Development of an Acetylcholinesterase-Based Detection Kit for the Determination of Organophosphorus and Carbamate Pesticide Residues in Agricultural Samples

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The objective of this study was to develop a rapid, simple, and qualitative acetylcholinesterase (AChE)-detection kit, based on a modification of the Ellman and ELISA methods, for the detection of organophosphorus (OP) and carbamate (CB) pesticide. The developed kits were used to screen a large number of agricultural samples (spiked and real) for OP and CB pesticide residues. AChE was extracted from the heads of honeybees (*Apis mellifera* L.) using Triton X-100, and was purified through 3 steps: diethylaminoethyl-cellulose chromatography (DEAE), affinity chromatography and membrane filtering, and Mono-Q column chromatography. Epoxy-activated Sepharose 6B affinity chromatography was used for large-scale purification. The presence of OP and CB pesticide residues in agricultural samples was assayed on the basis of AchE inhibition value. The presence (6 bands) or absence of some colored bands on the test line indicated a negative or positive result, respectively. The limits of detection for measured organophosphorus (OP) and carbamates (CB) pesticide residues in standard pesticide solutions and fortified samples were ranged from 0.50 to 2.50 ppm and 0.50 to 4.75 ppm, respectively.

Key Words : Acetylcholinesterase, Organophosphorus pesticide, Carbamate pesticide, Strip kit

Introduction

Acetylcholinesterase (AChE) plays an essential role in neurotransmission at cholinergic synapses, where it rapidly hydrolyzes the excitatory neurotransmitter, acetylcholine, into choline and acetic acid.[1-4] In honeybees and other insects, most of the AChE activity is found in the central nervous system, rather than in the peripheral nervous system.[5] Many compounds including organophosphorus and carbamate insecticides are commonly used to quasi-irreversibly inhibit AChE.[6,7] AChE has attracted a great deal of attention, and many studies have explored how its alteration may relate to insect resistance. However, most of these studies have used unpurified AChE.[8-14] These crude extracts were contaminated with non-AChE factors such as carboxylesterases, which could affect the measurement of AChE and produce misleading results. AChE enzyme has been purified from *Diabrotica Virgifera* v*irgifera*,[7] *Aphis gossypii* Glover,[13] *Schizaphis graminum*,[15] *Galleria mellonella* L.,[16] *Musca domestica*,[17] *Lygus hesperus* Knight,[18] *Leptinotarsa decemlineata*,[19] and *Helico Verpa armigera*.[20] On the other hand, Hsiao and his colleagues extracted and purified AChE from the oriental fruit fly, *Bactrocera dorsalis* Hendel, using affinity chromatography.[21]

Currently, the methods of detecting pesticide residues involve gas chromatography (GC) and high-performance liquid chromatography (HPLC) with postcolumn derivatization. However, these methods are either expensive or time-consuming due to the complications of sample preparation and preconcentration. Sophisticated equipment is usually needed, although it is often unavailable in many analytical laboratories. Additionally, these methods are not suitable for analyzing large numbers of samples. Immunoassays are analytical methods based on the interaction of an analyte with an antibody that recognizes it with high affinity and specificity. However, this kind of determination requires some expensive equipment, for example, a microplate reader, a plate washer, and a benchtop orbital motion shake.[22,23] Because the majority of the insecticides used were acetylcholinesterase inhibitors (55% of them were organophosphates and 11% were carbamates), the goals of this study were to:

a) extract acetylcholinesterase from the head of *Apis mellifera* L.; b) purify the extract using DEAE cellulose chromatography, affinity chromatography, Mono-Q column chromatography, and Epoxy-activated Sepharose 6B affinity chromatography (large-scale purification); c) prepare and purify AChE Ab; d) develop a simple and qualitative detection strip; e) apply this strip for monitoring a large number of agricultural samples contaminated with organophosphorus and carbamate (the main groups of pesticides that are

[a]This paper is equally contributed by the two authors.

acutely toxic toward humans) residues.

Experimental Section

Chemicals, Reagents, and Standards. Acetylthiocholine iodide (ATC), 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB), phenylmethylsulfonyl fluoride (PMSF), tetraethylammonium iodide (Net_4I), procainamide, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), organophosphate pesticide standards were purchased from Riedel de Haen (Seelze, Germany) and bovine serum albumin were purchased from Sigma (St. Louis, Mo, USA). The National Institute of Agricultural Science and Technology, Republic of Korea kindly supplied carbamate pesticides. Epoxy-activated Sepharose 6B was purchased from Amersham Biosciences (Uppsala, Sweden). All other chemicals were of the highest grade available and obtained from commercial sources. Standard pesticide stock solution was prepared by dissolving appropriate amounts of the analytes in methanol to obtain a concentration of 100 ppm. Intermediary standard solutions were prepared by diluting the standard stock solutions with methanol to obtain concentrations ranging from 0.5 to 4.75 ppm in food commodities. The standard stock and intermediary standard solutions were prepared in the same manner and stored at 4 °C in dark standard bottles. The solutions remained stable under these conditions for at least 3 months.

