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Detection of Multi-class Pesticide Residues Using Surface Plasmon Resonance Based on Polyclonal Antibody

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Abstract The detection of carbamate (carbofuran, carbaryl, benfracarb, thiodicarb, and methomil) and organophosphate (diazinon, cadusafos, ethoprofos, parathion-methyl, and chlorpyrifos) pesticide residues with very low detection limits was carried out using surface plasmon resonance (SPR) based equipment. The capacity to develop a portable SPR biosensor for food safety was also investigated. The applied ligand for the immunoassays was polyclonal goat anti-rabbit immunoglobulin (IgG) peroxidase conjugate. Concentration tests using direct binding assays showed the possibility of quantitative analysis. For ligand fishing to find a proper antibody to respond to each pesticide, acetylcholinesterase (AChE), and glutathione-s-transferase (GST) were tested. The reproducibility and precision of SPR measurements were evaluated. With this approach, the limit of detection for pesticide residues was 1 ng/mL and analysis took less than 11 min. Thus, it was demonstrated that detecting multi-class pesticide residues using SPR and IgG antibodies provides enough sensitivity and speed for use in portable SPR biosensors.

Keywords: carbamate, organophosphate, pesticide, surface plasmon resonance (SPR)

Introduction

During the last 60 years, large amounts of pesticides (herbicides, fungicides, and insecticides) have been used throughout the world, and millions of tons are used each year in agriculture, medicine, and industry. Many of them are highly toxic and their accumulation in living organisms can cause serious diseases (1-3).

In particular, carbamate and organophosphorous compounds are highly toxic, even though they demonstrate rather low environmental persistence. For most of these compounds, pesticidal action is the result of the inhibition of acetylcholinesterase (AChE) at nerve endings (cholinergic synapses) (4). This inhibition leads to severe impairment of nerve function or even death. Because of the effectiveness of this approach, insect AChE is the biological target of most insecticides used in agriculture (5). The detection of cholinesterase inhibitors (AntiChEs) is of concern to the regulatory agencies that deal with pesticide residues in food products and agricultural environments. However, AChE-based biosensors applied to the detection of organophosphate and carbamate pesticide residues measure the sum of toxic effects from all pesticides in a sample expressed as the paraoxon equivalent, but they do not measure the total pesticide concentration (6). It was reported that an optical biosensor was constructed for the detection of the major component in pesticides, atrazine by detecting the inhibition of glutathione-s-transferase (GST). The detection limit was 0.84 μM using extinction (7). In addition, pesticide residues such as chlorsulfuron, imidaclopird, methsulfuron-methyl, and simazine have been analyzed using amperometric sensors, photosensitive diode detectors, and surface plasmon resonance (SPR) devices with goat anti-rabbit immunoglobulin (IgG) peroxidase conjugates (8-11).

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Until recently, the identification and quantification of pesticide residues in water and other sources have been limited to the use of traditional gas chromatographic (GC), high performance liquid chromatography (HPLC), and spectroscopic methods. Although they are very sensitive, these sophisticated techniques are time consuming and require highly trained personnel and expensive apparatus (12,13). Furthermore, these methods are unsuitable for real-time, *in situ,* or on-line monitoring of pollutants.

A biosensor can be defined as a device revealing the association between a sensitive biological element and a transducer, which converts the biological signal into a measurable physical signal (14). Various types of biosensors have been developed in the field of environmental monitoring. Depending on the nature of the biological sensing element, they can be divided into immunosensors, enzyme sensors, organite-based sensors, and whole cell sensors. The presently available tranducers can be broadly divided into electrochemical, optical, mass sensitive, and thermal devices (15). The main optical immunosensors developed for environmental monitoring are based on SPR devices or the absorption or emission of light by the immunoreactants (9,16-18).

The used SPR device for detecting pesticides in this study was the BIAcore 3000 (BIAcore Co., Uppsala, Sweden), which works with an auto sampler allowing the continuous monitoring of a large number of samples. The robustness of this method was verified by the stability of the biospecific surface during 200 regeneration cycles.

It was therefore the objective of this study to test the possibility of simple and rapid detection of pesticide residues using SPR, to find a suitable enzyme substrate which reacts with pesticides, to clarify the optimal working conditions, and to evaluate measurement sensitivity to determine if it is practical to develop miniaturized biosensors on the basis of SPR. The conditions of antibody immobilization on the SPR sensor surface were analyzed through atomic force microscopy (AFM) imagery.

