Remote Biosensor for In-Situ Monitoring of Organophosphate Nerve Agents

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Abstract
A remote electrochemical biosensor for field monitoring of organophosphate nerve agents is described. The new sensor relies on the coupling of the effective biocatalytic action of organophosphorus hydrolase (OPH) with a submersible amperometric probe design. This combination results in a fast, sensitive, selective, and stable response at large sample-instrument distances. Such attractive performance is illustrated for direct measurements of micromolar levels of paraoxon and methyl parathion in untreated river water samples. Unlike multi-step inhibition biosensors, the remote OPH probe offers single-step direct measurements, and is thus highly suitable for the continuous monitoring task. Variables relevant to field operations are discussed, along with prospects for remote monitoring and early detection of nerve agents.

Keywords: Organophosphate compounds, Organophosphorus hydrolase, Nerve agents, Biosensor, Remote sensor

1. Introduction

Organophosphate (OP) compounds are among the most toxic substances and are thus commonly used as pesticides and nerve gases. The high toxicity of OP compounds creates the urgent needs for fast-responding analytical systems for their on-site monitoring. Such new field screening tools have relied on the use of inhibition enzyme electrodes [1, 2]. Until recently, cholinesterases, inhibited by OP compounds and carbamate insecticides, have been the only enzymes used for the biosensing of pesticides [1–5]. Noninhibition biosensors for direct monitoring of OP compounds based on the use of organophosphorous hydrolase (OPH) were introduced recently [6–10]. OPH has been shown to effectively hydrolyze a number of OP pesticides (e.g., paraoxon, parathion) and chemical warfare agents (e.g., sarin) [11]. The use of OPH is extremely attractive for the biosensing of OP substances that act as substrates for the enzyme, rather than exerting an inhibitory action. Several types of OPH-based biosensors have been introduced recently, including potentiometric [6–8], optical [9], and amperometric [10] ones.

In this article we report on the development of an OPH-based remote electrochemical biosensor for in situ monitoring of OP compounds. The ability to deploy submersible enzyme electrodes for in situ biosensing of toxic phenolic [12] or peroxide [13] substances has been demonstrated recently in our laboratory. Such remote biosensing technology offers a fast return of the chemical information in a timely, safe, and cost-effective manner [14]. The adaptation of inhibition biosensors for remote operations requires more complicated probes that are based on an internal delivery of the substrate [15]. In contrast, the new OPH-based submersible biosensor is based on the amperometric detection of OPH-catalyzed hydrolysis products, and offers the advantages of simpler, faster, and selective measurements of OP nerve agents. These biosensing considerations have been combined with the need for a compact device, large sample-instrument distances, and compatibility with field/in situ deployment. The new OPH remote bioelectrode thus offers an immediate warning in case of a sudden OP contamination or attack, while avoiding the errors and cost of laboratory-based analyses. The performance characteristics of the new submersible OP probe are reported in the following sections.

2. Experimental

2.1. Apparatus

Experiments were performed with the Bioanalytical Systems (BAS, West Lafayette, IN) Model CV-27 voltammetric analyzer, in connection with the BAS *X–Y–t* recorder. Experiments were performed in a 50 mL glass beaker, into which the probe (sensing) head was submersed. The probe design was similar to that of the previously reported remote phenol biosensor [12]. The electrode assembly, housed in a PVC housing tube, was connected to a 50-ft long shielded cable via three-pin environmentally sealed rubber connectors. The assembly included the OPH-modified carbon-paste working electrode, a Ag/AgCl reference electrode (BAS, Model RE-4) and a platinum wire counter electrode. Two female coupling connectors, fixed with epoxy in the PVC tube, served for mounting the working and reference electrodes; brass screws, located within these connectors, provided the electrical contact.

The carbon paste electrode was prepared by thoroughly hand mixing 120 mg mineral oil and 180 mg graphite powder. The resulting paste was packed firmly into the electrode cavity (3 mm diameter, 1 mm depth) of a 4-cm long Teflon sleeve. Electrical contact (to the inner part of the paste) was established via a stainless steel screw, contacting the brass screw within the female connector on the PVC housing. The paste surface was smoothed on a weighing paper. The enzyme OPH was immobilized by casting a 10 μL droplet, containing 5 μL (of 108 IU/μL) OPH and 5 μl Nafion (in 1 % ethanol) onto the carbon surface, and allowing the solvent to evaporate. The OPH-modified carbon paste electrode was stored at 4 °C until use.

