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Development of a sensitive competitive indirect ELISA for parathion residue in agricultural and environmental samples

Yihua Liu, Chunmei Wang, Wenjun Gui, Jincheng Bi, Maojun Jin, Guonian Zhu *

Institute of Pesticide and Environmental Toxicology, Zhejiang University, Hangzhou 310029, China

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ABSTRACT

A sensitive competitive indirect enzyme-linked immunosorbent assay (ELISA) for the insecticide parathion was developed. The optimal immunogen was 5-(ethoxy(4-nitrophenoxy)phosphorothioylamino)pentanoic acid. In addition, five competitors were applied for development of a heterologous competitive indirect ELISA. Then several physicochemical factors (organic solvent, ionic strength and pH) that influence assay performance were studied and optimized. The IC_{50} and IC_{10} of the optimized ELISA were 0.95 and 0.15 ng/mL, respectively, which meant almost 86-fold and 6-fold improvement in the assay sensitivity in comparison with the homologous assay (81.74 ng/mL) and the non-optimized heterologous assay (5.60 ng/mL). Finally, the assay was applied to the analysis of parathion in spiked agricultural and environmental samples without extraction or cleanup. The average recoveries of parathion added to water, soil, cucumber, rice and corn were between 78.57% and 107.67%. The limit of detection (LOD) for water and soil samples was 5 ng/mL, and the LOD for cucumber, rice and corn samples was 10 ng/mL.

1. Introduction

Environment contamination from pesticides is raising concerns for the public and regulatory agencies. Routine monitoring of these pollutants is required since they are chemical hazards to human health and environmental. In China, about 1 million tons of various pesticides are used every year, and 25% of them are organophosphates, such as parathion and methyl-parathion. Parathion (*O*,*O*-diethyl-*O*(4-nitrophenyl)thiophosphate) belongs to the organophosphorus group of pesticides. Parathion can deactivate the enzyme acetylcholinesterase, disrupt nerve function, and result in paralysis and death (Buratti et al., 2003). Due to its high toxicity and persistence, it was reported that parathion residue in food may pose a potential health hazard to people and animals (Buratti et al., 2003; Fenske et al., 2002). Recently, some new policies have been issued and enforced to reduce and limit the employment of parathion and other highly organophosphorus pesticides in vegetables and fruits in China and other countries. The conventional methods involving gas and liquid chromatography for parathion analysis (Toral et al., 2002; Zambonin et al., 2002) are accurate, but they are generally expensive, time-consuming and labor-intensive. There is the need for a fast, convenient and reliable analytical method to monitor parathion residues at very low concentrations in agricultural and environmental samples. To this end, immunoassays are known to be rapid, sensitive, specific and cost-effective.

Ercegovich et al. (1981) were pioneers in development of a radioimmunoassay for parathion in which parathion was coupled by diazotization (reduced parathion) with protein at the nitro group. They produced a polyclonal antiserum that was specific for parathion and utilized it to measure pesticide levels in fortified plasma and plant tissues, with a limit of detection (LOD) of 0.1 μg/mL. Since then, many researchers followed the same method (Garrett et al., 1997; Ibrahim, 1994; Zeng et al., 2005, 2007). Ibrahim et al. (1994) produced a monoclonal antibody (Mab) to parathion using the same conjugation procedure for the immunogen and coating conjugate. Garrett et al. (1997) produced a recombinant anti-parathion antibody (ScFv). The resulting assay did detect parathion, but the LOD was very high. Subsequently, Zeng et al. (2005, 2007) produced a monoclonal antibody to parathion using the same immunogen, the reduced parathion conjugated with a carrier protein, bovine thyroglobulin (BTG). The sensitivity was estimated as the IC_{50} value (360 ng/mL), with a practical working range between 47 and 6000 ng/mL. Though the sensitivity of the enzyme-linked immunosorbent assay (ELISA) was improved appreciably, the requirement of many countries for parathion could not be up to par. For example, The 1991 JMPR recommended a maximum residue limit (MRL) of 50 ng/mL for parathion in apple. The MRLs for parathion set by the European Union in 2004 on fruit, vegetables and meat were less than 50 ng/mL. In 2005, China set the MRLs for parathion on fruits and vegetables to the even more

* Corresponding author. Fax: +86 571 86971220.
E-mail address: zhugn@zju.edu.cn (G. Zhu).

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conservative 10 ng/mL level. Consequently, the ELISA method must be significantly improved to meet the practical requirement of pesticide residue determination.

It has been reported that an increase in detectability can be achieved by introducing a certain degree of heterology in the chemical structure of the competitor (Galve et al., 2000). Some authors have suggested that coating antigen/As combinations may modify the immunoassay selectivity (Galve et al., 2002a, b; Nichkova et al., 2002a, b). Moreover, immunoassays for pesticides are usually carried out under physiological conditions, and frequently little effort is made to optimize them for factors such as pH, ionic strength and presence of detergent (Liang et al., 2007; Nichkova et al., 2002b; Zhu et al., 2008). However, these factors can directly affect the assay sensitivity by changing the interaction of the antibody with the conjugated hapten. Thus, optimization provides information on the immunoassay performance that may be of great usefulness in solving problems arising during validation studies with real samples.

