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Assessing the potential toxic effect of one persistent organic pollutant: Non-covalent interaction of dicofol with the enzyme trypsin

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ABSTRACT

Because of the widespread concern that persistent organic pollutants (POPs) may be adversely affecting the health of humans, reliable assessing their toxic effects is urgently needed. We selectively study the interaction between dicofol (DCF) and trypsin by steady state and time resolved fluorescence quenching measurements and UV–visible absorption spectroscopy under physiological conditions as well as applying molecular docking method to establish the interaction model. The fluorescence results indicate DCF can spontaneously form a complex with trypsin mainly by hydrogen bond with only one binding site, which had been validated in molecular docking. The conformational change of trypsin was proved by UV–visible absorption and synchronous fluorescence spectroscopy indicating a red shift of carbonyl absorption peak. All the results indicated DCF had potential toxic effects on both the structure and activity of the enzyme trypsin and the effects enhanced with the increasing concentration of DCF.

1. Introduction

Information of biomacromolecule interaction plays a role of importance in interpreting the cellular processes including signal transduction, gene regulation and enzyme reactions [1]. The water-soluble globular protein, trypsin (EC 3.4.21.4, structure shown in Scheme 1) is a serine protease present as a zymogen (trypsinogen) in the pancreas of all vertebrates. Trypsin, which functions in digestion and other essential biological processes, has a molecular mass of 23,300 Da and consists of 223 amino acid residues. A trypsin molecule consists of two domains of nearly equal size, the major constituent of each being a set of six anti-parallel strands of polypeptide chain laced together into a β-sheet unit by a network of H-bonds [2,3]. Intake of any contaminants, however, potentially affects the activity of the enzyme in vivo.

The environmental chemistry and ecotoxicology of persistent organic pollutants (POPs) are fascinating areas of scientific research [4]. Among the important classes of POP chemicals are different organochlorine pesticides, which continue to be of public and scientific concerns because they might contribute to raising the risk of breast cancer incidence in several populations through their estrogenic actions [5]. Dicofol (DCF), trade name Kelthane, is a nonsystemic acaricide extensively used for controlling mites that damage cotton, fruit trees, and vegetables [6]. Its acaricidal active ingredient is 2,2,2-trichloro-1,1-bis (4-chlorophenyl) ethanol. It is usually synthesized from technical DDT (1,1′-(2,2,2-trichloroethylidene) bis [4-chlorobenzene]) [7], and is structurally similar to DDT (see Scheme 2). Besides, the commercial product of DCF contains a certain amount of o,p′ and p,p′ isomers of DDT, DDD (1,1′-(2,2′-dichloroethylidene)bis[4-chlorbenzene]), etc. [5]. Although some researchers reported that DCF could induce eggshell changes in a species of the American kestrels (Falco sparverius) [8] and had caused the failure in recruitment of alligators on Lake Apopka [9], DCF is still used as insecticide in many countries including the United States and China. However, in vitro study about the potential toxicity of DCF at the molecular level has not been reported yet. In vivo assays permit the detection of effects resulting from multiple mechanisms but may give indications only of gross effects and reveal little about mechanisms of activity. In vitro assays, however, can provide valuable insights on mechanisms of the action. In this work, the effect of DCF on the activity and conformation of trypsin and the interaction mechanism of DCF with this enzyme were investigated with the help of a number of techniques such as UV–vis absorption, fluorescence spectroscopy and molecular modeling methods. Such processes have been performed to elucidate the effect of DCF on the enzyme and its relationship to human health risks.

Abbreviations: DCF, dicofol; Trp, tryptophan; Cys, cysteine.

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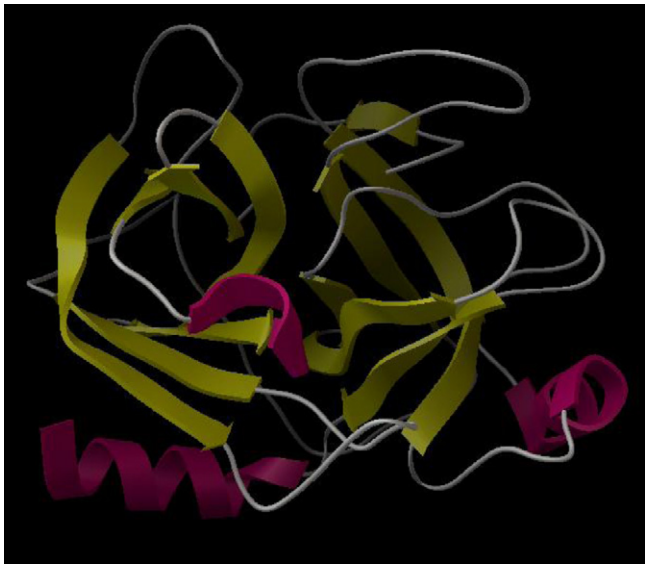


Scheme 1. Molecular structure of trypsin (PDB code 2ZQ1). Different types of the secondary structure of trypsin are color-coded as follows: α-helix: pink, β-pleated sheet: yellow, β-turn and random coil: white. (For interpretation of the references to color in this scheme legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Material

The globular protein, trypsin from bovine pancreas was a crystalline powder, a product of AMRESCO. Trypsin was dissolved in ultrapure water to form a 5×10^{-5} mol/L solution, then preserved at 0–4 °C and diluted as required.