Materials and Methods

Materials Anti-rabbit IgG (whole molecule)-peroxidase antibody produced in goat, acetylcholinesterase (AChE) from electric eel (EC 3.1.1.7; 1.1 mg/mL), glutathione-s-transferase (GST) from schistosoma japonicum (EC 2.5.1.18; 1 mg/mL), phosphate-buffered physiological saline (PBS), pH 7.4, and standard carbamate (carbofuran, carbaryl, benfracarb, thiodicarb, and methomil) and organophosphate (diazinon, cadusafos, ethoprofos, parathion-methyl, and chlorpyrifos) pesticides were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). IgG antibody was solid phase adsorbed with normal human serum proteins to ensure minimal cross reactivity in tissue or cell preparations. The affinity purification of IgG and Fab fragments has been described by Wilson and Nakane (19) and Mouvet *et al.* (20,21). Working standard solutions were prepared daily by dilution in PBS solution Tween 20-pH 7.4 (PBS: 10 mM). Carboxylated dextran matrix based (CM5) sensor chips and amine coupling kits that included 70% glycerol, BIAdesorb solution 1, BIAdesorb solution 2, and 1 M ethanolamine pH 8.5 were purchased from BIAcore Co. Common chemicals used in sensor surface immobilization were also purchased from Sigma-Aldrich: 100 mM *N*-hydroxysuccinimide (NHS), and 400 *N*-ethyl-*N'*-(3-dimethylamino-propyl) carbodiimide hydrochloride (EDC).

SPR device SPR device used in this study was a commercial SPR instrument, the BIAcore 3000. Biospecific interaction analysis (BIA) is able to measure biospecific interactions (e.g., antigen-antibody binding) in 'real-time'. The commercially available instrument (BIAcore) employs the principle of SPR (22) to continuously detect changes in the refractive index of an antibody and antigen solution close to the surface of the sensor chip. Antibody and antigen (toxin) are allowed to flow continuously over the surface. As antibody binds to the conjugate, the refractive index of the buffer in contact with the sensor chips changes. Continuous monitoring of the resonance angle gives a change in the refractive index of the buffer solution close to the metal film surface (23). This change is then detected and quantified (in resonance units, RU) by the instrument as a sensorgram. Approximately 1,000 RU is equivalent to a mass change in the surface concentration of 1 ng/mm^2 (24). After the binding interaction occurs, the bound antibody can be removed using chaotropic reagents, which allow the sensor surface to be used over 100 times repeatedly. BIAcore has been used for applications such as kinetic analysis (25).

Optimum pH buffer for immunoassay An immunoassay for a biochemical is a test that measures the level of a substance in a biological liquid using the interaction of an antibody with its antigen. Monoclonal antibodies derived from a single cell line, although homogenous and useful for specific and accurate testing, have some limitations including the need for highly skilled individuals, complicate screening procedures, and high cost of production (26). Instead, polyclonal antibodies were tested which are derived from different B-cell lines and bind to more than one site on multiple molecules to see the suitable antibodies

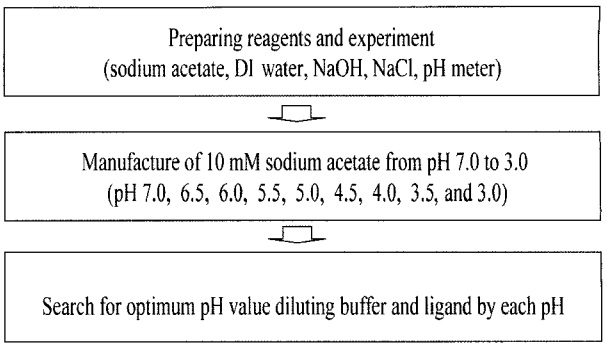

Fig. 1. Protocol for preconcentration.

reacted with pesticide residues. These antibodies, IgG, AChE, and GST are typically produced by the immunization of a suitable animal, such as a goat, rabbit, electric eel, or *schistosoma japonicum*.

The most crucial element of the detection strategy is a highly specific antibody-antigen interaction. First, a preconcentration test was performed to find the optimum pH value of the buffer, and then the antibodies were immobilized using the amine-coupling method. Then ligand fishing was performed to identify a proper antibody to respond to the pesticides, followed by AFM image analysis and immunoassay by serial dilution for detecting pesticides.