***Apis mellifera* L.** Female worker honeybees were collected from farm hives located in a clean, rural area of Jollanam-do, Dam-Yang Gun, Republic of Korea between April and June in the years 2004 and 2005. Approximately 20,000 insects were collected once each month. After collection, the insects were acclimatized for one night in the laboratory. During this period, old and weak insects died and highly active ones were directly stored in a deep freezer maintained at −70 °C.

Extraction of AChE from the Heads of Honeybees. After the honeybees were killed in a −70 °C deep freezer, the head was decapitated from the body for extraction of AChE. The heads were homogenized in ice-cold phosphate-buffered saline (PBS, pH: 7.4) containing 10 mM PMSF (in propanol) and 0.5% Triton X-100 (Sigma St. Louis, MO, USA) at 20,000 rpm for 2-3 min using Polytron Devices (NJ, USA). The homogenate was filtered through cheesecloth (Sigma St. Louis, MO, USA) and centrifuged at 6000 g for 15 min. The supernatant was centrifuged again at 6000 g for 30 min, and then precipitated with ammonium sulfate to obtain a protein fraction, which corresponds to 60% saturation. The precipitated fractions were dialyzed against PBS at 2 h intervals. The recovered contents were centrifuged at 6000 g for 60 min. The final supernatant was used as an enzyme source to assay the AChE activity according to the method described by Ellman *et al.*[24] using acetylthiocholine iodide (ATC) as a substrate. All procedures were performed at 4 °C.

The optimum pH and temperature for AChE activity were determined by assaying the enzyme at various pH and temperatures ranging from 4.5 to 9.0 and 5 °C to 55 °C, respectively. The condition for AChE extraction was further optimized by the addition of Triton X-100 at various levels (0.1 to 1.6%). The procedures mentioned above were then repeated under the optimized conditions.

Purification of AchE. After extraction by Triton X-100, AChE was purified using diethylaminoethyl-cellulose (DEAE-cellulose, Sigma St. Louis, MO, USA). DEAE-cellulose column with a height about 60 cm and diameter of 1.5 cm was equilibrated with PBS after loading the AChE extract from 150 g of heads (30 mL). The column was eluted with 0.1 M NaCl and the collected fractions were dialyzed against PBS.[25] After dialysis, the samples were purified by affinity chromatography using procainamide as a ligand specific for the choline-binding site. The procainamide affinity column was prepared according to the instructions of the manufacturer and stored at 4°C using ECH Sepharose 4B as the coupling gel and EEDQ for ligand immobilization.[15,26,27] The procainamide-coupled Sepharose 4B was poured into to a glass column (1 cm × 7.5 cm, Sigma St. Louis, MO, USA) and washed successively with 50 mM phosphate buffer containing 0.05% (v/v) Triton X-100 and 50 mM NaCl (PTS) until the protein content was below 0.01 absorbance at 280 nm. The enzyme was then eluted with 30 mM Net_4I in PTS and then dialyzed against 50 mM phosphate buffer (pH 7.4) with six changes of the buffer every 2 h. After dialysis, the sample was applied to a Mono-Q column chromatography. A Mono-Q HR5/50 column (Amersham Pharmacia Biotech AB, Uppsala, Sweden) was equilibrated with PBS.[28] The active fractions were loaded onto the column and eluted with a linear NaCl gradient (gradient volume, 20 mL) from zero to 0.5 mol/L at a flow rate of 45 mL/h. All steps were conducted at 4 °C.

Large-scale Purification of AChE using Epoxy-activated Sepharose 6B Affinity Chromatography. The active IgG fractions were coupled to the Epoxy-activated Sepharose 6B gel to obtain an immobilized AChE gel by following the instructions of the manufacturer (Amersham Pharmacia Biotech AB, Sweden). In brief, suitable amounts of Epoxy-activated Sepharose 6B powders were swollen in distilled water (200 mL distilled water per gram free-dried powders). After filtration using a G3 glass filter, the swollen gel was shaken in 50 mL of 1 M ethanolamine (pH 8.0) containing 400 μm/mL of AChE at 40-50 °C overnight. The immobilized AChE gel was washed repeatedly with acetate buffer (0.1 M, pH 4.0) and 0.1 M Tris-HCL buffer (0.1 M, pH 8.0), and was then blocked with 1 M glycine overnight under the same conditions. After alternating washes with solution containing 0.5 M NaCl of high and low pH, the immobilized AChE gels were collected for further use.