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2.2. Reagents

Organophosphorus hydrolase (OPH) (7250 IU/mg protein, 15 mg protein/mL) was produced and purified according to the methods described by Mulchandani et al [16]; paraoxon and methyl parathion were obtained from Sigma Chemical Co. (St. Louis, MO) and Supelco Inc. (Belefonte, PA), respectively. The graphite powder (grade #38) was obtained from Fisher Scientific (Pittsburgh, PA), while Aldrich (Milwaukee, WI) provided the mineral oil and the Nafion (5 % wt.) solution. The Rio Grande river water samples were collected in Las Cruces, NM.

2.3. Procedure

Chronoamperometric experiments were conducted by applying a potential step (from open circuit to +0.85 V) and recording the resulting transient current. The quantitative information was obtained by sampling the current after 60 s. All experiments were performed at room temperature.

3. Results and Discussion

The new remote biosensor relies on the immobilization of pure OPH (isolated and purified from recombinant *E. coli* cells [16]) onto the carbon-paste transducer, and the amperometric monitoring of the liberated *p*-nitrophenol products. A similar transduction principle was recently employed for the development of disposable screen-printed OP electrodes [10]. Such OPH-recognition/amperometric transduction has been combined in the present study with an environmentally compatible submersible probe, connected to a 50 ft long shielded cable, to allow continuous monitoring of OP nerve agents at large sample-instrument distances.

The potential of the OPH-based remote biosensor for detecting OP compounds in untreated environmental samples, in connection to a large sample-instrument distance, is illustrated in Fig. 1. The figure displays chronoamperograms for an unaltered river water sample containing increasing levels of paraoxon (Fig. 1A) and methyl parathion (Fig. 1B) in 4.6×10^{-6} M and 1.5×10^{-6} M steps, respectively (a–j). The submersible bioprobe yields well-defined amperometric signals that permit convenient quantitation of these micromolar concentrations. Despite the 50 ft long cable, and the full submersion of the sensor head, the noise level is very low. (Note the sensitive current scale.) The response of the unspiked water sample (dotted line) is also very small, indicating minimal interferences of coexisting oxidizable compounds. Such coupling of favorable signals towards the OP substrates with low noise and background levels results in detection limits of 4×10^{-7} M methyl parathion and 9×10^{-7} M paraoxon ($S/N = 3$). While the paraxon signal is linear over the entire ($4.6–46 \times 10^{-6}$ M) range tested that of methyl parathion displays some curvature above 5×10^{-6} M (see insets for resulting plots). The slopes of the linear portions of these resulting plots correspond to sensitivities of 2.14 (methyl-parathion) and 1.45 (paraoxon) nA/μM. Under the chronoamperometric conditions of Figure 1, 60 repetitive measurements are possible every hour, thus providing a rapid warning in case of sudden OP discharge. Such a frequent response cannot be attained with inhibition (acetylcholine esterase) biosensors due to their slow multi-step protocol, involving addition of the substrate, incubation period and enzyme regeneration. Submersible inhibition biosensors also require an internal reservoir for the substrate solution [15]. Note, however, that inhibition biosensors allow lower (nM) detection limits, although not in a remote configuration.

