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Colorimetric detection of dichlorvos using polydiacetylene vesicles with acetylcholinesterase and cationic surfactants

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ABSTRACT

Widespread use of dichlorvos in agriculture has posed serious concern for food and water contamination. A new colorimetric method for the detection of dichlorvos based on polydiacethylene and acetylcholinesterase inhibition is developed. The blue-to-red color transition of poly(10,12-pentacosadynoic acid) vesicles can be induced by myristoylcholine which is enzymatically hydrolyzed by acetylcholinesterase to myristic acid and choline to prevent the color transition. In the presence of dichlorvos, the hydrolytic activity of the enzyme is inhibited that the blue-to-red color transition is restored with a linear correlation to the dichlorvos concentration. Using UV–vis absorption spectrometer, the limit of dichlorvos detection is 6.7 ppb. A naked eye detection of 50 ppb dichlorvos is achievable by using dimiristoylphosphatidylcholine to the diacetylene mixed lipid vesicles.

1. Introduction

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is an organophosphate pesticide which have been widely used for crop protection in agriculture and residential settings (Kerem and Kalender, 2012). Dichlorvos is highly toxic and possesses long term effects on mammals that include severe liver dysfunction (Binukumar et al., 2010) and cancer (Xu et al., 2010). As a potent inhibitor for acetylcholinesterase (AChE), an enzyme regulating acetylcholine neurotransmitter, dichlorvos can also cause acute neurotoxic sickness including nausea, vomiting, drowsiness, headache, convulsions, and coma at high concentrations (EPA, 2012). The World Health Organization (WHO) classified dichlorvos in group IIb, the extremely hazardous compounds (Duarte et al., 2012). A maximum residue limit (MRL) assigned by Environmental Protection Agency (EPA) for dichlorvos in natural water resources is 0.01 mg/L. In order to determine the level of dichlorvos in water resources and agriculture products, a convenient and sensitive method for determination of dichlorvos is indispensable.

In well-equipped laboratories, chromatographic methods such as GC and HPLC (Xu et al., 2010) are the method of choice for highly sensitive and accurate determination of dichlorvos. However, these methods require expensive instruments and skillful operators that is not suitable for on-site analysis (Jin et al., 2004; Kumaran and Tran-Minh, 1992; Roger et al., 1991; Skládal, 1992; Tran-Minh et al., 1990). To achieve the on-site application, sensors for organophosphates have been developed based on nanosensors (Liu et al., 2008), chemiluminescence (Liu et al., 2014), fluorescence (Thakur et al., 2012; Wang et al., 2009) and colorimetric method (Lee et al., 2012). The colorimetric method provides an advantageous feature for on-site screening in its potential to allow naked eye detection.

A polydiacetylene (PDA) is an ene-yne conjugated polymer which is formed via topological 1,4 addition polymerization of the corresponding monomer, initiated by ultraviolet (UV) light or γ-rays irradiation (Deckert et al., 1995; Chance, 1980; Lim et al., 2008; Rubner et al., 1987). It has been successfully used as colorimetric agents for various sensing applications such as temperature (Carpick et al., 2004, 2000; Chen and Yoon, 2011; Gou et al., 2010; Kim et al., 2005; Lee et al., 2002; Rubner et al., 1987; Wang et al., 2007; Ye et al., 2008), mechanical stresses (Kwon et al., 2010; Miyano and Hasegawa, 1991), pH (Charoenthai et al., 2011; Li et al., 2012; Seo et al., 2013), and chemicals (Jiang et al., 2010; Jung et al., 2006; Potisatityuenyong et al., 2006; Sabatani et al., 2008; Su, 2006; Su et al., 2004; Takami et al., 2005; Xia et al.,

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2010). The blue-to-red color transition of PDA have been attributed to a release of the conjugated backbone strain or disturbance of the ene-yne planarity in association with the side chain movement (Lee et al., 2002; Lio et al., 1997; Wacharasindhu et al., 2010; Wang et al., 2007).