Determination of Protein Concentration. By using bovine serum albumin (BSA) as a standard, the protein content was determined according to the method described previously by Bradford.[29] The spectrophotometric assays were carried out using a spectrophotometer at a wavelength of 595 nm.

Measurement of AChE activity. AChE activity was measured according to the method of Ellman *et al.*[24] In a standard assay, 0.5 mM acetylthiocholine iodide was utilized as substrate in the presence of 0.33 mM 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB, utilized as a coloring agent) in

0.1 M PBS and the AChE extracts as enzyme. A complete reaction mixture containing boiled enzyme was used as a blank. The change in absorbance was recorded at 405 nm against the same reaction mixture in the absence of enzyme. One unit of AChE activity was defined as 1 μmol of substrate hydrolyzed per min at 20 °C.

Electrophoretic Analysis of AChE. Nondenaturating polyacrylamide gel electrophoresis (PAGE) was carried out using a Bio-Rad dual gel electrophoresis system (Hercules, CA, USA) coupled with a cold water circulating system (4 °C).[30] Approximately 0.5 mL of active AChE fractions (obtained after each step) was mixed with 2.0 mL of sample loading buffer, and 50 μL of the mixture was loaded into each well of the gel. The gel, composed of 30% acrylamide in stacking (consisting of 3.75% total acrylamide (T) and 2.60% cross-linker (C), 0.5% detergent, and 62 mM Tris/HCl, pH 6.8) and separating (consisted of 70% acrylamide (7% T, 2.60% C), 0.5% detergent and 0.375 M Tris/HCl, pH 8.9) gels, was run at 80 V for 30 min and then 100 V for 3 h. The running buffer was composed of 0.5% detergent and 10 mM Tris/glycine, pH 8.3. For visualization, the gel was stained for 2 h at room temperature with Coomassie solution containing 0.125 g Coomassie blue R-250 (Bio-Rad, Hercules, CA, USA) in 50% methanol/10% acetic acid/40% water, and was destained with 50% methanol and 10% glacial acetic acid for 1 h, followed by further destaining with 10% methanol and 5% glacial acetic acid for 8 h.

Production and Purification of AChE Antibody. Five New Zealand White rabbits were immunized with Mono-Q column and/or Epoxy-activated Sepharose 6B affinity chromatography-purified AChE from honeybees, and the AChE Ab were produced by repeated immunization. Four injections of 300 mg purified AChE (dissolved in 300 μL PBS) each were given intradermally at 30, 14, 10, and 3 days before the final bleed on day zero. The first initial injection contained Freund's complete adjuvant (Sigma St. Louis, MO, USA), whereas the boosters contained Freund's incomplete adjuvant. Care and handling of the animals was conducted in accordance with guidelines laid down by the Animal Research Review Committee of the University. The AChE Ab titers were measured using ELISA, and active blood fractions were subsequently collected (from 15 to 20 mL). Purification of AChE Ab was performed using Protein-A-Agarose (Sigma St. Louis, MO, USA), phosphate buffer containing 0.1 M NaCl as wash/binding buffer, and 0.2 M glycine (pH 2.85) as an elution buffer. IgG fractions were dialyzed against phosphate buffer with six changes at 2 h intervals. Then this was lyophilized and stored at −20 °C. Immunodiffusion assay method was used to confirm the specificity of the purified AChE.[31]

Assemblage of the Strip. Based on antigen-antibody reaction, AChE (act as antigen) was covalently bound to the nitrocellulose membrane by means of IgG (act as antibody). The test strip was pasted onto plastic backing with adhesive. An automatic dispenser (NDS-600, Zeta Corporation, Seoul, Korea) was used to dispense a solution mixture of AChE and IgG at a ratio of 2:1 on the nitrocellulose membrane (Milipore, MA, USA) (Figure 1). The solution in its form and ratio was used to reduce both the concentration of antigen and antibody and the assemblage steps. The principle of the strip preparation method was based on modification of ELISA using carbonate buffer (pH 9.6) as the coating buffer (at 4 °C for 3 h), carbonate buffer (pH 7.6) as the washing buffer, and bovine serum albumin (BSA) as blocking buffer for 1 h. A glass filter (Milipore, MA, USA) was attached to both ends of the strip to prevent chlorophyll absorbance.

