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Novel surface plasmon resonance (SPR) immunosensor based on monomolecular layer of physically-adsorbed ovalbumin conjugate for detection of 2,4-dichlorophenoxyacetic acid and atomic force microscopy study

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Abstract

A rapid and simple optical immunosensor for detection of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) has been developed based on SPR technology. Functional sensing surface of the immunosensor is created by immobilizing an ovalbumin conjugate of 2,4-D (2,4-D-OVA) by simple physical adsorption on an SPR thin-film gold chip. It has been established that the Au surface of the sensor chip was completely covered by 2,4-D-OVA up to a monomolecular layer and that the 2,4-D-OVA immobilized sensor chip was highly resistive to non-specific binding of proteins. Selective binding of a monoclonal antibody against 2,4-D (2,4-D-Ab) is followed by an increase in SPR angle. The antibody complexed on the sensor surface could be removed simply by the flow of an acidic buffer (glycine.HCl; 0.2 M, pH 2.0) for less than 1 min, facilitating repeated use of a same sensor chip. A competitive immunosensing method has been applied for the detection of 2,4-D, in which binding of the antibody onto the sensor surface in the presence and absence of 2,4-D is investigated. When 2,4-D is present in sample solution, a competition is set off between 2,4-D in solution and 2,4-D-OVA conjugate on sensor chip for binding to 2,4-D-Ab. A lowest detection limit of 0.1 ng/ml 2,4-D is established. Calibration curve of this analytical system covers a wide concentration range of 0.1–300 ng/ml 2,4-D. One assay could be completed in 18 min (binding, 15 min; acidic eluent and followed carrier buffer, 3 min). Enzyme-linked immunosorbent assay (ELISA) measurements for the detection of 2,4-D using analogous antigen-coat format showed a detection limit of 500 ng/ml. The high sensitivity of the configured SPR immunosensor system and the differences between the performances of SPR and ELISA are discussed. Cross-reactivity of the SPR sensor against a few compounds structurally and environmentally relevant to 2,4-D is examined. The fabricated SPR sensor is found to be highly resistant to interference with a maximum cross-reactivity of only 4% for 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

Keywords: 2,4-Dichlorophenoxyacetic acid; Surface plasmon resonance sensor; Immunosensor; Monolayer; ELISA; 2,4,5-Trichlorophenoxyacetic acid; 2,4-Dichlorophenol

1. Introduction

Biosensing methods have been immensely explored for analysis of environmental, pharmaceutical and food samples because of their potential to detect trace amounts of the analyte of interest directly from complex test solutions owing to the use of highly selective and sensitive biorecognition elements. Especially immunoassay methods attract extensive attention because antibodies can differentiate the target molecule among structurally and chemically relevant molecules and the ability to develop antibodies against any specified organic compound. Despite the development of various immunoassays like radioimmunoassay (RIA) or ELISA, immunosensors derived by the integration of immunoassay methods with modern

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transducer technologies eliminate laborious time-consuming procedures for the separation of bound or unbound antibodies or antigens and the use of (radioactive) reporter labels associated with immunoassay methods [1–3]. Physical transducers responding to changes in mass (piezoelectric), electrical properties (impedance, capacitance, etc.) or optical properties (diffraction, SPR, etc.) could directly follow immunoreactions in real-time without employing any reporter molecule. Among various transducers, SPR technique is versatile owing to its outstanding attributes such as miniaturization, reliable portable instrumentation, automation, disposable sensor chips and high throughput microarray sensing, and thus research areas exploiting SPR technology are growing widely from medical diagnosis, drug discovery to biowarfare detection [4–13].

