodness of fits were assessed from χ^2 criterion (0.9–1.2) and visual inspection of the residuals of the fitted functions to the data. Average fluorescence lifetime (τ) for multiexponential function fittings were from the following [26]:

$$I(t)=\sum_i A_i e^{-t/\tau_i} \quad (1)$$

where τ_i is the fluorescence lifetime and A_i is their relative amplitude, with i as the variable from 1 to 3.

2.2.3. Site marker competitive experiments

Binding location studies between HSA and chlorantraniliprole in the presence of four classical site markers (warfarin, ibuprofen, digitoxin, and hemin) were executed using the fluorescence titration approach. The concentration of HSA and site markers were held in equimolar (1.0×10^{-6} M), then chlorantraniliprole was added to the HSA-site markers mixtures. An excitation wavelength of 295 nm was chosen and the fluorescence emission wavelength was determined from 300 to 500 nm.

2.2.4. CD spectra

Far-UV CD spectra were examined with a Jasco-810 spectropolarimeter (Jasco, Japan) equipped with a microcomputer, the instrument was calibrated with d-10-camphorsulfonic acid. The CD measurements were carried out at 298 K with a thermostatically controlled cell holder attached to a Neslab RTE-110 water bath with an accuracy of ±0.1 °C. Each spectrum was conducted