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Developing a novel sensitive visual screening card for rapid detection of pesticide residues in food

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ABSTRACT

A novel sensitive visual screening card for rapid detection of pesticide residues was developed based on the blue-green color intensity changes, which were generated as a result of indoxyl acetate hydrolysis catalyzed by acetylcholinesterase (AChE) and the inhibition of AChE activity by pesticides. The procedure for preparing the test card was optimized. The AChE was immobilized onto the Hybond N^+ nylon membrane using physical adsorption method. The immobilization temperature was set at 4 °C and the immobilization time was set to 30 min. The immobilized enzyme was freeze dried under vacuum conditions for 2 min. The indoxyl acetate was dissolved in methanol and diluted to 10 mM by phosphate buffer solution (pH7.5). The inhibition time and the color development time were set to 15 min and 10 min respectively. The final experimental results on determining various pesticides showed that the limit of detection (LOD) of this assay system was 1 μg/mL for omethoate, 0.1 μg/mL for dichlorvos, 2 μg/mL for methamidophos, 0.05 μg/mL for chlorpyrifos, 1.5 μg/mL for carbaryl, and 0.8 μg/mL for pirimicarb. Detection results of pesticide residues in real food samples of fruit juices and vegetable showed this visual screening card had high sensitivity, good reproducibility and stable-storage property, suggesting its great potential for practical application in rapid determination of pesticide residues.

1. Introduction

Pesticides are one kind of neurotoxic compounds, which are widely used to ensure high crop yields due to reducing the pests and eradicating the diseases. But they also have enriched toxicity to humans and animals (Dmello, 1993). Especially in the developing countries, since the pesticides are used extensively and are inadequately supervised, many serious problems of food safety have been raised (Atreya, Sitaula, Johnsen, & Bajracharya, 2011; Ecobichon, 2001). In China, the correlative studies showed that the food safety problems induced by pesticide residues in fruits and vegetables are high occurrence (Qian et al., 2011). The current state of pesticide residues in food in China has received extensive attention (Chen, Li, Chen, Chen, & Qian, 2009). According to the incomplete statistics, the application amount of organophosphorus (OP) pesticides and carbamate (CM) pesticides accounts for about 70% of the total application amount of pesticides. It is significant to ensure that the pesticides in fruits and vegetables had been completely degraded before the sale of agricultural products. So, fast, sensitive and cost-effective analysis of pesticide residues is essential to assure the quality of agricultural products.

The standard reference methods for the determination of pesticide residues in real samples are mainly based on gas chromatography (GC) or high performance liquid chromatography (HPLC) (Molina, Honing, & Barcelo, 1994). These two traditional methods have the same disadvantages, such as time-consuming procedures, complicated operations, and high-cost expenses. Hence, it is necessary to develop the simple, rapid, and low-cost methods for determination of pesticide residues. Recently, there were numerous reports about the studies of class-specific determination of OP and CM pesticides based on the inhibition of AChE enzyme activity (Miao, He, & Zhu, 2010). The major forms were focused on the enzymatic analysis using spectrophotometers (Ni, Deng, & Kokot, 2009), or biosensors (Andreescu & Marty, 2006; Hildebrandt et al., 2008; Ion, Ion, Culetu, Gherase, Moldovan, &Iosub, 2010; Mavrikou et al., 2008; Rekha & Murthy, 2008; Sun & Wang, 2010; Yin, Ai, Xu, Shi, & Zhu, 2009). Although these methods simplified the testing process and reduced the determination time, they still required electrical power and correlative equipment, leading to the inconvenience for the fast on-site

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measurement. In addition, there are still some problems of biosensors that require further investigation and improvement, such as the poor reproducibility and the weak stability for the detection of pesticide residues in real samples.

Some dipstick assays based on high specific immunological reaction using antibody-coated membranes were developed before for detection of the individual pesticides (Lisa, Chouhan, Vinayaka, Manonmani, & Thakur, 2009; Lisha, Anshup, & Pradeep, 2009; Shi, Zhao, Zhang, Hong, & Zhu, 2008; Shuo Wang, Zhang, & Mang, 2009; Wang, Allan, Hill, & Kennedy, 2002). However, farmers in China often use a variety of pesticides in practice, and the exact species of pesticides can not be predicted. So, it is difficult to select the right antibody-coated membrane to discriminate the unknown type of pesticides. From the perspective of food safety control, developing the sensitive and broad-spectrum screening tools to detect the lethal level of various pesticides in food items will have significant practical applications.

