Highly Sensitive Detection of Organophosphorus Pesticides Using 5,10,15,20-Tetrakis(4-hydroxyphenyl)porphyrin

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We describe a unique UV-visible absorption spectral property of 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (THPP) in the presence of organophosphorus (OP) pesticides. Upon titrating each 16 among total 40 different OP pesticides, the Soret band was significantly red-shifted, and a very intense Q band appeared. They were attributed to the diprotonation of THPP. A suitable solvent for this reaction was determined to be methanol. THPP would become a potential sensor molecule used to detect OP pesticides with high sensitivity in the concentration range of 10^{-6} – 10^{-4} M.

Keywords 5,10,15,20-Tetrakis(4-hydroxyphenyl)porphyrin, organophosphorus pesticides, UV-visible absorption spectroscopy, solvent dependence, diprotonation

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Introduction

Organophosphorus (OP) compounds are powerful inhibitors of cholinesterases. They attach to a serine residue of acetylcholinesterase, while preventing the binding of acetylcholine.¹ Various OP compounds are widely used as ingredients of pesticides. About 70% of the insecticides used in the US in 2001 were OP pesticides according to Centers for Disease Control and Prevention.² The efficacy is based on the phosphoryl (P=O) or thiophosphoryl (P=S) groups in organophosphates. On the one hand, their toxicity causes serious human health concerns. OP pesticides have poisoned thousands of people all over the world.^{3,4} In 2008, hundreds of Japanese citizens became addicted after consuming Chinese-produced pork dumplings (jiaozi) tainted with dichlorvos (2,2-dichlorovinyl dimethyl phosphate), methamidophos, and parathion. In 2013, 23 school children died after eating school lunch contaminated with monocrotophos in India. Moreover, OP pesticides are often used for suicide because of their easy availability. Gunnell *et al.*⁵ estimated that there were over 230000 deaths by pesticide self-poisoning in the world each year. This number corresponds to approximately 30% of all suicides. Furthermore, OP compounds include lethal nerve agents and chemical warfare agents, such as VX, soman, and sarin, which show extremely high toxicity. In the case of the poisoning affairs using OP compounds, an immediate determination of the causative agents would be the first priority to secure conclusive evidence. Several detection methods of OP compounds have been developed based on the use of

chromatography,^{6,7} mass spectrometry,^{8,9} immunoassays,^{10,11} *etc.* Nevertheless, these systems suffer from limitations, such as a slow response time, operational complexity, and a lack of portability. Thus, there is great demand for the development of quick, simple, and reliable methods for detecting OP compounds.

Porphyrins are well-known heteromacrocycles in nature, and also in the chemical industry. They have a highly conjugated 18π -electron system, and show very intense absorption bands in the visible region, the Soret and Q bands. The Soret band has a molar absorption coefficient on the order of several hundred thousandths at 400 – 500 nm. The Q bands of free-base porphyrin consist of four distinct peaks in the 500 – 650 nm region. These absorption bands are sensitively influenced by changing the electronic states of the porphyrin ring. Consequently, many analytical methods using porphyrins as an indicator have been reported, such as those for determining metal ions,^{12,13} organic anions,¹⁴ sensing gas,^{15–17} and environmental pollution compounds.¹⁸ Here, we report on the unique absorption property of 5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (THPP) in the presence of dichlorvos (Fig. 1). Dichlorvos is the typical OP pesticide used commonly in agriculture and residential landscaping. Unfortunately, it causes a number of accidental and intentional poisoning incidents in Japan every year. The most appropriate solvent for the color change of THPP has been selected, and a total of 40 different OP pesticides have been examined. THPP would become a potential molecule to detect OP pesticides with high sensitivity at low concentration.

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Fig. 1 Chemical structures of THPP, TCPP, and dichlorvos.

Experimental

Reagents

All reagents were purchased from a commercial source as analytical reagent grade, and were used without further purification. THPP was purchased from Sigma-Aldrich Co. (St. Louis, MO) and 5,10,15,20-tetrakis(4-carboxylphenyl)-porphyrin (TCPP) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). All OP pesticides were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All solvents of special grade were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). The stock solutions of THPP (2.3×10^{-4} M) and TCPP (1.9×10^{-4} M) in methanol were stable for six months under the dark condition. Working solutions of THPP and TCPP (2.0×10^{-6} M) were prepared by diluting from stock solutions at room temperature.

