Reusable fiber-optic-based immunosensor for rapid detection of imazethapyr herbicide

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Abstract

Imazethapyr is a herbicide belonging to the imidazolinone class of compounds. It is effective in weed control by virtue of its ability to inhibit branched-chain amino acid biosynthesis [1]. The most effective use of imidazolinone herbicides depends on the knowledge of the metabolism of these compounds in different plants as well as the residual level of compound in the soil [2]. Soil residue analysis by ELISA has been developed [3]. The method requires some pretreatment of the soil extract before processing by ELISA. An alternate detection method for the ELISA system such as evanescent-excited fluorescence [4], piezoelectric oscillation [5,6], and surface plasmon resonance [7], or through a silicon sensor-based pH detector [8,9] may be useful approaches for the development of a simpler assay method for processing large quantities of soil samples.

We used a fiber optic evanescent fluorosensor as described by Rogers et al. [10] for the development of the imazethapyr immunosensing system. In this communication, we demonstrate an antibody sensor using immune sheep antibody immobilized on quartz fibers. Since fluorescein-linked
imazethapyr analog bound to antibody generates signals, imazethapyr can be quantified by affecting dissociation or association of fluorescent signal on the fiber with a dynamic concentration range of $10^{-3}$ to $10^{-9}$ M. This immunosensor system is fast, requires a small sample volume, and is quite transparent to sample matrix, thus requires minimum sample preparation. Another important feature of the sensor is that it is reusable.

EXPERIMENTAL AND RESULTS

Reagents

Polyclonal antibody was generated in sheep using an imidazolinone compound conjugated to bovine serum albumin [11]. The IgG fraction, purified by passing the serum through a protein A column was used to coat quartz fibers for the assay. Analytical grade imidazolinone compounds were synthesized at the American Cyanamid Co. All other agrochemicals were commercial products. The fluorescent tag was prepared by reacting 5-formyl imazapyr with 5(2-carboxyhydrizinomethyl)thio)acetylaminofluorescein to form the fluorescein hydrazino methylene imazapyr (FHMI) as shown in Fig. 1.

Instrumentation

The portable fluorometer described by Rogers et al. [12], and manufactured by ORD (North Salem, NH) was used to measure the fluorescence signal. Quartz fibers (60 mm x 1 mm diam.) with polished ends were purchased from ORD. The fiber optic immunosensor made use of the evanescent wave effect by exciting a fluorophore bound within the evanescent zone as described by Block and Hirschfeld [13]. A portion of the resultant fluorophore emission trapped in the wave guide, was transmitted back up the fiber and detected after transmission through 510 LP and 530/30 nm filter. The flow cell had a volume of 46 μl which was exchanged every 12 s at a flow rate of 0.23 ml/min. Figure 2 shows a schematic drawing of the instrument.

Determination of antibody and FHMI concentration used

FHMI (50 nM) in PBS bound non-specifically to quartz fibers as measured by total internal
fluorescence. However, addition of a 0.1% casein in the perfusate eliminated this non-specific signal. When control sheep IgG was immobilized on the fiber, a very small base-line signal was observed when perfused with FHMI. When FHMI was added to a fiber coated with anti-imidazolinone antibody, a strong signal was generated. This indicated that the binding of the FHMI was specific for the antibody. Figure 3 demonstrates the dependence of signal on the FHMI concentration infused and shows the concentration dependent signal generation by varying the amount of antibody immobilized on the fiber. The optimal concentrations of 25 nM FHMI was used for all perfusions, while 25 μg/ml antibody was used for immobilization.

Format of immunoassay

The cartoon in Fig. 4 describes the two modes where the analyte imazethapyr can be quantified. Association mode allows imazethapyr to be added with the FHMI tag and compete for the antibody binding sites on the fiber as the solution is perfused through the chamber. This results in a dose dependent inhibition of signal binding. Dissociation mode prepared the fiber first by attaching a steady-state amount of FHMI before the analyte imazethapyr is introduced. The fluorescent signal is reduced as a result of the binding of the non-fluorescent imazethapyr. The does responses are presented in Figs. 5 and 6. In the association mode (Fig. 5) the effective dose range of imazethapyr was from 0.1 to 100 μM. For the dissociation mode of the assay (Fig. 6), fluorescence reaches a steady state level in approximately 5 min. If imazethapyr was added to the perfusion solution after steady state fluorescence was established, reduction of fluorescence signal was almost immediate. A concentration dependent displacement of 0.001–100 μM of imazethapyr was observed.