Monitoring of environmental pollutants has become an increasingly demanding field because of the dreadful health hazards caused to terrestrial and aquatic wildlife. 2,4-D is one of the most widely and extremely used herbicide and it has been associated with the occurrence of cancer, endocrine-disrupting activities and degenerative changes in nerve system [14–16]. Water regulatory bodies fix the tolerable concentration for an individual pesticide in potable water to 0.1 ng/ml [17]. To ensure such a low-detection-limit exclusively in a wide variety of food products, potable water, etc., there is a strong demand for developing fast, sensitive, field-portable and cost-effective analytical techniques. Various immunoassay methods based on light absorption [18], chemiluminescence [19], quartz crystal microbalance (QCM) [20], impedance [21] and atomic force microscopy (AFM) [22] were investigated for detection of 2,4-D, but most of them involve either laborious time-consuming procedures, trained personnel, conjugation with reporter molecules or laboratory-based expensive instrumentation. In this report, SPR technique is explored for the development of a high performance immunosensor for 2,4-D. SPR is extremely sensitive to refractive index changes occurring in close vicinity to the transducer surface caused by the interaction of an analyte. Despite numerous advantages for practical applications, SPR response weakens with decreasing molecular weight of the analyte, making it difficult for quantification of trace amounts of small molecules. In order to overcome the drawback associated with the detection of small-molecules, an indirect competitive immunoreaction principle was introduced with which the SPR immunosensors become capable to detect very low concentration of low-molecular-weight (lmw) analytes of environmental, biomedical or pharmaceutical interest [23–26].

In the present work, development of a regenerable SPR immunosensor for highly sensitive and selective detection of 2,4-D (MW = 221) is described. Efficient integration of a biorecognition element with transducer platform is the prime factor in the fabrication of high performance sensor surfaces. A variety of methods were employed for functionalizing the sensor surface. In general, commercially available dextran polymer-based sensor chips were widely used as the base in affinity-based SPR sensor applications [4,27–31]. Recently, surface coatings with poly(ethyleneglycol)-based materials comprised into either three-dimensional polymer networks or ordered two-dimensional monolayer systems were exposed to be an alternate for the fabrication of sensor chips having superior sensor-performance characteristics [32–35]. We investigated a simple physical adsorption of bovine serum albumin–antigen conjugates for the fabrication of active sensor surfaces and found that the obtained sensor surfaces were highly sensitive and can accomplish simultaneous detection of different lmw environmental toxics from mixed analyte solutions [11] and that the base sensing layer was stable even to the treatment of highly acidic solutions employed for regeneration of active sensor surface [36–38]. The physical adsorption method is extended here to an ovalbumin conjugate for sensor chip fabrication, and wider applicability of the physical adsorption method has been demonstrated. Surface morphology of the formed 2,4-D-OVA conjugate layer was examined by AFM investigation. Stability and regeneration of the sensor surface for repeated use in multiple determinations of 2,4-D are demonstrated. Resistivity of the configured immunosensor to compounds structurally similar to 2,4-D and superior-selectivity and -sensitivity of SPR sensor compared to ELISA measurements are discussed.

2. Experimental

2.1. Chemicals and immunoreagents

2,4-D (Aldrich), 2,4,5-T (Wako), 2,4-dichlorophenol (2,4-DP; Aldrich), 2,4-dichlorobenzoic acid (2,4-DBA; Wako), 4-chlorophenoxyacetic acid (4-CP; Wako), OVA (Sigma), bovine serum albumin (BSA; Sigma), peroxidase-labeled anti-mouse IgG goat antibody (ICN) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)/H_2O_2 substrate solution (Wako) were used as supplied. All other reagents were of analar grade and were purchased from Kanto Chemical Co. Distilled deionized water (resistance = 18 MΩ) was used to prepare all aqueous solutions.

2.2. Synthesis of conjugates and anti-2,4-D antibody

The protein conjugates of 2,4-D (2,4-D-OVA and 2,4-D-BSA) were synthesized similar to our procedure reported elsewhere [39]. Briefly, isobutylchloroformate (10 μl) and *N*-triethylamine (10 μl) were added to a solution of 2,4-D (5 mg in 1 ml dioxane), and the mixture was stirred at room temperature for 2 h. It was added drop-by-drop to an aqueous solution of OVA or BSA (5 mg in 2 ml) and was kept stirring for 16 h. The reaction mixture was then dialyzed at 4 °C and lyophilized to get the conjugates.

A monoclonal antibody against 2,4-D (abbreviated as 2,4-D-Ab) was developed from a hybrid cell resulting from the fusion of an aminopterin-sensitive myeloma cell line and the spleen cell of mice immunized with 2,4-D-BSA [39]. Molecular weight (MW) of the conjugates was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using JMS time-of-flight mass monitor. Samples for the spectrometry were prepared by incorporating these proteins in a matrix of sinapinic acid.