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Study on the interaction of catalase with pesticides by flow injection chemiluminescence and molecular docking

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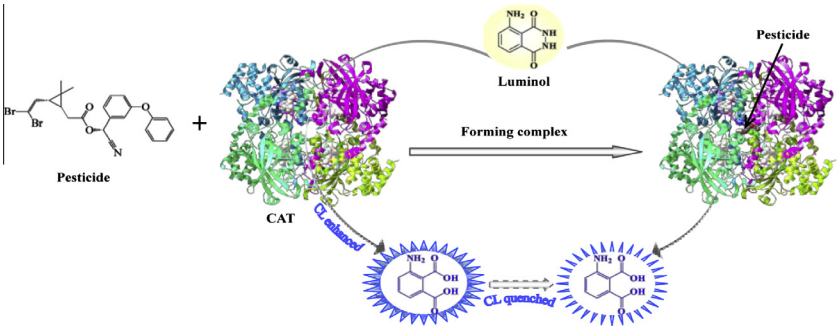
HIGHLIGHTS

- The luminescence behaviors of CAT–pesticides are studied by FI–CL & MD analysis.
- The binding and thermodynamic parameters of pesticides to CAT are given.
- The relationship of binding ability *vs.* structure of pesticide to CAT is analyzed.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction mechanisms of catalase (CAT) with pesticides (including organophosphates: disulfoton, isofenphos-methyl, malathion, isocarbophos, dimethoate, dipterex, methamidophos and acephate; carbamates: carbaryl and methomyl; pyrethroids: fenvalerate and deltamethrin) were first investigated by flow injection (FI) chemiluminescence (CL) analysis and molecular docking. By homemade FI–CL model of $\lg[(I_0 - I)/I] = \lg K + n\lg[D]$, it was found that the binding processes of pesticides to CAT were spontaneous with the apparent binding constants K of 10^3–10^5 L mol^{-1} and the numbers of binding sites about 1.0. The binding abilities of pesticides to CAT followed the order: fenvalerate > deltamethrin > disulfoton > isofenphos-methyl > carbaryl > malathion > isocarbophos > dimethoate > dipterex > acephate > methomyl > methamidophos, which was generally similar to the order of determination sensitivity of pesticides. The thermodynamic parameters revealed that CAT bound with hydrophobic pesticides by hydrophobic interaction force, and with hydrophilic pesticides by hydrogen bond and van der Waals force. The pesticides to CAT molecular docking study showed that pesticides could enter into the cavity locating among the four subdomains of CAT, giving the specific amino acid residues and hydrogen bonds involved in CAT–pesticides interaction. It was also found that the lg$K$ values of pesticides to CAT increased regularly with increasing lg$P$, M_r, MR and MV, suggesting that the hydrophobicity and steric property of pesticide played essential roles in its binding to CAT.

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1. Introduction

The massive usage of pesticides in agriculture leads to the widely spread of pesticides into the environment ranging from soil to foodstuff, resulting in continued wildlife and human exposure (Köck-Schulmeyer et al., 2012; Rutsaert et al., 2013; Abrantes et al., 2010). Organophosphate (OP), carbamates (CM) and pyrethroid (PY) are primarily utilized pesticides to protect crops or gardens from insects (Gupta and Milatovic, 2012; Schleier and Peterson, 2011). OP and CM, which are commonly known as anticholinesterase agents, are being phased out of use gradually due to biomagnification or high non-target toxicity, and PY as neurotoxic agents have been widely used nowadays. Because the long-term pesticide exposure might pose the potential risks of health effects on no-targets mainly via the interactions of proteins with pesticides, it is of great importance to investigate the interaction behavior between protein and pesticide at molecular level.

The protein–small molecule interaction has become a hot spot in the fields of biology (Azami-Movahed et al., 2013; Agostino et al., 2013), medicine (Khan et al., 2012; Yoshimura et al., 2013; Tan and Song, 2014), environment (Xie et al., 2010; Akiyoshi et al., 2012) and chemistry (Dobretsov et al., 2013; Saha et al., 2013; Wang et al., 2013) in recent decades. Catalase (CAT, MW ~240 kDa) presents in the perixisomes of nearly all aerobic cells and serves to protect tissues against damage from hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water without the production of free radicals (Schroeder et al., 1982). It is also one of the first enzymes proposed to be an effective marker of oxidative stress (Livingstone et al., 1993). CAT exists as a dumbbell-shaped tetramer of four identical subunits, each subunit formed by a single polypeptide chain with a heme as a prosthetic group (Reid et al., 1981). The interaction of CAT with small molecule has been studied *in vitro* by approaches including spectrometry (Zhao et al., 2007; Li et al., 2008; Zhang and Jin, 2008), calorimetry (Zhao et al., 2007) and equilibrium dialysis (Ruso et al., 2001), etc. Yet, no report on the interactions of CAT with pesticides using chemiluminescence (CL) method combined with flow injection analysis (FIA) has been described. In this current work, it was first found that pesticides (including disulfoton, isofenphos-methyl, malathion, isocarbophos, dimethoate, dipterex, methamidophos, acephate, carbaryl, methomyl, fenvalerate and deltamethrin, Scheme S1) obviously quenched the CL intensity from luminol–CAT system and the CL intensity decrements were proportional to the logarithm of pesticides' concentrations within ranges from 0.3 to 30 nmol L^{-1}. The binding parameters of CAT with pesticides were obtained using the FI–CL model of protein–small molecule interaction, $\lg[(I_0 - I)/I] = \lg K + n\lg[D]$ (Wang and Song, 2010), giving the binding ability of pesticides to CAT, and the major interaction force was speculated by the thermodynamic parameters of CAT–pesticides association process.