Preconcentration is a procedure carried out to give a high local protein concentration at the sensor chip surface and to determine the optimal immobilization buffer and pH. In this way, the immobilization of the protein to the sensor chip surface is more efficient (27). Preconcentration was done with the dextran-carboxyl group based sensor chip, CM5, which was designed to allow detailed quantitative studies on concentration, binding ratios, interaction kinetics, and affinity. In preconcentration experiments, the ligand was diluted in several immobilization buffers with a pH range of 7.0-3.0, each differing by 1.5 pH units (Fig. 1).

Surface activation and enzyme immobilization The first step in interaction analysis is the immobilization of one of the interactants on the sensor chip surface. Choosing an immobilization method depends primarily on the nature of the ligand. Amine coupling is generally the most applicable coupling chemistry and is recommended as the first choice. Most macromolecules contain amine groups, which can be used in amine coupling. Covalent coupling through amine groups produces stable surfaces.

Details of the immobilization protocol are described in Fig. 2. A mixture of NHS/EDC (0.1/0.4 M in water) was pumped over the gold-coated sensor surface in a volume of 35 μL. EDC converts the carboxylic acid of the alkanethiol into reactive intermediates (NHS esters), which react with the free amine groups of the IgG (50 μg/mL in 10 mM sodium acetate buffer, pH 5.0 selected by preconcentration test) protein conjugate. The AChE and GST protein were tested also. Injection of ligand leads to electrostatic attraction and coupling to the surface matrix. At this point, the ligand solution is still in contact with the sensor surface, and includes both immobilized and non-covalently bound ligand. The immobilization process concluded with

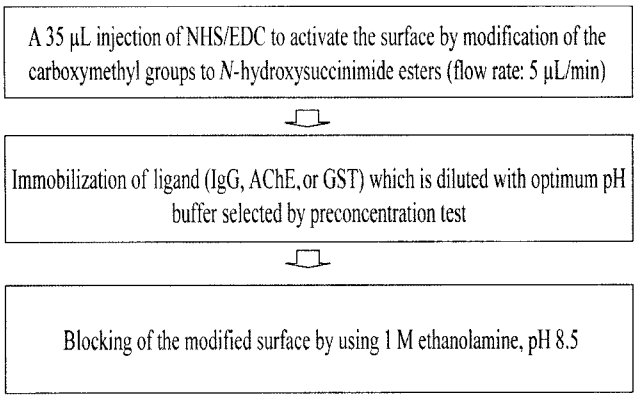

Fig. 2. Protocol for enzyme immobilization.

the blocking of the modified surface with 1 M ethanolamine, pH 8.5. This procedure ensures both the elimination of the non-covalently bound protein conjugate and the deactivation of all unreacted NHS-esters remaining on the sensor surface. Running and dilution buffer (10 mM HEPES-NaOH, pH 7.4) was used in all binding experiments and for regeneration.

An immobilized protein receptor can be used to screen or 'fish' for orphan ligands. This type of experiment can be used to identify binding activities in conditioned cell media, lysates, etc. (28). It also allows on-line detection of binding events and the direct quantification of bound material on biological surfaces of interest (29).

Ligand fishing was performed in order to find the proper receptor to react with the carbamate and organophosphate residues. Antibody-antigen reactions between proteins (IgG, AChE, and GST) and pesticides were tested for ligand fishing, and the best receptor was chosen in each case.

Concentration and AFM image analysis There are two principal assay formats for concentration analysis using SPR biosensors: direct binding assays and inhibition assays (30). Because the measured response is proportionally related to the concentration of analyte in the samples and the response time, direct binding assays for these experiments were used. All pesticide samples were dissolved and diluted in running buffer in the range of 0.0001-10 μg/mL to minimize solution refractive index changes so that the sensor would respond primarily to binding.

AFM images provide information regarding surface topological changes to identify captured proteins on protein arrays (31). Label-free methods such as SPR are capable of detecting and quantifying bound proteins onto arrays by using changes in the refractive index of the surface (32, 33). Therefore, these two methods should be combined to completely analyze the antibody immobilization.

