Biosensors and Bioelectronics 35 (2012) 452–455

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Short communication

Highly selective fluorescent sensing of fenitrothion using per-6-amino-β-cyclodextrin:Eu(III) complex

Kuppusamy Kanagaraj, Abdullah Affrose, Subbaiah Sivakolunthu, Kasi Pitchumani*

School of Chemistry, Madurai Kamaraj University, Madurai 625021, India

ARTICLE INFO

Article history:
Received 23 December 2011
Received in revised form 16 February 2012
Accepted 20 February 2012
Available online 28 February 2012

Keywords:
Per-6-ABCD:Europium(III) complex
Fluorescent chemosensor
Organophosphorus pesticides
Fenitrothion

ABSTRACT

A unique, efficient, highly sensitive and selective fluorescent chemosensor for fenitrothion has been reported for the first time using per-6-amino-β-cyclodextrin:Eu(III) complex. Among the various pesticides, the sensitivity response is found to be in the order, fenitrothion ⋙ quinalphos > methylparathion > parathion > methylparaoxon > paraoxon > fenchlorphos > profenofos > malathion. A detection limit as low as 1×10^{-12} M for fenitrothion sensing is realized with a 2.4% relative standard deviation (RSD) of three consecutive runs. The per-6-amino-β-cyclodextrin:Eu(III):pesticide complexes and their sensing mechanism are evidenced from emission, NMR, FT-IR, binding constant measurement, Job's plot, ICD spectra, ESI-MS, lifetime measurements and molecular modeling studies. The proposed sensing is a consequence of Absorption Energy Transfer Emission (AETE) process as a result of better encapsulation of fenitrothion inside the cavity of per-6-amino-β-cyclodextrin:Eu(III) complex. The remarkable sensitivity and selectivity of fenitrothion compared to other OPs, is attributed to a more deeper binding and tighter fit of fenitrothion inside the CD cavity, which is evident from binding constant values and molecular modeling studies. This tighter fit ensures the replacement of two coordinating water molecules on Eu(III) ion, which may have contributed to the more selective sensing of fenitrothion.

1. Introduction

Pesticides play an important role in contemporary agricultural operations by increasing crop yields while stabilizing global food supply through the protection of plants from disease and pests (Arias-Estevez et al., 2008; Gonzalaz-Rodriguez et al., 2008). However, pesticide residues can persist in the environment, within and/or on some plant tissues consumed by humans or animals, which has generated significant public concern (Hunt et al., 2006; Pan et al., 2008; Zhou et al., 2009). Pesticide control, thus has been widely recognized as important for public health.

Organophosphate pesticides (OPs), a group of commonly used neurotoxic pesticides in agriculture, can disrupt the cholinesterase enzyme and lead to cholinergic dysfunction and death, which endanger the health of both humans and animals (Rosenberry, 1975; Fennouh et al., 1997; Guerrieri et al., 2002; Liu and Lin, 2005; Zhao et al., 2009). Owing to the inherent toxicity of the OPs neurotoxins, there is considerable interest in developing highly sensitive, selective, rapid and reliable methods for their detection. Applications include the protection of water resources, food supplies, in effective defence against terrorist activity and monitoring detoxification processes (Knapton et al., 2006; Zourob et al., 2007; Burnworth et al., 2007; Obare et al., 2010; Orcutt et al., 2010). Several techniques (Knapton et al., 2006; Burnworth et al., 2007; Obare et al., 2010; Orcutt et al., 2010), such as chromatography, immunoassay, and enzyme biosensors based on inhibition of cholinesterase activity (Zourob et al., 2007), for monitoring OPs have been reported (Lei et al., 2004). Although sensitive, these methods are time-consuming, expensive, and require skilled personnel and therefore unsuitable for on-line or field monitoring (Sherma, 1993). Therefore, a fast, sensitive, selective and reliable assay/determination of these pesticides is essential.