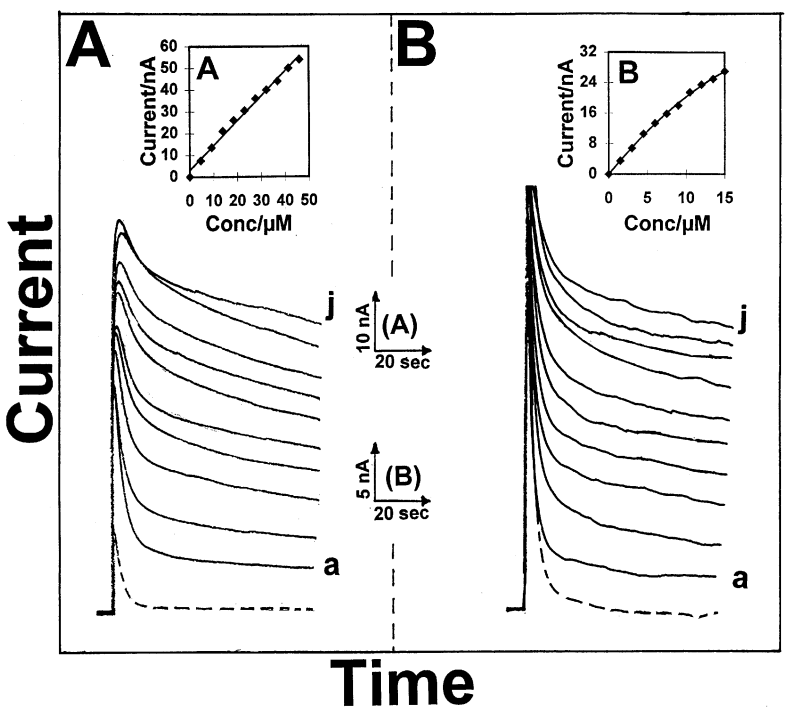

Fig. 1. Chronoamperometric response to an untreated river water sample containing increasing levels of paraoxon (A) and methyl parathion (B) in 4.6×10^{-6} M and 1.5×10^{-6} M steps, respectively (a–j). Dotted lines represent the response of the unspiked samples. Potential step to +0.85 V.

Remote sensors for nerve agents should respond rapidly to sudden changes in the OP concentration. As indicated from Figure 2, the submersible OPH bioprobe responds rapidly to dynamic changes in the concentration of paraoxon (Fig. 2A) and methylparathion (Fig. 2B). No apparent carry over is observed between river water sample containing 4.6×10^{-6} M and 23.0×10^{-6} M paraoxon or for samples spiked with 1.5×10^{-6} M and 7.5×10^{-6} M methyl parathion. High stability is another important requirement for an in-situ OP sensor. Figure 3 displays the response for river water samples containing 2.3×10^{-5} M paraoxon (Fig. 3A) and 7.5×10^{-6} M methyl parathion (Fig. 3B) over prolonged periods of 280 min and 160 min, respectively. For both OP compounds the response remained nearly unchanged throughout these series (following an initial 10 min stabilization). The relative standard deviations over these series are 3.9 (A) and 5.8 (B)%. The data of Figure 3 reflect the reusable character of OPH bioelectrodes (as opposed to inhibition biosensors) and indicate great promise for continuous monitoring of nerve agents. These results also indicate no apparent surface fouling upon prolonged submersion in the river water sample. The chronoamperometric operation provides the necessary baseline for in situ measurements, as compared to potentially drifting and not-measurable one for fixed-potential measurements.

The adaptation of OPH biosensors for in situ monitoring poses several challenges. Unlike laboratory-based sensing applications, where the solution conditions can be adjusted for optimal performance, submersible probes rely on the use of the natural conditions (i.e., untreated samples). Figure 4A examines the influence of the ionic strength upon the sensor output. A similar sensitivity is observed for buffer concentrations ranging from 0.005 to 0.05 M; the signal decreased by 30 % upon lowering the concentration to 0.001 M. While displaying a broad ionic-strength activity the response is strongly dependent upon the sample pH (Fig. 4B). It rises nearly linearly upon increasing the pH from 5.5 to 7.4, and then more slowly. Such pH profile reflects the pH dependence of OPH [16]. The sensor is thus suitable for environmental matrices with pH higher than 6.0. Field calibrations (using the target sample) or programmable pH compensation (common to water profilers) should be useful for addressing the pH profile of Figure 4B.