Results and Discussion

Optimal working conditions for immobilization With most chemical coupling methods, preconcentraiton of ligand on the chip surface is important for the efficient immobilization of macromolecules. Preconcentration is accomplished by the electrostatic attraction between

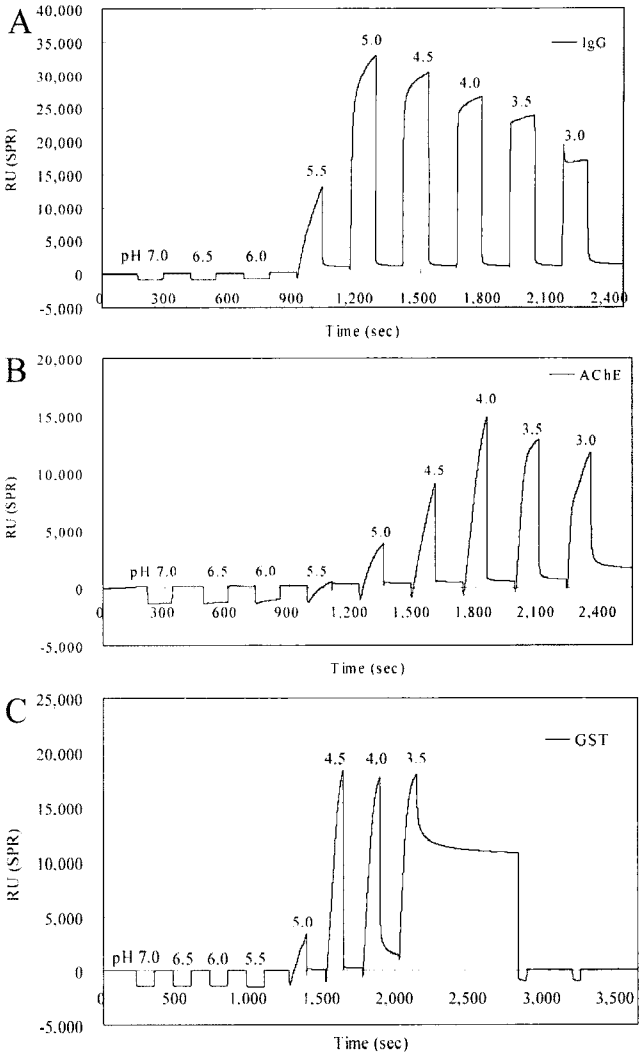

Fig. 3. Results of preconcentration tests with various proteins. IgG (A), AChE (B), and GST (C).

negative charges on the surface matrix and positive charges on the ligand at suitable pH values, and allows efficient immobilization from relatively dilute ligand solutions. A preconcentration test was performed from pH 7.0 to 3.0 to select the optimum buffer pH. The results showed high RU values from pH 5.0 to 4.0. Sometimes preconcentration was efficient although the covalent linkage was not due to the low pH. The dextran matrix on the gold surface lost its preconcentration capacity at a pH lower than 3.0. Thus, to use higher pH values, pH 5.0 for IgG, pH 4.0 for AChE, and pH 4.5 for GST were chosen (Fig. 3).

Enzyme immobilization To activate the surface by converting the carboxymethyl groups to *N*-hydroxysuccinimide esters, NHS/EDC (0.1/0.4 M in water) was mixed and injected onto the sensor surface for a period of 7 min (step a of Fig. 4). The surface of the reference cell, flow cell 1, was not coated to compare with the sensograms of flow cell 2. The *N*-hydroxysuccinimide esters react with the amines on the ligand to form covalent links on flow cell 2 (step b of Fig. 4), and this reaction was carried out multiple times until enough antibodies were immobilized. Unreacted NHS-esters were deactivated by adding 1 M ethanolamine