In our previous manuscript (Liu et al., 2007), the application of molecular modeling in hapten designing was investigated. The best immunogen and competitor were computed by using molecular modeling method. By using a polyclonal antibody, an indirect competitive ELISA for parathion was finally selected. The immunoassay had an IC_{50} value of 4.79 ng/mL. But the published manuscripts included neither the optimization of the selected ELISA, nor the application of the ELISA to determine the parathion residue in real samples. Based on the optimal immunogen, a monoclonal antibody had been produced, and a heterologous indirect ELISA was developed by using another competitor with IC_{50} of 2.94 ng/mL (Wang et al., 2009). As we all know, monoclonal antibody has higher specificity than polyclonal antibody, but the production of polyclonal antibody is easier and simpler. So the polyclonal antibody is often adopted by the commercialized ELISA kit or other immunochemical tools. In this research, we selected several typical haptens to go into the advantages of the optimal immunogen and competitor. Our work resulted in the development of a heterologous ELISA method for parathion, which was optimized and utilized for the determination of parathion in some agricultural and environmental samples.

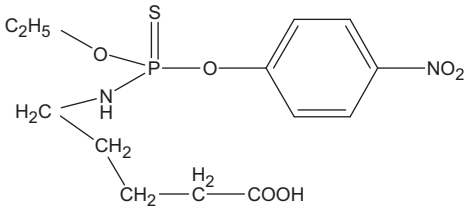
2. Materials and methods

2.1. Chemicals

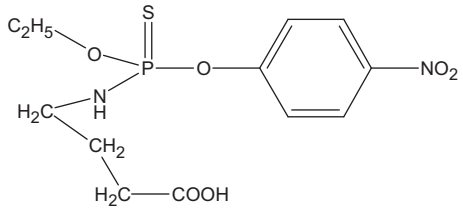
All reagents were of analytical grade unless specified otherwise. Parathion was obtained from the National Standards Company (Beijing, China). Starting products for hapten synthesis were provided by Hangzhou Pesticide Manufactory (Hangzhou, China). Thin-layer chromatography (TLC) was carried out on 0.2 mm precoated silica gel F_{254} (100–200 mesh, particle size) on glass sheets. Ovalbumin (OVA, MW 45,000), bovine serum albumin (BSA, MW 67,000), *N*-hydroxysuccinimide (NHS) and *N,N′*-dicyclohedxylcarbodiimid (DCC) were all obtained from Sigma (Steinheim, Germany). Complete and incomplete Freund's adjuvants were obtained from Institute of Bio-products (Beijing, China). *o*-phenylenediamine (OPD), tri-*n*-butyl amine (TBA), triethyl amine (TEA), iso-butyl choroformate and other chemical reagents were purchased from Shanghai Chemical Reagents Company (Shanghai, China). The ELISA was carried out in 96-well polystyrene microplates (COSTAR, New York, USA).

2.2. Instrumentation and equipment

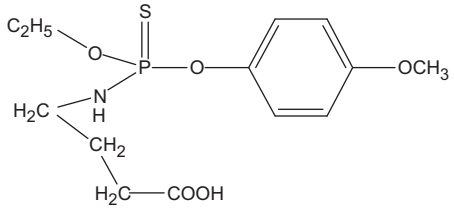
^{1}H nuclear magnetic resonance (NMR) spectra were obtained with an AVANCE DMX 500 spectrometer (Bruke, Berlin, Germany), operating at 400 MHz for solutions in $CDCl_3$. Chemical shifts were given relative to tertramethylsilane (TMS). Electron spray ion (ESI) mass spectra were measured using an Esquire-LC00075 mass spectrometer (Bruke, Berlin, Germany). Electron ion (EI) mass spectra were obtained with a HP 5890/5973 mass spectrometer (Agilent, Wilmington, DE, USA). Ultraviolet–visible (UV–Vis) spectra were recorded on a spectrophotometer



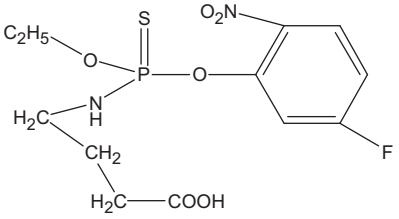
Hapten 1 (immunogen)



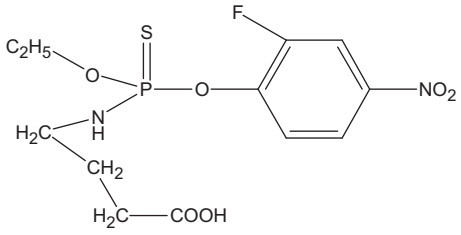
Hapten 2 (competitor 1)



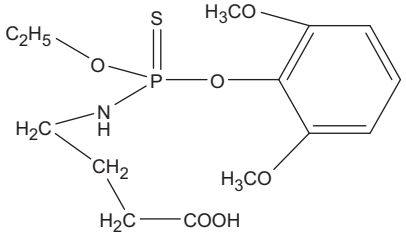
Hapten 3 (competitor 2)



Hapten 4 (competitor 3)



Hapten 5 (competitor 4)



Hapten 6 (competitor 5)

Fig. 1.