Titration experiment

Aliquots of OP pesticides were titrated using a micro-syringe to a 3-mL methanol solution of THPP (2.0×10^{-6} M) in a 1×1 cm quartz cuvette, which was sealed by a rubber septum. UV-visible absorbance spectra were recorded on a V-650 spectrophotometer (JASCO Corp., Tokyo).

Results and Discussion

Titration with dichlorvos

The addition of dichlorvos to a THPP solution in methanol (2.0×10^{-6} M) at 25°C induced a significant red-shift of the Soret band ($418 \text{ nm} \rightarrow 445 \text{ nm}$) with an isosbestic point of 429 nm (Fig. 2A). The original Q band absorptions decreased, and an intense Q(I) band appeared at 687 nm . Accordingly, the color of the solution changed from pale-pink to light-green. The spectrum became saturated at 6.0×10^{-5} M dichlorvos (Fig. 2A, inset). The limit of detection (LOD = 3σ ; σ is the standard deviation of the Soret band) of dichlorvos was determined to be 2.8×10^{-6} M. The same experiment was performed with TCPP for a comparison (Fig. 2B). Surprisingly, almost no spectral change was observed in the range of $0 - 6.0 \times 10^{-5}$ M dichlorvos. The 3.5×10^{-3} M dichlorvos was required for a complete spectral change of TCPP (Fig. 2B, inset). The Soret band at 415 nm was red-shifted to 436 nm , and the broadened Q band appeared at 649 nm . Several research groups¹⁹⁻²¹ reported that inter-molecular complex formation of porphyrin and dimethyl methylphosphonate (DMMP) occurred,

(A) THPP

(B) TCPP

Fig. 2 UV-visible absorption spectral changes of (A) THPP (2.0×10^{-6} M) and (B) TCPP (2.0×10^{-6} M) titrated with dichlorvos in methanol. The insets show the absorbance changes at (A) 445 nm (B) 436 nm. Fig. 3 Decreasing ratio $(A_0 - A)/A_0$ of the Soret band of THPP (2.0×10^{-6} M) in the presence of 3.7×10^{-3} M DMMP in each solvent.

which is an OP model compound, revealed by UV-visible absorption spectroscopy. However, our results clearly demonstrated that the obtained absorption pattern of THPP in the presence of dichlorvos was identical to that of the diprotonated porphyrin.²² Moreover, the addition of other OP

Table 1 LODs determined by the Soret band absorption change of THPP in methanol and the structures of OP pesticides^a

OP pesticides	LOD/M	X	Y	R ₁	R ₂	R ₃
Dioxabenzofos	1.4×10^{-6}	S	O	*b	*b	OMe
Pirimiphos-methyl	1.8×10^{-6}	S	O	2-Diethylamino-6-methylpyrimidin-4-yl	OMe	OMe
Naled	1.9×10^{-6}	O	O	1,2-Dibromo-2,2-dichloroethyl	OMe	OMe
Dichlorvos	2.8×10^{-6}	O	O	2,2-Dichlorovinyl	OMe	OMe
Profenofos	4.7×10^{-6}	O	O	4-Bromo-2-chlorophenyl	S(<i>n</i> -Pr)	OEt
Quinalphos	6.1×10^{-6}	S	O	Quinoxalin-2-yl	OEt	OEt
Vamidothion	9.4×10^{-6}	O	S	2-(1-Methylcarbamoylthio)ethyl	OMe	OMe
Diazinon	1.0×10^{-5}	S	O	2-Isopropyl-6-methylpyrimidin-4-yl	OEt	OEt
Triazophos	1.2×10^{-5}	S	O	1-Phenyl-1 <i>H</i> -1,2,4-triazol-3-yl	OEt	OEt
Formothion	1.7×10^{-5}	S	S	Formyl(methyl)carbamoylmethyl	OMe	OMe
Trichlorfon	2.0×10^{-5}	O	—	2,2,2-Trichloro-1-hydroxyethyl	OMe	OMe
Edifenphos	4.6×10^{-5}	O	S	Phenyl	SPh	OEt
Azinphos-methyl	6.0×10^{-5}	S	S	3,4-Dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl	OMe	OMe
Cyanophos	3.4×10^{-4}	S	O	4-Cyanophenyl	OMe	OMe
Malathion	6.0×10^{-4}	S	S	1,2-Bis(ethoxycarbonyl)ethyl	OMe	OMe
Fenitrothion	1.3×10^{-3}	S	O	4-Nitro- <i>m</i> -tolyl	OMe	OMe