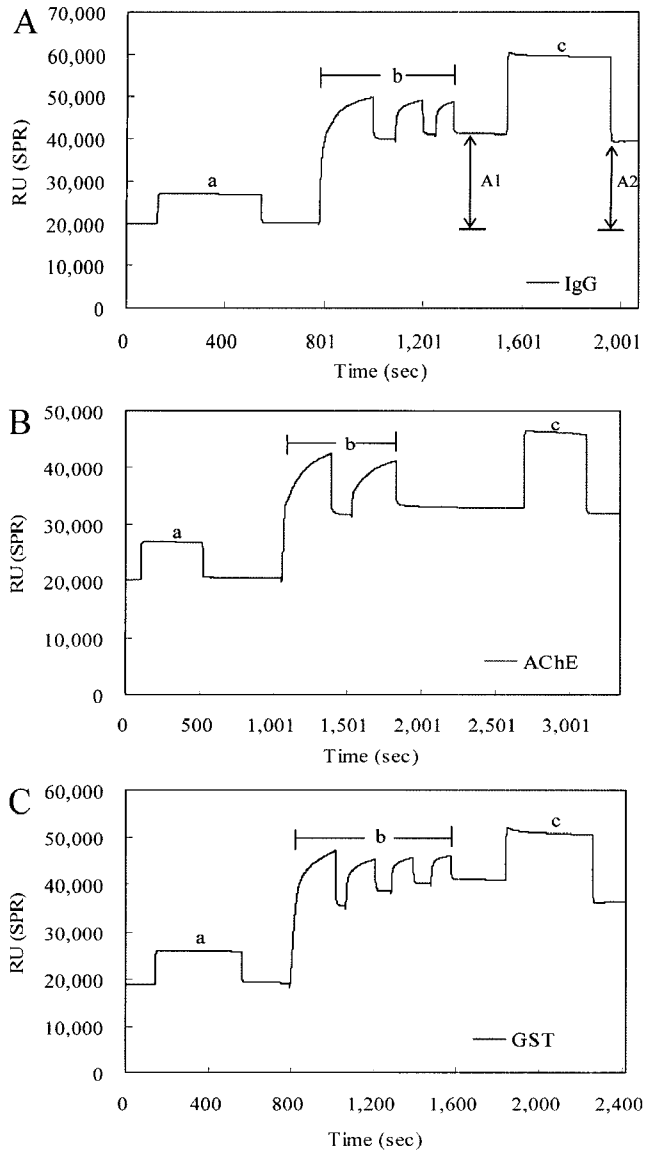

Fig. 4. Enzyme immobilization. (A) IgG, (B) AChE, and (C) GST.

hydrochloride (step c of Fig. 4). Typical quantities of immobilized ligand before and after deactivation are shown in A_1 and A_2 of Fig. 4. The measured amounts of immobilized IgG, AChE, and GST proteins were 19,257, 11,372, and 17,240 RU, respectively.

Selection of appropriate ligand The results of ligand fishing with the various ligands (IgG, AChE, and GST) and analytes (carbamate and organophosphate pesticide) are shown in Table 1. Among the pesticides, methomil was not detected due to nonspecific reaction based on its small molecular weight of 162.21 Da. IgG and GST were able to detect pesticide samples well with the exception of methomil. However, AChE showed a much higher degree of nonspecific reaction than the other analytes. Because of the high degree of nonspecific reaction, the signal from the AChE interaction with carbofuran could not be distinguished from the nonspecific signal; much like the methomil signal seen with all analytes. Because IgG provides better reactivity, economical efficiency and universality relative to GST, IgG protein is chosen as a proper ligand. In addition, GST and IgG can both be activated using the same procedure.

Table 2. The analysis of antibody-antigen reactions

Items	Surface roughness (nm)	Average cell pitch (nm)	Average cell size (nm)
Dextran	4.04	0.44	54.08
IgG ligand	8.85	1.48	102.20
Pesticide analyte	70.63	2.52	140.51

AFM image analysis Several photographs were taken of the carboxylated dextran matrix based (CM5) gold chip surface, IgG protein immobilized on the dextran surface, and interactions between IgG ligand and pesticide analytes using AFM. The size of AFM images used for analysis was 5(L)×5(W) μm and 5 spots were analyzed and averaged. The features analyzed were surface roughness, average cell pitch, and average cell size. The surface roughness of the CM5 chip was 4.04 nm. Immobilizing a layer of IgG protein onto the surface increased the roughness to 8.85 nm, however when pesticide analytes were captured by the immobilized IgG, the surface roughness soars to 70.63 nm (Table 3). This shows that antibody and antigen reacted briskly. The average cell pitch and size of pesticide analyte were also shown to be larger than dextran and IgG.

Association, dissociation, and regeneration reactions by concentration The association and dissociation phases provide a lot of information about the detection of pesticide residues and the kinetics of the analyte-ligand interaction. The equilibrium phase provides information about the affinity of the analyte-ligand interaction, which can be used for quantitative analysis. Thus, the information from concentration tests was analyzed using direct binding

Table 1. Results of ligand fishing using IgG, GST, and AChE[1)]

Antibody \ Antigen	Carbamate					Organophosphate				
	Carbo.	Carba.	Benfu.	Thiodi.	Metho.	Diaz.	Cadus.	Etho.	Parat.-Me.	Chlor.
IgG	+	+	+	+	-	+	+	+	+	+
GST	+	+	+	+	-	+	+	+	+	+
AChE	-	+	+	+	-	+	+	+	+	+
Mw (Da)	221.3	201.2	410.5	353.46	162.21	304.4	270.4	242.3	263.2	350.6
MRL (μg/mL)	0.01	0.2	0.22	0.05	0.05	0.05	0.01	0.005	0.01	0.05

[1)]+, specific reaction; -, nonspecific reaction.

