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Understanding the fate of human serum albumin upon interaction with edifenphos: Biophysical and biochemical approaches

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ABSTRACT

Edifenphos (EDF), an important organophosphate fungicide used in agriculture, is a great threat to human health and environment. To assess the toxicity of EDF at the level of protein molecule, the effect of EDF on human serum albumin (HSA) was investigated by biophysical and biochemical approaches. EDF-HSA complex is formed as a result of static quenching as revealed by the intrinsic fluorescence analysis. Thermodynamic analysis of the binding data suggests involvement of hydrophobic interactions in EDF-HSA complex formation, which is in line with molecular docking results. Moreover, thermodynamic parameters of binding between EDF and HSA suggest entropy-driven spontaneous interaction, presumably dominated by hydrophobic forces. Further, binding site of EDF seems to have been located within sub-domain IIA of HSA. EDF binding to HSA decreases its alpha helical content as analyzed by CD spectra. Marked micro-environmental changes around tryptophan/tyrosine residues in HSA upon EDF binding were recorded via three-dimensional fluorescence spectroscopy. Substantial release of protein carbonyl from HSA as a result of EDF treatment suggested involvement of ROS in EDF induced protein damage. This work is expected to provide some leads toward EDF induced toxicity in humans and would be helpful in reinforcing the check on food safety.

1. Introduction

Edifenphos [O-ethyl *S*, *S*-diphenyl dithiophosphate] (Fig.1) is an organophosphate pesticide (OP) which has been reported to be accumulated in many agricultural products, such as fruits, vegetables, and grains especially rice [1,2]. OPs have been categorized as toxicological Class1 toxicants by the U.S Environmental Protection Agency [3]. Despite the beneficial effects of edifenphos (EDF) in food crops production, potential toxicity risks are also associated with EDF exposure such as decreased blood cholinesterase activity, fatty degeneration of liver and immune suppression [4,5]. Moreover, EDF induces clastogenic effects in bone marrow cells [6] and chromosomal aberrations [7] in mouse. The widespread use of fungicides has generated increasing concerns about their detrimental effects on human health and nature [8,9]. We have recently studied the interaction of DNA with EDF and elucidated its detailed mode of binding [10].

In recent years, numerous studies have reported the binding of various pesticides with HSA and a triazole fungicide has also been shown to induce some changes in structure and function of the protein [11–16]. Organophosphate fungicides get easily absorbed in human body through lungs, skin and gastrointestinal tract and act primarily by disrupting the protein phosphorylation pathways associated with the metabolic regulation, hormone signaling, neuronal functions, cell survival and cell death [17–19].

HSA is the major and most abundant soluble protein in the human circulatory system with varying physiological functions, such as maintaining the osmotic pressure and blood pH and as carrier to transport various exogenous and endogenous substances [20,21]. Crystallographic studies revealed HSA to be a monomeric heart shaped protein with three homologous α-helical domains (I, II and III), each composed of subdomains (A and B) and the primary ligand-binding sites that were located in hydrophobic subdomains IIA and IIIA [22,23].

To the best of our knowledge, not a single study has been carried out to evaluate the toxic effects of EDF at the molecular level of protein. Therefore, our aim in this study was to investigate the EDF-HSA interaction and to decipher the binding mechanism to gain an insight into the toxicological mechanism at the molecular level. In order to achieve this, we employed various spectroscopic and biochemical techniques.

2. Materials and methods

2.1. Materials

Human serum albumin (99%) and edifenphos (99.7%) were

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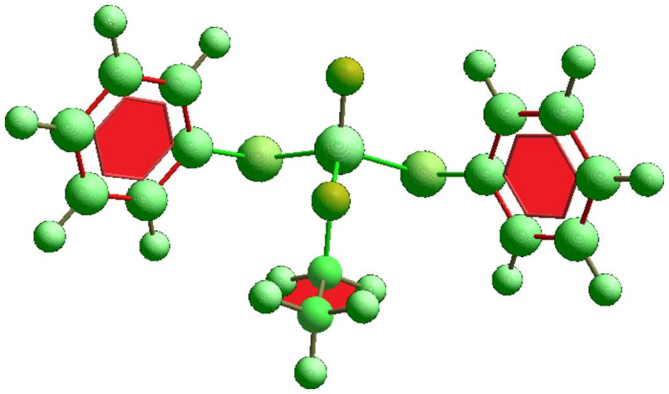

Fig. 1. Chemical structures of edifenphos(EDF) fungicide.

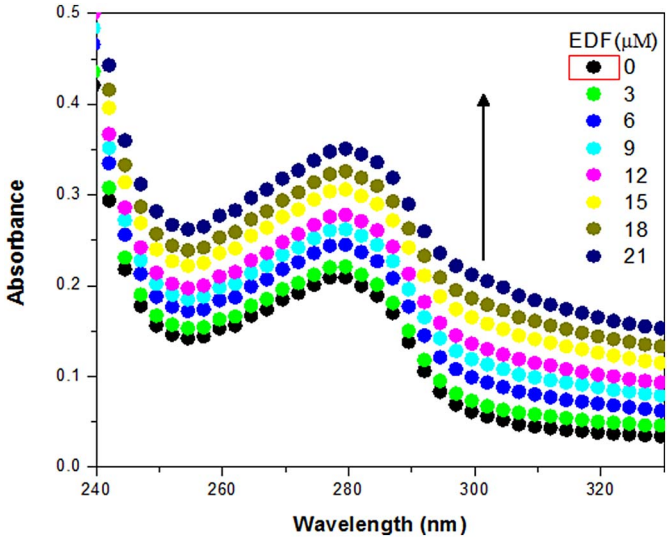

Fig. 2. The UV–visspectra of HSA (2 μM) in the presence of different concentrations of EDF (3–21 μM) at 25 °C and pH 7.4.