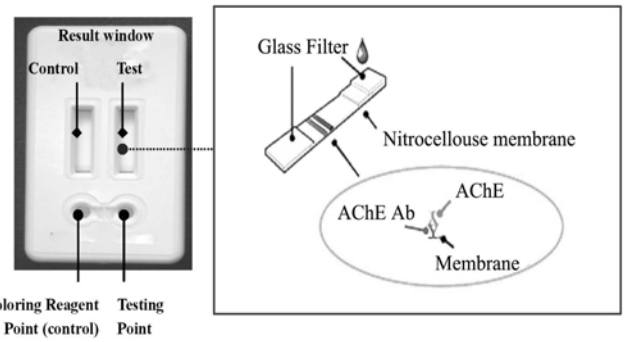

Figure 1. Composition of simple pesticide residue detection strip.

Sample Preparations. Four to five real samples (weighing approx. 1.0 to 2 kg each) for each of the food commodities used in this study (vegetable and fruits) were bought from a large market located in the Gwangju region, Republic of Korea. The blank fruits and vegetables were bought from a special market (selling organic vegetables and fruits) and confirmed by GC-ECD analysis. Once it was confirmed that the samples did not contain any pesticide residues, they were used to determine the limit of detection (ranging from 0.50 to 4.75 ppm). Samples of each concentration were assayed five times (n = 5). All samples were chopped, mixed, packed separately in plastic bags, labeled, and stored at −20 °C until analysis. Approximately 1 g of sample was mixed with 2.5 mL coloring reagent (ATC and DTNB) and left for 5 min. For detection, one drop of the liquid samples and coloring reagents was pipetted into the sample pad, and the sample began to migrate upward. After 10 min, results of testing were assessed. Traditionally, AChE activity was determined with endpoint measurement, in which the enzyme was incubated with substrate, and excessive DTNB was added to colorize only after the reaction was stopped.

Results and Discussion

Optimization Conditions for AChE Extraction. The AChE enzyme activity was gradually increased with temperature, reaching its peak at 37 °C. The activity suddenly decreased from 49 °C onwards (data not shown). This might be due to partial inactivation and/or irreversible denaturation of the purified AChE, which has been reported previously.[21] The optimal pH value for AChE extraction was indicated by an increased enzyme activity, reaching its peak at pH 7.6 (data not shown). The enzyme activity was also increased by increased Triton X-100 concentrations, and reached a maximum at 0.5% (v/v), remaining stable thereafter (data not shown).

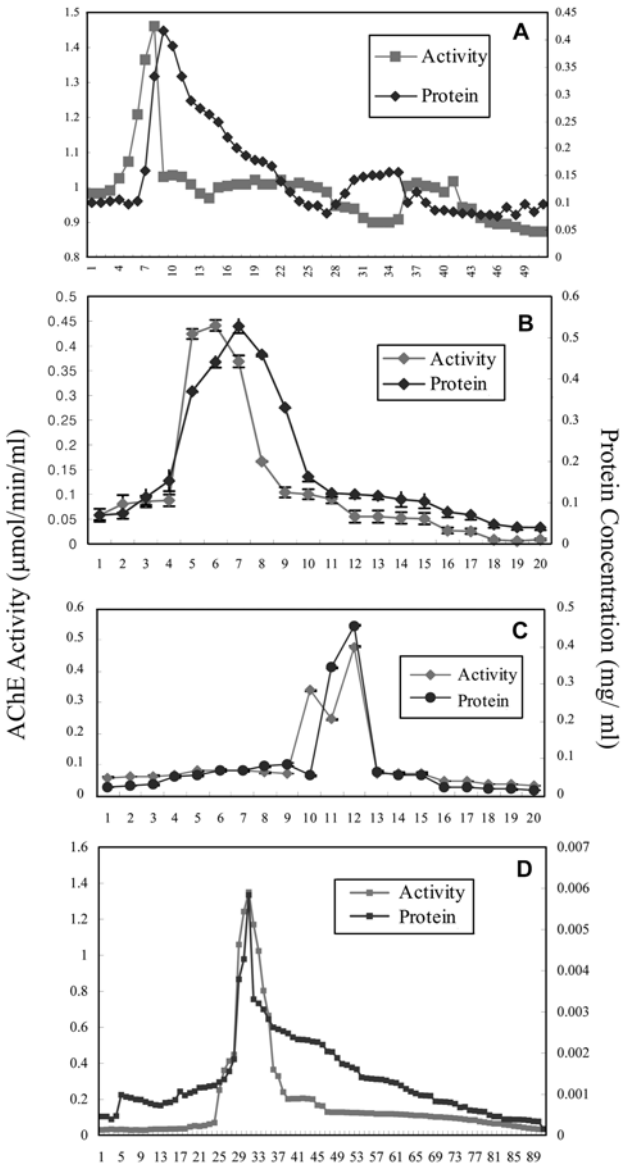

Figure 2. Profiles of AChE activity and total proteins detected using DEAE cellulose chromatography (A), affinity chromatography (B), Mono Q-column chromatography (C), and Epoxy-activated Sepharose 6B (D).