Table 3. Regressions of each composition from 1 ng/mL to 1 μg/mL

Classes	Pesticides	Regressions	R^2	LODs (μg/mL)	Average standard error	Average chi^2
Carbamate	Carbofuran	Y=6.4595X−5.9665	0.985	0.001-1	1.80	0.77
	Carbaryl	Y=4.9010X−5.7870	0.980	0.001-1	1.59	0.68
	Benfracarb	Y=5.0160X+3.7213	0.955	0.001-1	1.99	0.85
	Thiodicarb	Y=5.6531X−2.9159	0.977	0.001-1	2.10	0.90
Organophoshate	Diazinon	Y=4.9267X−3.0416	0.982	0.001-1	1.05	0.45
	Cadusafos	Y=5.3562X+3.0851	0.981	0.001-1	1.36	0.58
	Ethoprophos	Y=4.6596X+2.1526	0.975	0.001-1	1.01	0.43
	Parathion-Methyl	Y=5.3873X+0.3028	0.986	0.001-1	1.03	0.44
	Chlorpyrifos	Y=6.5476X−4.9313	0.990	0.001-1	0.91	0.39

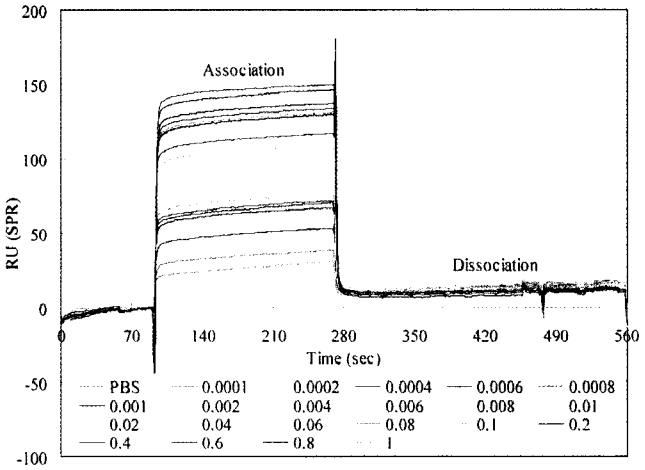

Fig. 5. Association, dissociation and regeneration reactions by concentration.

assays grouped by class of pesticide:carbamate and organophosphate. Regeneration of the sensor chip surface must totally remove the analyte without damaging the immobilized ligand, IgG protein. The 10 mM NaOH for regeneration was chosen to use. The reaction time in total was 636 sec (10.6 min): preparation (90 sec), association (180 sec), dissociation (288 sec), and regeneration (78 sec) (Fig. 5).

Evaluation of the measurement sensitivity To analyze the sensitivity of the SPR device for the detection of different pesticide classes, an experiment to determine the detection limit was performed using the direct binding assay with samples ranging from 0.0001 to 10 μg/mL (Fig. 6). The plots have a positive slope relative to increasing concentration with similar inclinations. These results provided an opportunity for quantitative analysis by concentration using a polyclonal ligand as shown in Fig. 6a and 6b. Unfortunately, the thiodicarb graph show a strange lack of consistency, which was considered to be the result of pipetting errors.

Regressions, coefficients of determination (R^2), and limit of detections (LODs) of each analyte are described in Table 3. The R^2 ranged from 0.95 to 0.99. The repeatability test for pesticide compounds was carried out with various pesticide concentrations. The saturation point was reached