pesticides also changed the THPP spectrum in the same manner (described below). We concluded that the drastic color change of THPP is caused by diprotonation of the porphyrin ring, and excluded the formation of an intermolecular complex. The difference of sensitivity between THPP and TCPP is due to the substituent effect to the 18π -electronic conjugated structure of the porphyrin. The electron-donating hydroxy group can increase the electron density of the porphyrin ring, although the carboxyl group has an electron-withdrawing effect.^{22,23} Thus, THPP was easily protonated compared to TCPP.

Solvent dependence

To clarify the solvent effect of the diprotonation, DMMP was added into a DMSO, DMF, acetone, toluene, ethyl acetate, acetonitrile, chloroform, and methanol solution of THPP (2.0×10^{-6} M). The absorption spectra were measured until the spectral changes were saturated. Figure 3 shows the decreasing ratio $(A_0 - A)/A_0$ (A_0 ; the initial absorbance of THPP, A ; the absorbance in the presence of DMMP) of the Soret band in each solvent. The absorption decreased significantly in methanol compared to in other solvents; the ratio was ascertained to be 0.842. We could not find any difference in other solutions; 0.005 in DMSO, 0.007 in DMF, 0.018 in acetone, 0.022 in ethyl acetate, 0.029 in toluene, 0.052 in acetonitrile, and 0.096 in chloroform. These differences are probably based on the Lewis basicity of the solvent; the acceptor number of methanol is extremely higher (41.3) than others. Therefore, methanol could stabilize the diprotonated THPP generated by DMMP.

Titration with several OP pesticides

We examined total 40 different OP pesticides including dichlorvos,²⁴ and found that 16 compounds are effective to change the THPP spectrum. The final absorption patterns after the titrations were all the same as that observed after the addition of dichlorvos. The LOD of each OP pesticide is summarized in Table 1. We found that (i) the 15 compounds, except for fenitrothion, demonstrated micromolar order LOD, and (ii) dioxabenzofos, pirimiphos-methyl, and naled showed lower values in comparison to dichlorvos.

In general, OP pesticides are easily hydrolyzed by undergoing

a nucleophilic substitution of phosphate ester; $(OR)_3P=X \rightarrow (OH)(OR)_2P=X$ ($X=O$ or S).²⁵ We supposed that the LOD value could be dependent on the hydrolysis rate of OP pesticide. Okajima and Maegawa²⁶ reported the relationship between the hydrolysis reactivity and the structure of OP compounds by estimating the activation energies ($\Delta E^\ddagger$) for nucleophilic attacking to the $P=X$ double bond. Their calculation suggested that the reactivity has an inverse relation to the $\Delta E^\ddagger$ values. (1) $\Delta E^\ddagger$ of OP compounds having $P=O$ are relatively smaller than those having $P=S$, (2) the sulfur atom in a phosphoester linkage generally increases $\Delta E^\ddagger$, and (3) the electron-donating and electron-withdrawing substituents affecting the electrophilicity of the phosphorus atom in $P=X$ increases and decreases $\Delta E^\ddagger$, respectively. The LODs of OP pesticides seem to depend on their chemical structures (Table 1). For instance, naled, dichlorvos, and profenofos, which are substituted with halogens, showed lower LODs. Dioxabenzofos and pirimiphos-methyl were out of this assumption, but they were more easily hydrolyzed than any other OP compounds because of their low stability owing to the steric strain. If the LOD values of OP pesticides are correlated to hydrolysis reactivity, the source of protons in the titration experiment of OP pesticides to THPP could also be explained.

Conclusions

The UV-visible absorption spectral property of THPP in the presence of OP pesticides has been investigated. By the addition of dichlorvos, the Soret and Q bands of THPP showed bathochromic shifts, and thereby the color of the solution turned pale-pink to light-green immediately. The obtained spectrum corresponded to a typical diprotonated porphyrin, and methanol was the best solvent for this reaction. We found that 15 OP pesticides showed very low LOD values of 10^{-6} – 10^{-4} M. A detailed analysis of the protonation mechanism and the difference of the OP pesticide reactivity is now underway. The THPP has the potential of acting as a promising sensor molecule to detect OP pesticides with high sensitivity at very low concentration.

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