Recently, oxime functionalized 10,12 pentacosadiynoic acid (PCDA) was developed for sensing organophosphate with the detection limit for diisopropylfluorophosphate of ~160 ppb (Lee et al., 2012). In this article, we would like to present our work on the utilization of the AChE inhibition activity of dichlorvos in conjunction with the blue-to-red color transition of unmodified poly(PCDA) to develop a colorimetric sensor for dichlorvos. In our view, the utilization of AChE enzyme will provide not only specificity but also sensitivity amplification via its catalytic nature.

The strategy in this work is inspired by the report that the color transition of PDAs can be induced by certain cationic surfactants (Chen et al., 2009; Su et al., 2004, 2005). We hypothesized that the cationic choline ester derivative of a fatty acid could induce the blue-to-red color transition of poly(PCDA) vesicles. In the presence of AChE, the ester derivative should be enzymatically hydrolyzed to the corresponding fatty acid and choline (Wang et al., 2009). These hydrolytic products lack of surfactant properties and ability to induce the color change of poly(PCDA). If the system contains dichlorvos, the enzymatic activity of AChE will be inhibited and the color transition of poly(PCDA) will be restored with the colorimetric response proportional to dichlorvos concentration (Fig. 1).

2. Materials and experimental method

2.1. Materials

The diacetylene monomer, PCDA (10,12-Pentacosadiynoic acid, 97% purity), was purchased from Fluka, USA. The monomer was dissolved in CH_2Cl_2, filtered through No. 1 Whatman filter paper and evaporated to dryness prior to use. Other analytical grade solvents and reagent grade chemicals were purchased from Fluka and used as received. Dichlorvos (2,2-dichoroethenyl dimethyl phosphate, 98% purity), AChE (acetylcholinesterase, EC 3.1.1.7, V-S type from electric eel, 2000 units/mg protein) and DMPC (dimyristoylphosphatidycholine) were purchased from Sigma, USA. Generally, Milli-Q water with a resistance of 18.2 MΩ was used as the solvent for preparation and rinsing in all aqueous experiment.

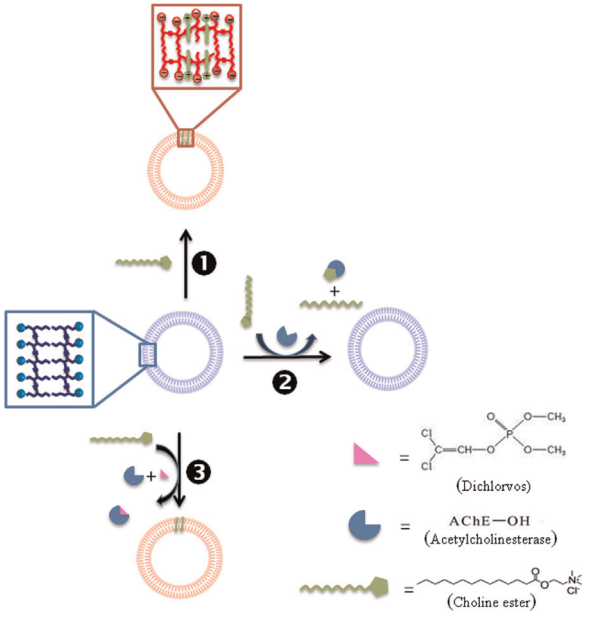

Fig. 1. Schematic illustration of dichlorvos detection based on poly(PCDA) vesicles/AChE/choline ester system: ❶ colorimetric response of poly(PCDA) to choline ester; ❷ colorimetric response of poly(PCDA) to mixture of choline ester and AChE; ❸ colorimetric response of poly(PCDA) to mixture of choline ester, dichlorvos and AChE.