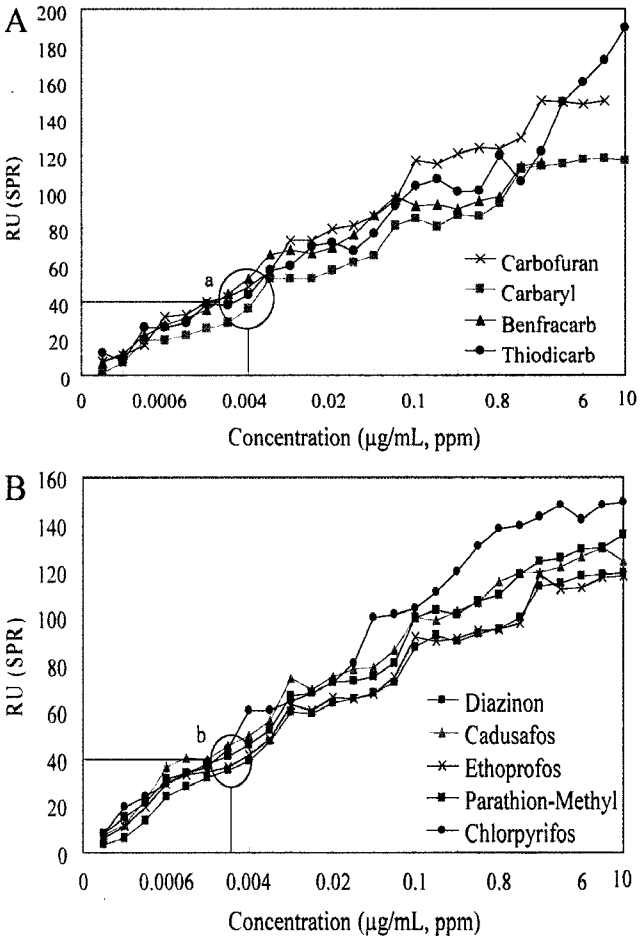

Fig. 6. Concentration tests by the direct binding assay. (A) Carbamate components and (B) organophosphate components.

at 1 μg/mL for all samples except for thiodicarb. Therefore, the range for detecting carbamate and organophosphate pesticides was from 1 ng/mL to 1 μg/mL allowing a safety coefficient of 10. These experiments satisfy the maximum residue limits (MRLs) established by the Korea Food & Drug Administration (KFDA) which sets MRLs for agricultural chemicals in agricultural produce, particularly produce entering the food chain (Table 1).

The tests were performed over 105 times per pesticide (Table 3), with each sample repeated 5 times. As the result,

the average standard error and chi^2 for RU values for each analyte decreased from 2.10 and 0.90 to 0.91and 0.39. These tests showed a close correlation between concentration and SPR signal (RU value). The fabricated protein IgG maintained its activity for over 200 trials. Therefore, detecting multi-class pesticide residues using SPR and IgG antibody provided enough sensitivity and real time capability for its use in a study of portable SPR biosensors. However, this assay is not suitable for the simultaneous assay of multiple samples due to the use of single flow cells, although the SPR device has enough sensitivity. Each sample was prepared at a known dilution in buffer, not from actual agricultural products. Therefore it is necessary to investigate the possibility of cross reaction using vegetable samples. Additional future studies will involve miniaturized SPR devices or biosensors which have multiple flow cells, and also automatic sample pretreatment systems which can extract pesticide residues from agricultural products.