Purification of AChE. To purify AChE from honeybees, we developed a three-step purification procedure including DEAE cellulose chromatography (Figure 2A), procainamide-based affinity chromatography (Figure 2B), and Mono-Q column chromatography (Figure 2C); these procedures were performed after Triton X-100 extraction and ammonium sulfate precipitation. The results of the present study clearly demonstrated that the AChE from heads of honeybees could be purified to apparent homogeneity using the 3-step procedure. The Mono Q-column chromatography appears to be an effective step. The overall purification factors were 5.759, 1327.593, and 2211.593-fold with yields of 39.961, 17.463, and 11.791, respectively, for the 3-steps (Table 1). The purification factor of honeybee heads was higher than that of AChE purified from *G. mellonella* heads by affinity chromatography followed by anion exchange chromatography (283-fold),[16] but smaller than those of *L. decemlineata* heads by affinity chromatography (39,000-fold),[19,32] and *D. virgifera virgifera* (20,000-fold) by affinity chromatography.[7] The activity yield was comparable to those found in previous purifications of other AChEs.[7,13,15,16,32-36] However, it was considerably higher than that recorded by Hsiao *et al*.[21] Approximately 17 μg of purified AChE with a specific activity of approximately 119.588-μmol/min/mg proteins was obtained from 4 g of honeybee heads. With ATC as a substrate, the specific activity of purified AChE has been reported to be 1350, 385, 50, 128, 5, 1456, 691, and 119.588 units/mg protein for *Drosophila* heads,[37] horn fly (by anion exchange chromatography),[38] Western tarnished plant bug,[34] Colorado potato beetle,[19] lesser grain borer,[6] cotton aphid,[13] oriental fruit fly,[21] and honeybee (by affinity chromatography). The variation in the specific activity of the purified AChE among different insect species may be partially due to the degree of purity of AChE, but might also be due to variation in the insect species. The purification of acetylcholinesterase by Epoxy-activated Sepharose 6B affinity chromatography (Figure 2D) is faster (2 days) and more specific than other chromatographic procedures (7 days), in addition to the satisfyingly high recovery rate. In this case, the enzyme was determined to be homogenous by PAGE with 2480.426-fold purification, a final recovery of 95.551%, and a specific activity of 133.943 ìmol/min/mg. The enzymes obtained by Mono-Q column and Epoxy-activated Sepharose 6B affinity chromatographies were used for subsequent biochemical characterization and mass production, respectively.

Characterization of AChE. The results of nondenaturating polyacrylamide gel electrophoresis showed only one major band, suggesting a single molecular and electrophoretically homogeneous form (Figure 3). This band corresponded to the Coomassie Brilliant Blue-stained protein band, which suggests that we had only one major molecular form of AChE in purified samples obtained from head of honeybees. A similar trend was illustrated in oriental fruit fly.[21] The

Table 1. Various purification steps for AChE obtained from honeybee heads

Procedures	Protein (mg/mL)	Total protein (mg)	Specific activity (μmol/min/mg)	Total activity (μmol/min)	Purification factor (fold)	Yield (%)
Triton X-100 extraction	6.390	319.500	0.054	17.242	1.000	100.000
Precipitation	6.062	73.930	0.112	8.260	2.074	47.906
DEAE-cellulose chromatography	0.964	22.180	0.311	6.890	5.759	39.961
Affinity chromatography	0.047	0.042	71.690	3.011	1327.593	17.463
Mono-Q column chromatography	0.011	0.017	119.588	2.033	2214.593	11.791
Epoxy-activated Sepharose 6B	0.006	0.123	133.943	16.475	2480.426	95.551

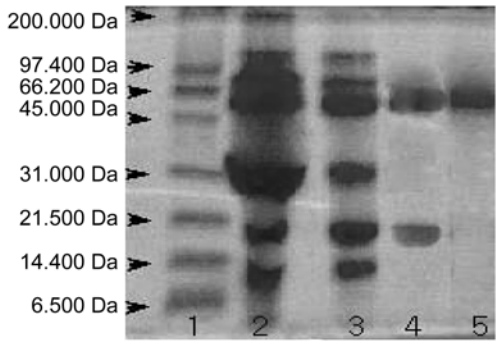

Figure 3. Nondenaturing PAGE analysis of the purified AChE. 1) Weight marker, 2) Extraction, 3) DEAE cellulose chromatography, 4) Mono Q-column chromatography and 5) Epoxy-activated Sepharose 6B.