2.2. Preparation of choline ester

The synthesis of choline ester typically involved a dissolution of the fatty acid chloride (9.14 mmol) in dichloromethane (10 mL) and trimethylamine (2 mL) in a round-bottom flask followed by an addition of excess dimethyl ethanol amine (2 mL). The reaction mixture was stirred at room temperature for 4 h before adding distilled water (20 mL). The organic layer was separated and the aqueous phase was extracted with dichloromethane (2 × 20 mL). The combined organic extractant was dried over anhydrous $MgSO_4$ and the solvent was removed by a rotary evaporator followed by a vacuum dry to afford 2-(dimethylamino)ethyl ester of the fatty acid (8.50 mmol, 93% yield). The 2-(dimethylamino)ethyl ester (8.50 mmol) was methylated with methyl iodide (1.57 g, 11.1 mmol) in acetronitrile (10 mL) by stirring the reaction mixture at room temperature for 30 min. The solution was evaporated to dryness to afford the choline ester as white powder. *Lauroyl choline* (3.48 g, 92% yield); ^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 4.54 (b, Hz, 2H), 3.75 (m, 2H), 3.25 (m, 9H), 2.40 (t, J=7.6 Hz, 2H), 1.62 (t, J=7.2 Hz, 2H), 1.28 (m, 16H), 0.89 (t, J=6.6 Hz, 3H). *Myristoyl choline* (3.29 g, 93% yield); ^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 4.56 (m, 2H), 3.78 (m, 2H), 3.27 (s, 9H), 2.42 (t, J=7.6 Hz, 2H), 1.64 (t, J=7.2 Hz, 2H), 1.30 (m, 20H), 0.91 (t, J=6.6 Hz, 3H). *Palmitoyl choline* (3.25 g, 95% yield); ^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 4.52 (m, 2H), 3.71 (m, 2H), 3.21 (s, 9H), 2.38 (t, J=7.6 Hz, 2H), 1.61 (t, J=7.2 Hz, 2H), 1.27 (m, 24H), 0.89 (t, J=6.8 Hz, 3H). *Stearoyl choline* (3.25 g, 95% yield); ^{1}H NMR (CD_3OD, 400 MHz): δ (ppm) 4.52 (m, 2H), 3.7 (m, 2H), 3.20 (s, 9H), 2.38 (t, J=7.6 Hz, 2H), 1.61 (t, J=7.0 Hz, 2H), 1.26 (m, 28H), 0.87 (t, J=6.8 Hz, 3H).

2.3. Preparation of polydiacetylene vesicles

2.3.1. Poly(PCDA) vesicles

PCDA (0.01 mmol) was dissolved in chloroform (2.5 mL) in a 50 mL test tube, and the solution was purged with N_2 gas to dryness. Water (10 mL) was added to the dry film of PCDA and the resulting suspension (1 mM) was sonicated in a sonicator bath (230 W) at 80 °C for 30 min. The suspension became translucent indicating the formation of a colloidal sol of PCDA vesicles. The PCDA sol was kept at 4 °C overnight before being irradiated with 254 nm UV light for 5 min at room temperature to generate a blue poly(PCDA) sol (Potisatityuenyong et al., 2008). The blue sol was filtered through No. 1 Whatman filter paper to remove any undesired large aggregates. For the preparation of a mixed lipid vesicle sol, a saturated fatty acid (i.e. lauric acid, myristic acid, palmitic acid and stearic acid) or a phospholipid (dimyristoylphosphatidycholine, DMPC) was mixed with PCDA at designated PCDA/lipid mole ratio in the dissolution step. The sonication of mixed lipid was performed at the same condition as described for the pure PCDA except for DMPC that was performed at 60 °C for only 10 min.

2.4. Colorimetric measurements

The colorimetric response in percentage term (%CR) was used to quantitatively evaluate the blue-to-red color transition. The %CR is $100 \times (PB_0 - PB)/PB_0$, where PB_0 and PB are the percent blue determined before and after the exposure to external stimulants calculated from $A_{blue}/(A_{blue}+A_{red})$. The A_{blue} and A_{red} refer to the