Acknowledgments

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References

1. Chun MH, Lee MG. Reduction of pesticide residues in the production of red pepper powder. Food Sci. Biotechnol. 15: 57-62 (2006)
2. Ivnitskii DM, Rishpon J. A potentiometric biosensor for pesticides based on the thiocholine hexacyanoferrate (III) reaction. Biosens. Bioelectron. 9: 569-576 (1994)
3. Kang SM, Lee MG. Fate of some pesticides during brining and cooking of Chinese cabbage and spinach. Food Sci. Biotechnol. 14: 77-81 (2005)
4. Pogaènik L, Mladen F. Determination of organophosphate and carbamate pesticides in spiked samples of tap water and fruit juices by a biosensor with photothermal detection. Biosens. Bioelectron. 14: 569-578 (1999)
5. Millard CB, Broomfield CA. Anticholinesterases: Medical applications of neurochemical principles. J. Neurochem. 64: 1909-1918 (1995)
6. Marty JL, Mionetto N, Lacorte S, Barcelo D. Validation of an enzymatic biosensor with various liquid chromatographic techniques for determining organophosphorus pesticides and carbaryl in freeze-dried waters. Anal. Chim. Acta 311: 265-271 (1995)
7. Andreou VG, Clonis YD. Novel fiber-optic biosensor based on immunobilized glutathione s-transferase and sol-gel entrapped bromcresol green for the determination of atrazine. Anal. Chim. Acta 460: 151-161 (2002)
8. Dzantiev BB, Yazynina EV, Zherdev AV, Plekhanova YV, Reshetilov AN, Changc SC, McNeil CJ. Determination of the herbicide chlorsulfuron by amperometric sensor based on separation-free bienzyme immunoassay. Sensors Actuat. B-Chem. 98: 254-261 (2004)
9. Harris RD, Luff BJ, Wilkinson JS, Piehler J, Brecht A, Gauglitz G, Abuknesha RA. Integrated optical surface plasmon resonance immunoprobe for simazine detection. Biosens. Bioelectron. 14: 377-386 (1999)
10. Song SJ, Cho HK. Enzyme immunoassay for on-line sensing of the insecticide imidaclopird residues. J. Korean Soc. Agric. Mach. 28: 505-510 (2003)
11. Xing WL, Ma LR, Jiang ZH, Cao FH, Ming-Hong JC. Portable fiber-optic immunosensor for detection of methsulfuron methyl. Talanta 52: 879-883 (2000)
12. Oh CH. Applicability of using GC-PDD (pulsed discharge detector) for multiresidual pesticides analysis. Food Sci. Biotechnol. 15: 959-966 (2006)
13. Yang GM, Cho NH. Sensing of the insecticide carbofuran residues by surface plasmon resonance and immunoassay. J. Biosystems Eng. 30: 333-339 (2005)
14. Coulet PR. Biosensor Principles and Applications. Marcel Dekker, Inc., New York, NY, USA. pp. 1-6 (1991)
15. Marty JL, Leca B, Noguer T. Biosensors for the detection of pesticides. Analusis 26: M144-M149 (1998)
16. Marco MP, Barceló D. Environmental applications of analytical biosensors. Meas. Sci. Technol. 7: 1547-1562 (1996)
17. Maurize E, Calle A, Lechuga LM, Quintana J, Montoya A, Manclús JJ. Real-time detection of chlorpyrifos at part per trillion levels in ground, surface, and drinking water samples by a portable surface plasmon resonance immunosensor. Anal. Chim. Acta 561: 40-47 (2006)
18. Mauriz E, Calle A, Abad A, Montoya A, Hildebrandt A, Barcelo D, Lechuga LM. Determination of carbaryl in natural water samples by a surface plasmon resonance flow-through immunosensor. Biosens. Bioelectron. 21: 2129-2136 (2006)
19. Wilson M, Nakane P. Immunofluorescence and Related Staining Techniques. Elsevier/North Holland BioMedical Press, Amsterdam, Netherlands. p. 215 (1978)
20. Mouvet C, Broussard S, Jeannot R, Maciag C, Abuknesha R, Ismail G. Validation of commercially available ELISA microtiter plates for triazines in water samples. Anal. Chim. Acta 311: 331-339 (1995)
21. Mouvet C, Amalric L, Broussard S, Lang G, Brecht A, Gauglitz G. Reflectometric interference spectroscopy for the determination of atrazine in natural water samples. Environ. Sci. Technol. 30: 1846-1851 (1996)
22. Quinn JG, O'Kennedy R. Transduction platforms and biointerfacial design of biosensors for 'real-time' biomolecular interaction analysis. Anal. Lett. 32: 1475-1517 (1999)
23. Stephen JD, Gary JK, Paul PD, Bernadette MM, Richard O, Heather AL, Michael RA. Development of surface plasmon resonance-based immunoassay for aflatoxin B1. J. Agr. Food Chem. 48: 5097-5104 (2000)
24. Stenberg E, Persson B, Roos H, Urbaniczky C. Quantitative determination of surface concentration of protein with surface plasmon resonance using radiolabeled proteins. J. Colloid. Interf. Sci. 143: 513-526 (1991)
25. Malmborg AC, Borrebaeck CA. BIAcore as a tool in antibody engineering. J. Immunol. Methods 183: 7-13 (1995)
26. Aga DS, Thurman EM. Immunochemical Technology for Environmental Applications. American Chemical Society ACS Symposium Series 657, American Chemical Society, Washington DC, USA. pp. 22-23 (1997)
27. Elwing H. Protein absorption and ellipsometry in biomaterial research. Biomaterials 19: 397-406 (1998)
28. Macromolecular interactions facility (MIF). Ligand fishing. Available from: http:// www.med.unc.edu/wrkunits/2depts/biochem/MACINFAC/biacore.html#10. Accessed Mar. 5, 2007.
29. Drewianka S. Biochips in drug development. Available from: http:// www.innovations-report.de. Accessed Mar. 5, 2007.
30. David W. The Immunoassay Handbook. 5th ed. Elsevier Press. Oxford. UK. pp. 289-291 (2005)
31. Zhu H, Snyder M. Protein chip technology. Curr. Opin. Chem. Biol. 7: 55-63 (2003)
32. McDonnell JM. Surface plasmon resonance: Towards an understanding of the mechanism of biological molecular recognition. Curr. Opin. Chem. Biol. 5: 572-577 (2001)
33. Yuk JS, Ha KS. Proteomic applications of surface plasmon resonance biosensors: Analysis of protein arrays. Exp. Mol. Med. 37: 1-10 (2005)