Table 2. Percentage of antibody binding to membrane surfaces

Membrane	Percent
Cellulose acetate	87
PTEF	101.5
Nitrocellulose	100
Nylon	117
PVDF	97
Polystyrene	107.5

molecular mass of the AChE subunit of honeybees was estimated to be approximately 57 kDa. This value corresponds very well with AChE purified from *Drosophila* (56 kDa),[37] lesser grain borer (56 kDa),[6] and horn fly (54 kDa),[41] and quite similar to those purified from waxmoth (60 kDa),[16]

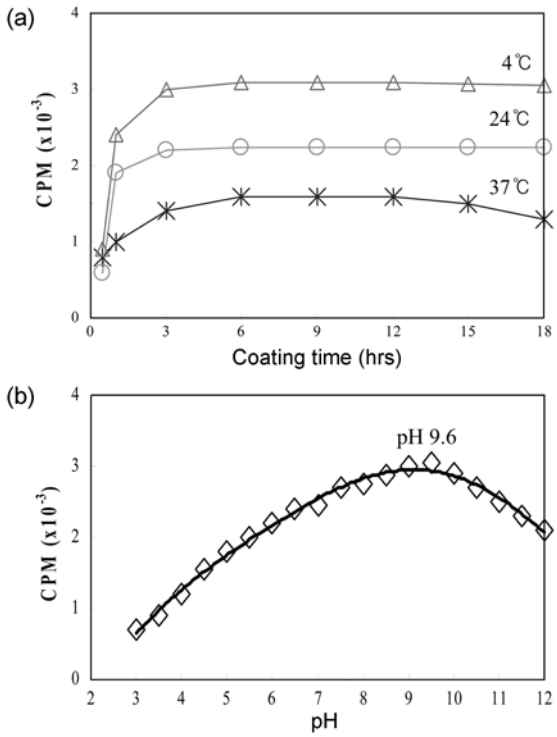

Figure 4. (a) Adsorption of IgG to nitrocellulose membrane as assayed by ELISA. (b) Effect of coating time and pH on IgG adsorption to nitrocellulose membrane.

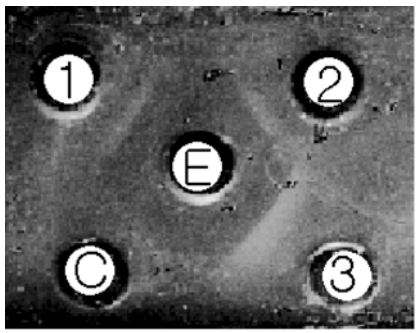

Figure 5. Immunodiffusion of AChE Ab (E-Purified AChE ; 1,2,3-AChE Ab obtained from three rabbits and (C) serum obtained from control rabbit).

Japanese quail brain (62.5 kDa),[36] Cotton aphid (63.5 kDa),[13] and Colorado potato beetle (65 kDa).[19]

Purification of AChE Antibody. A marked immune response was observed in all immunized animals from the first booster injection and the subsequent injections, and the presence of anti-AChE antibodies in the anti-sera was assessed using ELISA. The affinity of the adsorption of the ACh Ab (IgG) to surface membranes was tested in this study. Nitro-

Table 3. Detection limits of the standard pesticides using the AChE detection kit

No	Carbamates	Detection limit	Organophosphate	Detection limits
1	Aldicarb	0.75	Acephate	0.75
2	Bendiocarb	1.25	Azinphos-methyl	0.50
3	Benfuracarb	0.75	Bensultap	1.25
4	Carbaryl	0.50	Bifenazate	0.75
5	Carbofuran	1.50	Carbendazim	1.00
6	Fenobucarb	1.25	Chloropyrifos-methyl	1.50
7	Fenoxycarb	1.00	Demeton-S-methyl	0.75
8	Furathiocarb	0.75	Diazinon	0.50
9	Isoprocarb	0.75	Dichlorvos	0.75
10	Methiocarb	1.50	EPN	1.25
11	Methomyl	0.75	Ethion	1.25
12	Pirimicarb	2.50	Ethoprophos	0.50
13	Propoxur	1.25	Fenitrothion	0.50
14	Thiobencarb	0.50	Guthion	1.00
15	Thiodicarb	2.25	Malathion	0.50
16			Methyl-parathion	0.75
17			Omethoate	0.75
18			Phenthoate	1.25
19			Phosalone	1.00
20			Phosphamidan	1.25
21			Phoxim	1.25
22			Primiphos-methyl	0.50
23			Profenofos	1.00
24			Prothiofos	1.50
25			Pyraclofos	0.50
26			Pyridaphenthion	1.25
27			Quinaphos	1.00
28			Terbufos	0.75
29			Triazophos	0.50
30			Trichlorfon	1.50
31			Zolone	0.75