Fenitrothion (*O*,*O*-Dimethyl *O*-(3-methyl-4-nitrophenyl)-phosphorothioate) is one of the most hazardous and broad spectrum insecticide and acaricide used for pest control in rice, vegetables, wheat, cereals, and cotton (Rougier et al., 2010). It is also considered by World Health Organization (WHO) effective for the control of malaria. It has low mammalian toxicity but it is a known endocrine disrupting chemical. It has been suggested that less than 1% of the total applied pesticide reaches its target, the remaining material entering the water drainage areas *via* agricultural runoff and leaching (Rougier et al., 2011).

Cyclodextrins (CDs) are macrocyclic oligosaccharides possessing hydrophobic cavities that bind substrates selectively *via* noncovalent interactions (Takahashi, 1998; Sakuraba and Maekawa, 2006; Bhosale and Bhosale, 2007) and this property

* Corresponding author. Tel.: +91 452 2456614; fax: +91 452 2459181.
E-mail address: pit12399@yahoo.com (K. Pitchumani).

0956-5663/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2012.02.046

enables them to be used in various applications such as catalysis and enzyme mimics (Harano et al., 1991; Surendra et al., 2006). Selectively modified cyclodextrins are used as sensors as well as catalysts (Haider and Pikramenou, 2005; Ikeda and Ueno, 2006; Heck et al., 2002; David et al., 2007; Maisonneuve et al., 2008). Per-amino-CDs are homogeneous CD derivatives modified by persubstitution at the primary face with amino pendant groups, which display combined hydrophobic and electrostatic binding of guest molecules relative to native CDs. They have been employed as catalysts in various organic transformations (Suresh and Pitchumani, 2008a,b; Kanagaraj and Pitchumani, 2010; Kanagaraj et al., 2010), sensors (Suresh et al., 2010; Azath et al., 2011) and also form stable complexes with metal cations (Tabushi et al., 1977; Haskard et al., 1996; Mortellaro and Nocera, 1996). In the present work, per-6-amino-β-cyclodextrin:Eu(III) complex ($[Per\text{-}6\text{-}ABCD:Eu(III)]^{3+}\cdot 2H_2O$) (**2**) is employed for the first time as a highly selective fluorescent chemosensor for the detection of fenitrothion in water medium at a level as low as 1×10^{-12} M (with a 2.4% relative standard deviation (RSD) of three consecutive runs).

2. Materials and methods

2.1. Material

Per-6-amino-β-cyclodextrin (per-6-ABCD **1**) was synthesized and purified according to the procedure described in the literature (Ashton et al., 1996). The product was dried for 24 h under vacuum over phosphorus pentoxide at 60 °C and then stored in a vacuum desiccator. β-Cyclodextrin, Europium(III) nitrate pentahydrate, Paraoxon, Methylparaoxon, Parathion, Methylparathion, Fenitrothion, Profenofos, Fenchlorphos, Quinalphos and Malathion were purchased from Sigma Aldrich (India) as analytical grade reagents and used as received. All the measurements were carried out in double distilled water which was free from ions.

2.2. Methods

Nuclear magnetic resonance (NMR) spectra were acquired on a Bruker DRX-300 (300 MHz) instrument using TMS as an internal standard. Binding constants were calculated by non-linear regression using prism software (trial version) in an IBM compatible personal computer with Microsoft Windows XP service pack 2 operating system. All the absorption spectra were recorded in a JASCO V-550 double beam spectrophotometer with PMT detector. The emission spectra were recorded in a FLUOROMAX-4 spectrofluorometer (HORIBA JOBIN YVON) with excitation slit set at 2.0 nm bandpass and emission at 5.0 nm bandpass in 1 cm × 1 cm quartz cell. Induced Circular Dichroism (ICD) spectra were recorded in a JASCO J-810 spectropolarimeter with PMT detector, FT-IR were recorded in a JASCO FT/IR-410 spectrometer. Electrospray Ionization Mass Spectrometry (ESI-MS) analyses were recorded in LCQ Fleet, Thermo Fisher Instruments Limited, US. ESI-MS was performed in negative ion mode. The collision voltage and ionization voltage were −70 V and −4.5 kV, respectively, using nitrogen as atomization and desolvation gas. The desolvation temperature was set at 300 °C. The scan range of mass spectrum was 300–2000 *m*/*z*. The relative amount of each component was determined from the LC–MS chromatogram, using the area normalization method.

