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Spectroscopic investigation of the interactions of carbofuran and amitrol herbicides with human serum albumin

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ABSTRACT

In this study, various spectroscopic techniques including UV absorption, fluorescence and synchronous fluorescence spectroscopy were used to examine the interactions of carbofuran (CF) and amitrol (AMT) herbicides with human serum albumin (HSA). The results of spectroscopic experiments illustrated that CF was bound by HSA, on the other hand there was no interaction between HSA and AMT molecules. In HSA–CF system, static quenching mechanism was responsible for the fluorescence quenching of HSA. The Stern–Volmer constant and binding constant decreased with increasing temperature. This means that an increase in temperature reduces the stability of HSA–CF complex. In HSA–CF system, the number of binding site on protein was found to be one. From the thermodynamic parameters, enthalpy change (ΔH) and entropy change (ΔS) were calculated as -22.30 kJ mol^{-1} and -10.70 J mol^{-1} K^{-1}, respectively, which indicated that the interaction forces between HSA and CF molecules were mainly hydrogen bonding and van der Waals forces. The conformational change in the protein structure was investigated by synchronous fluorescence spectroscopy. According to the results of synchronous fluorescence analysis, there was a change in the protein structure owing to the interaction of CF with HSA.

1. Introduction

Herbicides are commonly used against crop diseases in many agricultural areas [1]. When these chemicals are applied on crops; the droplets of herbicides fall on soil, plant, and water. Although some part of herbicides stays in the applied area, the major part of them is transported to various environmental places [2]. Therefore, herbicides can contaminate surface and ground water and lead to environmental pollution [3]. When herbicides are taken directly or indirectly by humans via food and drinking water and through the air after aerosol application, these compounds may be harmful for human health [4]. The toxicokinetic behaviors of herbicides in human body mainly depend on the binding phenomena. The distribution and toxicity of hazardous chemicals such as herbicides are affected from the interactions of these substances with carrier plasma protein [5,6].

Human serum albumin (HSA) is the most abundant protein in blood and acts as the major transport protein for many molecules. It has a single polypeptide chain of 585 amino acids and only one tryptophan residue (Trp-214). Serum albumin contains 17 disulfide bridges. It is a globular protein composed of three structurally similar domains (I, II, and III). Each domain can be divided into two subdomains A and B. HSA has many physiological functions. This protein contributes significantly to colloidal osmotic blood pressure and is responsible for the maintenance of blood pH. It is the principal carrier of many compounds. Additionally, HSA performs other functions such as sequestering oxygen free radicals and inactivating various toxic lipophilic metabolites [7,8]. The distributions and metabolisms of small molecules in the body depend on the binding degree to serum albumin. Therefore, generally HSA has been used as a model protein for investigating the protein–ligand binding mechanisms.

HSA can control the extent and duration of herbicidal actions in human body [5]. The concentrations of various ligands such as herbicides can show a decrease in blood due to the binding of these substances to HSA. The substances bound to protein are inactive. The level of free herbicide concentration in blood determines the toxicity degree of herbicide. The interactions of herbicides with HSA are of toxicological importance, because the binding of herbicide molecules by HSA controls the free and active concentrations of herbicides in blood and therefore, affects the extent and duration of toxicological actions [9]. When the binding strength of herbicides to HSA is high, the free concentration of herbicides for the toxic action displays a decrease in parallel to increasing affinity degree. Generally, the binding phenomenon is a reversible process, so the strength of interaction between protein

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and herbicide molecules is very important [10]. Furthermore, the binding of various herbicides to a protein molecule may cause some conformational changes in the secondary structure of protein. When a conformational change occurs in the protein structure, some alterations may be observed in the normal functions of protein [11].

Spectroscopic techniques have become popular in protein–ligand interaction studies because of their high sensivity, rapidity and easy of implementation. UV–visible, fluorescence, synchronous fluorescence and circular dichroism spectroscopy, equilibrium dialysis and potentiometry have been used to investigate the binding of ligand molecules to proteins. Among these methods, equilibrium dialysis is used widely; but it requires the analysis of free and total herbicide concentration and takes a long time [12]. In potentiometric method, ion selective electrodes are used [13,14]. These electrodes have the lack of selectivity for herbicides. Fluorescence techniques are very helpful in protein–herbicide interactions, because they can provide some useful information about the binding of small molecules to protein such as binding mechanism, binding mode, binding constants and binding sites [12,15]. Synchronous fluorescence spectroscopy reflects the conformation changes of proteins after protein–ligand interaction [16].