It is well known that molecular docking (MD) is a method to predict and understand molecular recognition, find the predominant binding mode and binding affinity between the protein and ligand (Brink and Exner, 2009; Lie et al., 2011), and give a three-dimensional structural explanation of the protein–ligand interaction (Gumede et al., 2012; Hou et al., 2013). In this paper, by MD the specific binding sites of pesticides on CAT and the binding mode were obtained, which was a beneficial complementary explanation to the CL results for understanding the interaction mechanism of CAT with pesticides.

2. Material and methods

2.1. Reagents

All reagents were of analytical pure grade, and the deionized water used in this work was passed through a Milli-Q system (Millipore, Bedford, MA, USA, 18.2 MΩ cm). Luminol (Fluka, Biochemika, Switzerland) and CAT from bovine liver (C40, Sigma–Aldrich, St. Louis, MO, USA) were used as received without further purification. Pesticides (OP: disulfoton, isofenphos-methyl, malathion, isocarbophos, dimethoate, dipterex, acephate and methamidophos; CM: carbaryl and methomyl; PY: fenvalerate and deltamethrin) with a concentration of 5 mg mL^{-1} (ethanol as solvent) were supplied by Material Evidence Identifying Center of Xi'an Public Security Bureau, China.

Luminol stock solution of 2.5×10^{-2} mol L^{-1} was prepared by dissolving 0.44 g luminol in 100 mL of 0.1 mol L^{-1} NaOH solution in a brown calibrated flask. The stock solution of 5.0×10^{-6} mol L^{-1} CAT was prepared by dissolving 30.0 mg lyophilized powder in 25.00 mL deionized water. All stock solutions of pesticides with the concentration of 1.0×10^{-4} mol L^{-1} were prepared in deionized water. Working standard solutions of pesticides were prepared daily from the above stock solutions by appropriate dilution as required. All of the stock solutions were stored at 4 °C.

2.2. Apparatus

The FI mode was shown in Fig. S1. The FI–CL apparatus (Xi'an Remex Analysis Instrument Co. Ltd., Xi'an, China) consisted of the sampling system (IFFM-E), the photomultiplier tube (PMT), and the PC with an IFFM-E client system (Remax, Xi'an, China). Poly Tetra Fluoro Ethylene (PTFE) tubing (1.0 mm i.d.) was used to carry and deliver the solutions. A six-way valve with a loop of 100 μL was used for quantitatively injecting luminol into carrier stream. The CL detector contained a flow cell made by coiling 15 cm of colorless glass tube (1.0 mm i.d.) into a spiral disk shape with a diameter of 2.0 cm and placed close to the PMT, and it is important to ensure that the sample compartment and PMT were light tight. The CL signal produced in flow cell was detected by the PMT without wavelength discrimination, with output recorded by computer. The temperature of the solutions was controlled in a water bath ($T \pm 0.1$ °C).

The F-4500 fluorophotometer (Hitachi, Kyoto, Japan) was applied to fluorescence measurements of CAT with pesticides (Supplementary Material).

2.3. General procedures

Each solution was placed in a water bath to control the temperature. Before the running step was started, the whole flow system was washed using deionized water until a stable baseline was recorded. With a flow rate of 2.0 mL min^{-1} on each flow line, 100 μL luminol was quantitatively injected into the carrier stream by the six-way valve and then merged with the mixed solution of CAT and pesticide. The whole mixture was thereafter delivered into the flow cell in an alkaline medium to produce CL emission. The CL signal was detected by the PMT with the negative voltage of 700 V. The concentration of pesticide was quantified by the decrement of CL intensity.

2.4. The optimization of CL experimental conditions

The effect of luminol concentration on the CL intensity was tested over the ranges of 5.0×10^{-7} to 5.0×10^{-4} mol L^{-1}, it was found that the CL signal increased steadily with increasing luminol concentration until 2.5×10^{-5} mol L^{-1}, and tended to be stable thereafter. Therefore, 2.5×10^{-5} mol L^{-1} luminol was chosen as the optimum concentration. Due to the alkaline medium-dependent nature of the luminol CL reaction, NaOH was introduced into the luminol solution to enhance the sensitivity of the system. A series of NaOH solutions over the ranges of 5.0×10^{-3} to 0.2 mol L^{-1} was examined, and the concentration of