The used DCF was prepared to a stock solution (5×10^{-4} mol/L) by dissolving 0.0187 g DCF (Aladdin Chemistry Co. Ltd.) in 100 ml of methanol.

BAEE (N-α-benzoyl-L-arginine ethyl ester, from Sinopharm Chemical Reagent, BR) was dissolved in ultrapure water to form a 1.0×10^{-2} mol/L solution.

Phosphate buffer (0.2 mol/L, mixture of $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 12H_2O$, pH 7.6) was used to control pH. $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 12H_2O$ were of analytical reagent grade, obtained from Tianjing Damao Chemical Reagent Factory.

2.2. Apparatus and methods

The fluorescence emission spectra of tryptophan residue in trypsin–DCF systems were measured using an F-4600 spectrofluorimeter (Hitachi, Japan). Spectrophotometric measurement was made using a UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan). The pH measurements were made with a pHs-3 C acidity meter (Pengshun, Shanghai, China).

2.2.1. UV–visible absorption spectra

UV–visible spectra were collected in the range of 190–350 nm with 1.0 cm × 1.0 cm quartz cuvettes used for sample holding.

2.2.2. Fluorescence measurements

The fluorescence measurements were carried out as follows: 1.0 mL of 0.2 mol/L phosphate buffer (pH 7.6) and 1 mL 5×10^{-5} mol/L trypsin were added to each of a series of 10 mL test tubes, respectively. Samples were equilibrated by allowing them to stand at room temperature for 30 min. The spectra were then measured with fluorescence excited at 278 nm and emission wavelengths scans recorded from 285 to 450 nm. The synchronous fluorescence spectra were measured at $\lambda_{ex} = 310$ nm, $\Delta\lambda = 15$ nm, and $\Delta\lambda = 60$ nm.

The fluorescence lifetimes of trypsin in the absence and presence of DCF were measured ($\lambda_{ex} = 278$ nm, $\lambda_{em} = 330$ nm) on the FLS920 Combined Fluorescence Lifetime and Stead State Spectrophotometer (Edinburgh, England).

2.2.3. Determination of trypsin activity

The activity of trypsin was measured using BAEE as the substrate. Trypsin can catalyze the hydrolysis of BAEE into N-benzoyl-L-arginine, the UV absorption of which is far stronger than that of BAEE. The enzyme activity was determined based on the increase in absorbance at 253 nm in 2×10^{-2} mol/L buffered phosphate medium (pH 7.6) containing 1×10^{-3} mol/L BAEE before and after the addition of trypsin.

The velocity of the enzymatic reaction (ν, mmol L^{-1} s^{-1}) was calculated using the following equation [10]

$$\nu = \frac{2 \times A_{253\ nm}}{\varepsilon \times 10^{-3} \times 60} \quad (1)$$

where $A_{253\,nm}$ is the absorbance of the reaction system within 30 s, deducted the absorbance of DCF when DCF exists in the system. ε is the molar extinction coefficient of BAEE.

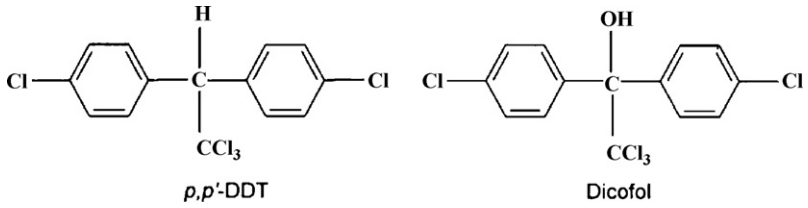
2.2.4. Molecular modeling study

The PDB file of trypsin was taken from RCSB Protein Data Bank (PDB ID 2ZQ1) and the initial structure of dicofol molecule was gained from ZINC Database for Virtual Screening (ZINC ID 2041041). Docking calculations were carried out using AutoDock software. The MMFF94 force field was used for energy minimization of the ligand molecular (dicofol) using Docking Server. Gasteiger partial charges were added to the ligand atoms. Nonpolar hydrogen atoms were merged, and rotatable bonds were defined.

3. Results and discussion

3.1. Conformation investigation

Spectroscopy is an effective and efficient method to investigation the conformational changes in proteins for it allows non-intrusive measurements of substances in low concentration under physiological conditions [11]. The intrinsic fluorophores are



Scheme 2. The structure of p,p′-DDT and dicofol.