In this study, to investigate the protein–herbicide interactions, carbofuran (CF) and amitrol (AMT) have been selected as model herbicides, because these chemicals are commonly used in agricultural applications and have been frequently detected as water contaminant in America, Europe and Asia [3,17]. From these herbicides, CF, a carbamate group pesticide, is widely used to control soil and leaf-feeding insects and nematodes [18]. It has an important usage in various crops such as potatoes, corn, rice, alfalfa, grapes and other foodstuffs [1]. CF is an active acetylcholinesterase inhibitor [17]. Its toxicity is very high in animals [17,18]. This herbicide decreases the micronucleus formation in mouse erythroblast and causes weak mutagenic response in hamster ovary cells. In addition, CF induces chromosome aberrations, micronucleus formation and sperm abnormalities in mouse [3]. Owing to its common use in agriculture and relatively good solubility in water, CF can contaminate water, foods, soil and air [3,18]. In addition, CF is known to be more persistent than other carbamate or organophosphate insecticides [19]. The half live of CF depends on the pH value of water and found to be 690 days at pH 5 and 7 days at pH 9. CF is also nonbiodegradable [20]. Amitrol (AMT) is a toxic herbicide used to control weeds in agriculture [21]. It has very high solubility in water [22]. AMT leads to liver cancer in animals and is resistant to degradation by abiotic hydrolysis and aqueous photolysis.

CF and AMT herbicides have potential toxicological risk to public health [23]. Therefore, it is necessary to investigate the interactions of CF and AMT with HSA. Although CF and AMT are widely used in agricultural applications and detected as water contaminant in many countries, the interactions of these herbicides with HSA protein have not been investigated. Therefore, in this study, to evaluate the toxicity of CF and AMT at the protein level, the interactions of these herbicides with HSA have been characterized by spectroscopic methods [4].

The aim of this study is to determine the affinity of CF and AMT herbicides to HSA protein, to calculate binding constants, the numbers of binding sites and thermodynamic parameters, to explain the interaction mechanisms between protein and herbicide molecules and also to examine the effect of herbicide molecules on the structure of HSA.

2. Materials and methods

2.1. Materials

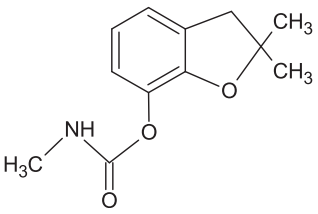
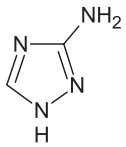
Human serum albumin (HSA), carbofuran (CF), amitrol (AMT), tris(hydroxymethyl)aminomethane and NaCl were purchased from Sigma-Aldrich. The average molecular weight value of 66,500 g/mol was used in the preparation of protein solutions. The properties of herbicides are given in Table 1. Prior to each experiment, all solutions were prepared freshly in tris(hydroxymethyl)aminomethane buffer solution (0.05 M, pH 7.4) containing 0.1 M NaCl. Deionized water was used in the preparation of buffer solution.

2.2. Absorption and fluorescence measurements

Firstly, the stock solutions of HSA (2.0×10^{-5} M), CF (5.0×10^{-4} M) and AMT (1.0×10^{-3} M) were prepared with 0.05 M tris (hydroxymethyl)aminomethane buffer solution containing 0.1 M NaCl to obtain physiological pH value of 7.4 and the ionic strength of solutions was adjusted. Afterwards, these solutions were kept in a refrigerator at 4–6 °C. Then, a series of HSA–herbicide mixture solution containing various CF or AMT concentrations was prepared from the stock solutions of protein and herbicides to investigate the interactions between protein and herbicide molecules. In protein–herbicide interaction experiments, the concentration of HSA was kept constant at 2.0×10^{-6} M and herbicide concentrations were varied in the range of $0.0–50\times10^{-6}$ M.

UV absorption measurements of HSA in the absence and presence of herbicides were made by a Varian Cary 5000 NIR–UV–vis spectrophotometer using a matched pair of quartz cells (path length: 1 cm) in a peltier thermostatted cell holder at 288.15, 298.15, 310.15 and 318.15 ($\pm$0.10) K.

Table 1
Characteristics of carbofuran (CF) and amitrol (AMT).

Property	Carbofuran	Amitrol
Chemical name (IUPAC)	2,3-dihydro-2-2-dimethyl-7-benzofuranol N-methylcarbamate	1,2,4-triazol-3-amine
Chemical formula	$C_{12}H_{15}NO_3$	$C_2H_4N_2$
Molecular weight (g/mol)	221.25	84.08
Application	Herbicidal	Herbicidal
Solubility in water at 25 °C	0.33 g/L	280 g/L
Log K_{ow}[a] at 25 °C	1.7	−0.97
Chemical structure		

[a] K_{ow}: Octanol–water partition coefficient.