cellulose, which gave a 100% binding capacity, was chosen because it is cost-efficient soft, safe, and the movement of the immunoreagent on it occurred quickly (Table 2). The minimum IgG volume adsorbed to the membrane was 9 μg/mL at 4 °C (coating temperature) for 6 h (coating time) using carbonate buffer, pH 9.6 (Figure 4). Approximately 13.02 μg of purified AChE Ab with a specific activity of approximately 31.94 μmol/min/mg protein was obtained from 15 mL of blood. Immunodiffusion confirmed specificity of the purified AChE and the antibodies as shown in Figure 5.

Monitoring of Pesticide Residues in Samples. Korean cabbages, cucumbers, garlic, fresh green peppers, lettuce, onions, welsh onions, squash, perilla leaves, sweet peppers, carrots, dropworts, apples, pears, grapes, kiwifruits, mandarin oranges, oranges, and peaches were chosen as model matrices to evaluate the suitability of the developed kits for the analysis of 15 carbamate and 31 organophosphate pesticides (Table 3) in vegetables and fruits. Five replicates of each pesticide standard at the range of 0.50 to 2.50 ppm or fortified matrices at the range of 0.5 to 4.75 ppm were examined. The limits of detection for the standard pesticides are shown in Table 3. Some of these levels were below the maximum residue limits (MRLs), while the majority of the concentrations were higher than the MRLs. For simplicity, we presented the limits of detection in a few representative fortified (Table 4) and real samples (Figure 6). The limits of detection (LOD) were determined by the presence of five bands in the tested region, and six bands indicated no negative results. It should be noted that the limits of detection using standard pesticides and fortified samples were different due to the matrix effect. In Table 4, it is shown that both carrot and dropwort had higher LOD compared to others. The phytochemicals such as β-carotene and chlorophyll, present in carrot and dropwort, respectively, may be the reason that the coloring reagents of the developed kits showed some disturbances. As shown in Figure 6, diazinon was not detected in pear, but chlorpyrifos-

Table 4. Detection limits of some OP and C pesticides in fortified blank samples using AChE detection kits

Compounds	Carrot		Dropwort		Garlic		Pear		Perilla leaves		Korean cabbage	
OP	MRL	LOD	MRL	LOD	MRL	LOD	MRL	LOD	MRL	LOD	MRL	LOD
carbendazim	1.00	4.75	x	4.00	1.00	1.50	2.00	1.50	5.00	1.75	x	2.00
chloropyrifos-methyl	0.50	4.25	x	3.75	0.50	1.25	0.50	1.00	1.00	1.00	1.00	1.25
diazinon	0.50	3.75	x	4.75	0.10	0.75	0.10	0.75	x	1.25	0.10	0.50
Dichlorvos	0.30	4.50	x	4.50	0.30	1.50	0.10	0.50	x	1.00	0.30	1.00
malathion	0.50	4.75	x	3.75	2.00	3.00	0.50	0.50	2.00	1.75	0.50	1.00
phenthoate	x	4.00	x	4.00	x	1.75	0.20	0.50	x	1.75	x	1.25
pirimiphos-methyl	0.50	4.50	x	4.50	1.00	1.75	1.00	0.75	x	2.75	2.00	0.75
prothiofos	x	2.75	x	3.00	x	1.75	0.05	0.50	x	1.25	0.05	0.50
C												
carbaryl	0.50	4.25	x	3.75	x	0.75	0.50	0.50	x	1.00	0.50	0.75
carbofuran	x	4.75	x	3.75	x	0.75	0.10	0.50	x	1.00	x	1.00
methomyl	0.20	4.25	x	4.00	0.20	1.00	x	0.75	x	2.00	0.50	0.75

X: MRL not authorized by the Korea Food and Drug Administration (KFDA).