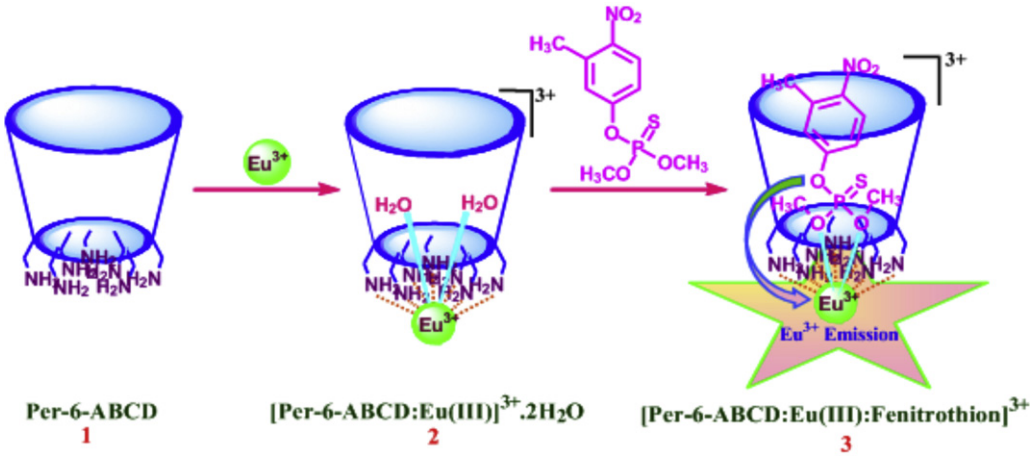
3. Experimental

Stock solutions of per-6-ABCD (**1**) (0.0028 g) and $Eu(NO_3)_3\cdot 5H_2O$ (0.0011 g) were prepared separately in 25 mL SMF. Aqueous solution of the complex $[per\text{-}6\text{-}ABCD:Eu(III)]^{3+}\cdot 2H_2O$ (**2**) was prepared *in situ* from the reaction of per-6-ABCD (1×10^{-6} M) and $Eu(NO_3)_3\cdot 5H_2O$ (1×10^{-6} M) by sonication for 1 h.

$[Per\text{-}6\text{-}ABCD:Eu(III):pesticide]^{3+}$ (**3**) complex was prepared by titrating a known volume of complex **2** (1×10^{-7} M) with different concentrations of pesticides (1×10^{-12} to 1×10^{-7} M). These solutions of complex **3** were equilibrated at room temperature for 10 min and then sensing was measured by recording the emission spectra.

4. Results and discussion

Per-6-ABCD (**1**) is synthesized according to reported procedure (Ashton et al., 1996). Aqueous solutions of complex **2** were prepared *in situ* from the reaction of **1** and $Eu(NO_3)_3\cdot 5H_2O$, and characterized by NMR, FT-IR, Job's plot, and ESI-MS (see Figs. S2–S10 and S57 in Supporting Information (SI)). The asymmetric bending frequency of–NH_2 increased from 1618 cm^{-1} to 1632 cm^{-1}, indicating that Eu(III) ions bind to the amino groups of **1**. Fig. 18a (in SI) shows the UV–Vis absorption spectra of **2** and its fenitrothion complex (indicated as **3** in Scheme 1) and fluorescent spectra of **3** ($[Per\text{-}6\text{-}ABCD:Eu(III):Fenitrothion]^{3+}$) ($\lambda_{exc}$ = 276 nm) in water. The binding constant value of **2** (2562 M^{-1}) is calculated by non-linear curve fit method (Fig. S8 in SI), which is much higher than that of Eu(III) with native *β*-CD (152 M^{-1}). The lifetimes of the Eu(III) $^5D_0$ excited state was 1.61 ms in D_2O and 0.38 ms in H_2O, consistent with the presence of two coordinated water



Scheme 1. Mechanism of fenitrothion sensing by **2** in water.