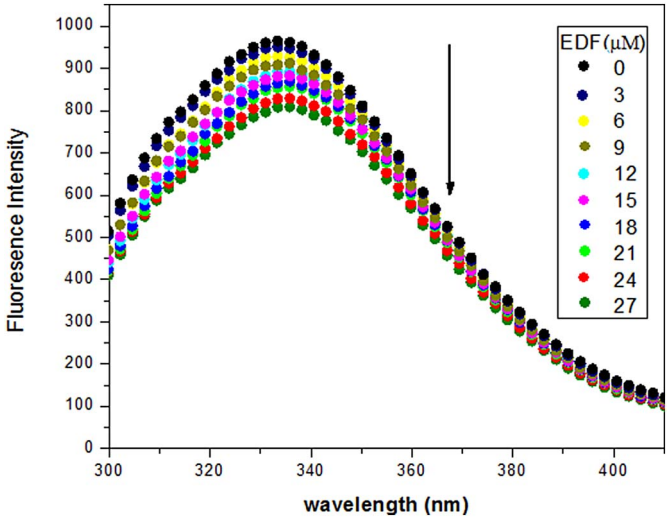

Fig. 3. Emission spectra of HSA (2 μM) in the presence of different concentrations of EDF (3–27 μM) at 25 °C and pH 7.4.

purchased from Sigma-Aldrich (Germany). Warfarin and ibuprofen were the products of Ranbaxy Laboratories Ltd., India. Other chemicals and reagents were of analytical grade. The HSA stock solution (250 μM) was prepared in Tris–HCl buffer solution (0.2 M Tris, pH 7.4) and

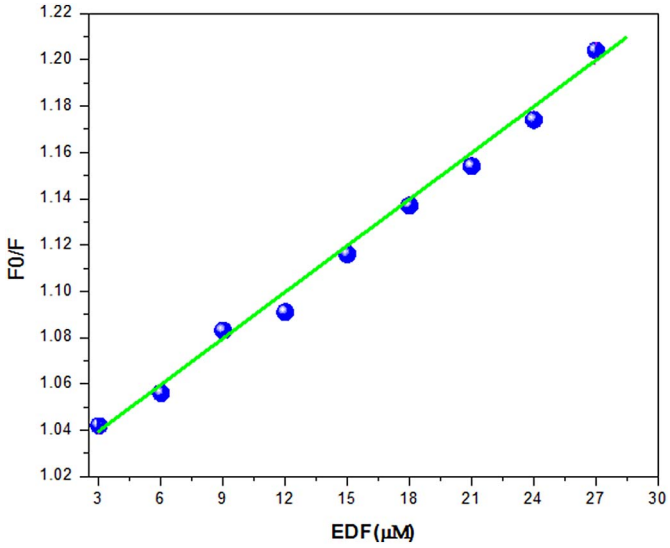

Fig. 4. Stern-Volmer plot for fluorescence quenching of the EDF-HSA system.

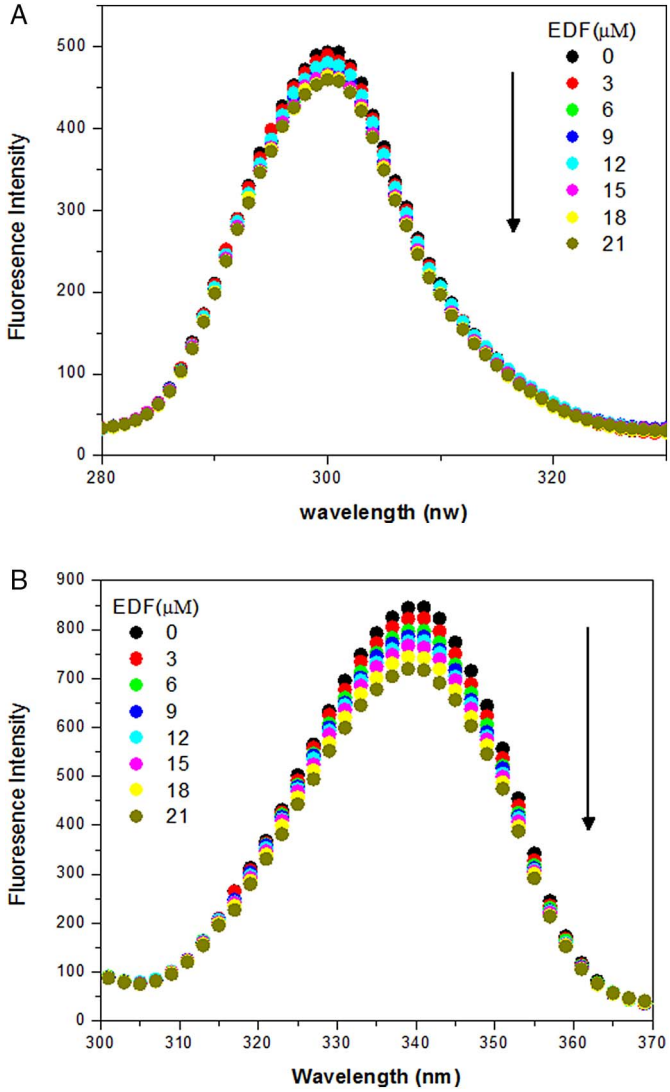

Fig. 5. Synchronous fluorescence spectra of HSA (2 μM) with incremental addition of EDF (3–21 μM) at (A) Δ λ = 15 nm and (B)Δ λ = 60 nm at 25 °C.