In this study, a novel rapid visual screening card was developed for the detection of pesticide residues, which contained the enzyme-coated membrane and substrate solution. The mechanism of the visual color change on the test card was based on the inhibition of AChE activity by pesticides, and the enrichment of chromogenic reagent on the membrane surface owing to its rapid evaporation. A similar membrane chip had been developed based on the reaction between the enzymatic reaction-generated thiocholine and 5,5′-dithiobis-2-nitrobenzoic acid, which induced a color change from white to yellow on the chip surface (Nagatani et al., 2007). For visual determination of pesticide residues, one most important issue is to enhance the color resolution so as to improve the accuracy. The research results on visual acuity showed that the visual cells of humans are most sensitive to the wavelength which varies near the blue-green (Hunt, 2004). For this reason, we proposed indoxyl acetate as chromogenic reagent. As reported in an earlier paper (Villatte, Bachman, Hussein, & Schmid, 2001), indoxyl acetate was a good chromogenic substrate of AChE for determining the activity of enzyme. The hydrolysis of colorless (lavender) indoxyl acetate can be catalyzed by AChE to produce indoxyl (Fig. 1). The indoxyl is then quickly oxidized by air yielding indigo, which is just right blue-green and can be well distinguished with the naked eye. Therefore, according to the color intensity change induced by the inhibition of AChE activity, the concentration of pesticide residues can be simply analyzed by qualitative or semi-quantitative method. The as-prepared screening card will provide a more sensitive candidate for fast on-site detection of pesticide residues in real samples.

2. Material and methods

2.1. Material

Acetylcholinesterase (AChE, V-S type from electric eel, 1000–2000 units/mg protein), indoxyl acetate and bovine serum albumin (BSA) were obtained from Sigma–Aldrich (Shanghai, China). Positively charged nylon membrane, Hybond N^+ (pore size, 0.45 μm) was obtained from Amersham Int. Plc. Immobilon PVDF membrane (pore size, 0.45 μm) was purchased from Millipore (Shanghai, China). PVC backing card (thickness, 0.4 mm) was obtained from Kinbio Tech Co., Ltd (Shanghai, China). Pesticides were standard commercial products. Glutaraldehyde (GA), phosphate buffer solution (PBS) (0.1 M), Tris–HCl buffer (20 mM, pH8.0) and other regents were of analytical grade. Lettuce and fruit juices were purchased from a local supermarket in China.

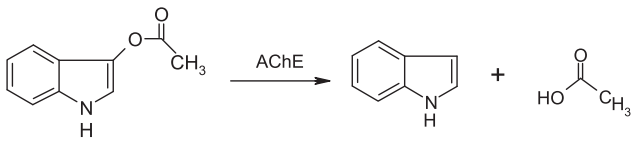

Fig. 1. The reaction mechanism of indoxyl acetate hydrolysis catalyzed by AChE.

2.2. Apparatus

UV Probe-2450 spectrometer (Shimadzu Corporation, Japan) was used to measure the absorbance (Abs) for determination of AChE activity. Vacuum freeze dryer FD-1A-50 (Beijing boyikang Lab Instrument Co., Ltd, China) was adopted to dry the immobilized enzyme. Colorimeter XZ-S (Xinye Optoelectronic Engineering Co., Ltd, China) was utilized to measure the color intensity changes for the visual comparisons. Gas chromatography and mass spectrometry (GC–MS) instrument Agilent 7890 (Agilent Technologies, Inc., Santa Clara, USA) was used to compare with the rapid visual screening card for quantitative determination of pesticide residues in real food samples.

2.3. Preparation of test cards

The membrane and PVC backing card were both cut into square pieces (1 cm × 1 cm), so that the membrane could be directly mounted onto the PVC backing card. 5 μL of the diluted AChE enzyme was immobilized onto the center of the membrane. After that, the enzyme-coated membrane was dried, followed by rinsing with PBS (pH8.0) for several times. Finally, the membrane was dried again for standby application.

2.4. Measurement the recovery activity of enzyme-coated membrane

The UV spectrophotometer was used to measure the immobilized enzyme activity (Ellman, Courtney, Andres jr, & Featherstone, 1961; Villatte et al., 2001). First, the enzyme-coated membrane was immersed into 3 mL PBS (pH 7.5) and was incubated at 30°Cfor 20 min. Then 30 μL indoxyl acetate solution (200 mM) was quickly added and blended. After that, the absorbance change of the mixture was measured every 20 s at 650 nm for 15 min. The activity of the immobilized enzyme, ΔA_1 (Abs/min) was calculated by using the best linear 3-min data. And the activity of the frozen enzyme, ΔA_0 (Abs/min) was measured using the similar method, just taking the equivalent dose of enzyme solution instead of the immobilized enzyme. The recovery rate of enzyme activity (R) can be calculated with the following equation:

$$R(\%) = \frac{\Delta A_1}{\Delta A_0} \times 100\% \quad (1)$$

2.5. Performance of the assay system

The organophosphorus pesticides (omethoate, dichlorvos, methamidophos, and chlorpyrifos) and carbamate pesticide (carbaryl and pirimicarb) were diluted to several concentration levels by PBS (pH7.5). At each time, 50 μL pesticides sample solution was dropped onto one test card. After incubation for 10 min, 10 μL substrate solution of indoxyl acetate was dropped onto the pesticides-inhibited test card. The results were obtained after 10-min chromogenic reaction.

2.6. Analysis of omethoate in real food samples

Three kinds of fruit juice samples (grape, orange and apple) were tested using the as-prepared screening cards. The pH of all