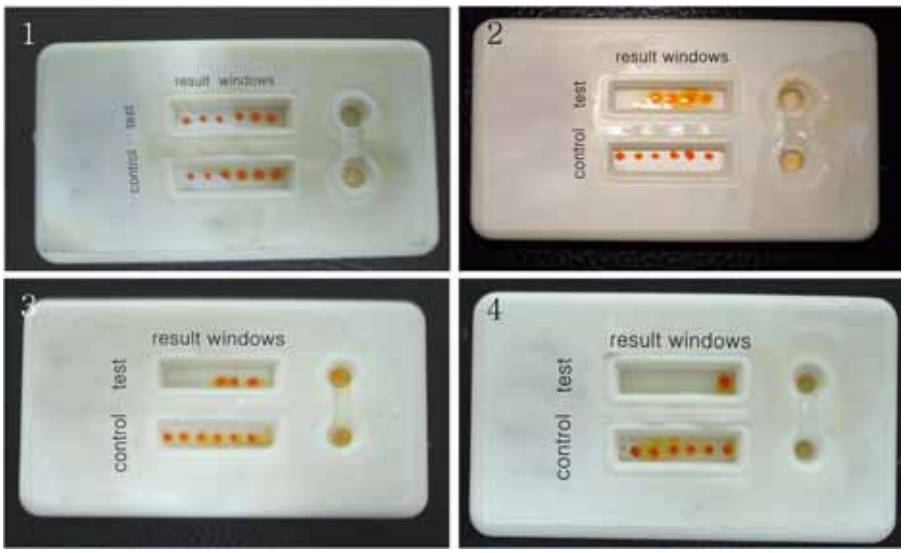

Figure 6. Pesticide residues in real samples 1) diazinon not detected in pear, 2) carrot with diazinon residues of 4.25 ppm, 3) perilla leaves with chlorpyrifos-methyl ranging from 1.25-1.50 ppm, and 4) dropwort with phenthoate at a residue of 4.5 ppm using AChE detection kits.

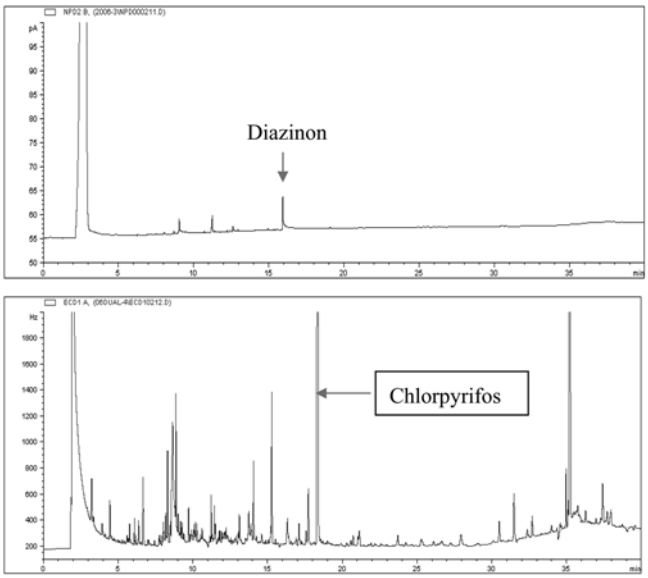

Figure 7. Chromatograms of diazinon (0.05 ppm) in pear and chlorpyrifos (1.325 ppm) in perilla leave as determined by GC-NPD or ECD, respectively.

methyl was detected at concentrations ranging from 1.25 to 1.50 ppm. This finding was confirmed by GC-NPD/ECD (Figure 7). Using GC-NPD, diazinon was detected at a very low concentration, 0.05 ppm. This low concentration is out of margin of detection for the developed kits. Additionally, chlorpyrifos-methyl was detected at a residue level of 1.325 ppm, which strongly supports our finding. Currently, an experimental work is being undertaken to quantify all of the pesticide residues in various food matrices and to correlate the findings with GC-ECD/NPD.

The simplicity, cost-effectiveness, and field-portability of the developed kits make them very promising as analytical tools for use in pesticide monitoring programs, particularly for those chemicals that are difficult and/or costly to investigate by conventional chromatographic techniques or for specific pesticides that deserve special attention because of their toxicity, extensive use, or high frequency of appearance in foodstuffs.

Conclusion

Results of this work clearly prove that the developed kit is capable of analyzing various pesticides belong to the C and OP pesticide groups in a variety of fruits and vegetables at appropriate levels of detection. AChE detection kit requires minimal equipment and is easy to use even for unskilled people. An additional issue of major importance is the ability to determine pesticide levels from crude samples, which has several practical consequences. First, the amount of time required for analysis is significantly decreased, allowing a higher sample throughput. Second, the cost of analysis is reduced, as is organic solvent consumption. The developed kits might be easily included as a complementary method for use in pesticide regulation programs.

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