Reusability of the fiber optic sensor

One of the most significant features of this sensor is the ability to use the same sensor for multiple measurements without significant loss of sensitivity. This is due to reversible binding of both FHMI and imazethapyr to the antibody coating the fiber. A single antibody coated fiber was perfused until a steady-state equilibrium was reached. Then, a perfusate containing 1 μM free imazethapyr was introduced. A reduction in fluo-
rescent signal was observed almost immediately due to the displacement of FHMI from the fiber by free imazethapyr (Fig. 7). When free imazethapyr was removed from the perfusate, the optical signal reversed to its steady-state level. In several examples, single fibers were used to obtain multiple measurements over a span of several hours.

Specificity of the fiber optic sensor

The effect of six agrochemicals (Fig. 8) on the immunosensor was investigated. Three of the compounds: imazapyr, imazaquin, and imazamethabenz methyl belong to the same imidazolinone class. The others: chlorimuron ethyl, sethoxydim, and primisulfuron are herbicides with different chemical structures. When 10 μM concentrations of the compounds were added to the perfusion solution after a steady-state binding of the FHMI was established, the three imidazolinone compounds displaced FHMI from the quartz fiber, whereas the three unrelated chemicals did not (Fig. 9). These results indicate the generic nature and specificity of the polyclonal antibody for detecting imidazolinone herbicides [11].
Fig. 5. Inhibition of FHMI association by imazethapyr. Varying concentrations of imazethapyr were added to the perfusion solution (25 nM FHMI in PBS casein). Each perfusion solution reacted with a new antibody coated fiber. All the fibers were prepared on the same day.

Detection of imazethapyr in untreated soil extracts

Two types of soil (Plan0 and Sassafras) with different organic matters and clay content were extracted in water at a soil to water ratio of 1:1.

Fig. 6. Displacement of bound FHMI by imazethapyr. A 25 nM FHMI in PBS casein perfusion solution was used to establish a steady state level of fluorescence. After the steady state level was reached, the perfusate was changed to contain different concentrations of imazethapyr (0.001 to 100 μM). The decrease in fluorescence is measured in seconds.

Fig. 7. Reusability of the fiber sensor. A 25 nM FHMI in PBS casein was perfused to reach a steady state of binding (about 5 min), at the point indicated as "ON", 1 μM imazethapyr was introduced in the perfusate. As considerable amount of FHMI was displaced, the perfusion solution was switched back to 25 nM FHMI alone (indicated as "OFF"). The ON and OFF process was repeated.

Fig. 8. Chemical structures of three imidazolinone class compounds and three non-imidazolinone agrochemicals used to demonstrate the specificity of the fiber optic sensor.
After sedimenting the particles and filtering through a 0.45-μm filter, the extracts were neutralized with 1/10 the volume of 10X PBS buffer and subjected to assay. The perfusion solution was prepared in the soil extraction instead of the PBS and dose response curves with imazethapyr were established. The dose response curves for imazethapyr present in PBS and the two soil extracts were superimposable (Fig. 10), indicating that the sensor system is quite transparent to matrix in the samples.

**Conclusions**

A fiber optic evanescent fluoro sensor was successfully used for the detection of imazethapyr herbicide in buffered solution and in soil extracts. An IgG fraction of a polyclonal antibody was immobilized on the fiber and direct competitive binding of FHMI and free imazethapyr was monitored by total internal fluorescence transmitted through the optic fiber. The dose response curves obtained from either the binding or the displacement modes indicated that the assay sensitivity was better in the displacement mode (0.001 μM for displacement and 0.1 μM for binding). The reversibility of binding of both FHMI and imazethapyr suggests that this immunosensor may be useful for large sample handling for screening purposes. Automation of the assay system can also be envisioned. Since the samples are perfused through the flow-cell, thus, a short interaction time existed on the fiber probe, allowing the material with the highest affinity for the immobilized antibody be bound to the sensor. Matrix materials, which may present problems in a normal ELISA where incubation time is prolonged, may have a minimal effect in this system. This evanescent-excited fluorescence sensor offers the advantage of speed, sensitivity, and matrix transparency.

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![Graph](image)

**Fig. 10.** The matrix transparency of the fiber sensor. Soils Plan0 and Sassafras were extracted with water at 1:1 ratio (v/w). The extracts were centrifuged at 1700 g for 10 min. The clear supernatant was adjusted to neutral pH by the addition of 1/10 the volume of 10X PBS buffer. After filtering through a 0.45-μm filter, the soil extraction solutions were used in place of PBS to prepare the perfusion solutions. FHMI displacement format was used to obtain the dose responses with imazethapyr. Each plot of% inhibition of the soil extracts [Plan0 (●) and Sassafras (○)] and PBS buffer (□) against imazethapyr concentrations represent an average of two measurements.
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REFERENCES