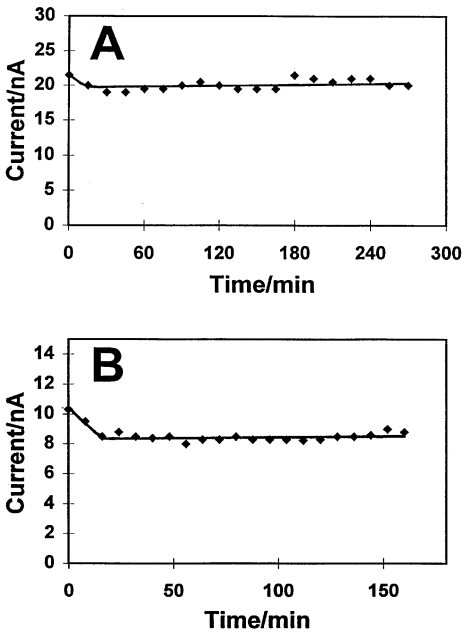

Fig. 3. Stability experiment. Untreated river water samples, containing 23.0×10^{-5} M paraoxon (A) and 7.5×10^{-6} M methyl parathion (B). Other conditions, as in Figure 1.

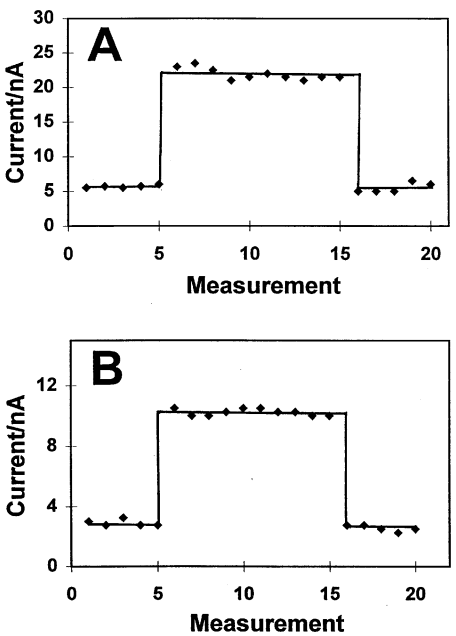

Fig. 2. Carry over experiments. Untreated river water samples, containing 4.6×10^{-6} M and 23.0×10^{-6} M paraoxon (A) or 1.5×10^{-6} M and 7.5×10^{-6} M methyl parathion (B). Other conditions, as in Figure 1.

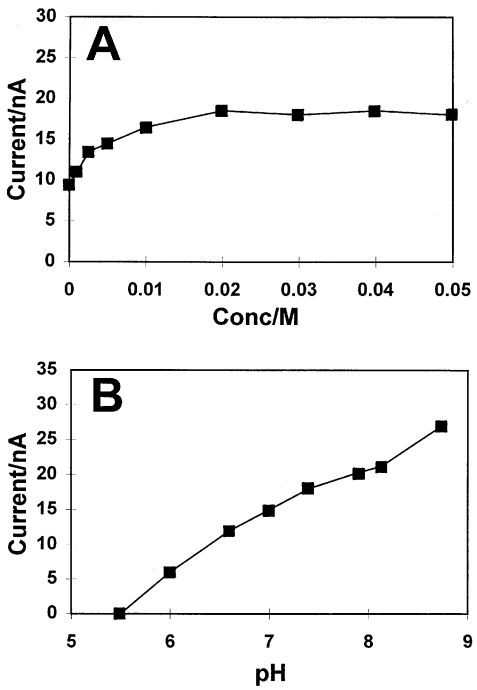

Fig. 4. Effect of solution conditions, including buffer concentration (A) and pH (B) upon the response to 2.3×10^{-5} M paraoxon. Potential step to +0.85 V; medium: phosphate buffer of pH 7.4 (A) and 0.05 M concentration (B).

4. Conclusions

The above experiments have illustrated that toxic OP compounds can be monitored continuously, at large sample-instrument distances, by incorporating a reagentless OPH electrode into a newly designed remote probe assembly. Such remote monitoring capability is coupled to a sensitive, selective, and reversible response. Attention should be given to the presence of oxidizable compounds in actual samples that may lead to false-positive measurements. Further improvements in the detectability are also desired. While the concept has been presented within the framework of OP pesticides, the remote OPH probe should be attractive also for monitoring of phenol-forming OP warfare agents of defense relevance. Compact, low-power, 'on-cable' amperometric microanalyzers (with 'smart' data processing and signal-transmission capabilities) are currently being developed in this laboratory for supporting such environmental and defense surveillance activities. Work is in progress also towards the development of other remote sensors and 'microlaboratories' on a cable platform.

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6. References

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