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Current-Driven Ion Fluxes of Polymeric Membrane Ion-Selective Electrode for Potentiometric Biosensing

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We report here on a novel polymeric membrane ion-selective electrode (ISE) for potentiometric sensing of enzymes and their inhibitors. Diffusion of the substrate ions across the ISE membrane can be controlled precisely by applying an external current. The substrate ions released at the membane−sample interface are consumed by reaction of the enzyme in solution or immobilized on the surface of the membrane, which can be sensed potentiometrically. Applications of this approach include the detection of both free and labeled enzymes in biosensors and enzyme immunoassays and the measurement of bioanalytes involved in enzymatic systems.

Electrochemical control of ion transport through polymeric membranes is an emerging field of research that offers the possibility of programmable tuning of ion fluxes and lowering of the detection limits of potentiometric polymeric membrane ISEs.[1] Current-driven ion fluxes across ISE membranes have been extensively investigated and have been applied in solid-contact ISEs,[2] polyion sensors,[3] pulstrodes,[4] controlled-current coulometry,[5] chronopotentiometric flash titrations,[6] and backside-calibration potentiometry.[7] Although sensitive detection of peptidase activities and surface-confined proteins using chronopotentiometric polymeric membrane electrodes has been reported,[2,8] the use of ISEs with current-controlled release of reagents for sensitive detection and quantification of biomolecules has not been realized to date.

Recently, we developed a promising detection system that makes use of outward ion fluxes through an ISE membrane (i.e., fluxes in the direction of the sample solution) to provide controlled-release substrates for in situ biosensing of enzymes and their inhibitors.[9] This system can be used as a simple and effective potentiometric biosensor based on disturbance of transmembrane diffusion fluxes of ion-selective electrodes under near-zero-current conditions. Such an ISE membrane not only serves as a polymer matrix for reagent release but also works as a transducer for sensitive potentiometric detection, making the ISE membrane very attractive for sensor miniaturization. However, once the substrate ions released at the sample−membrane interface are consumed by the enzyme, especially at high concentrations, the recovery time may be rather long. Reproducible results can only be obtained if a constant release of primary ions in the direction of the sample solution is available before each measurement. Herein, we introduce a novel potentiometric biosensing system using pulsed-current-driven reagent delivery, which addresses such limitations. It will be shown that the current-controlled release of substrate from the ISE enables sensing of enzyme activities and screening of different enzyme inhibitors in a rapid and reproducible way.

As a proof-of-concept experiment, a butyrylcholine (Buch)-selective membrane containing heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (β-CD) as an ionophore was constructed. In addition to the conventional ingredients,[9] ETH 500 was incorporated into the ISE membrane. This lipophilic electrolyte was used to decrease the membrane resistance and thereby the IR voltage drop inside the current-polarized membrane.[10] Notably, spontaneous Buch extraction is often suppressed by working with membranes that possess no ion-exchange properties.[11] In the present work, the cation exchanger provides anion sites that can induce the formation of Buch−ionophore complexes in the membrane. During a short current pulse (e.g., 1 s), the Buch−ionophore complex dissociates, thus resulting in repeatable delivery of Buch cations from the ISE inner solution into the sample solution for in situ biosensing of butyrylcholinesterase (BuchE), which is a major target enzyme of cocaine, organophosphorus pesticides, and chemical warfare agents.

As illustrated in Figure 1, macro-command-controlled procedures for switching between the potentiostatic and galvanostatic steps were designed for substrate release with parallel reading of the system properties (i.e., zero-current potential and current-dependent potential). A controlled voltage was used to refresh the membrane for multiple consecutive measurements. The amount of Buch released in each applied current pulse was determined from the ISE and varied depending on the amount of applied current through the ISE membrane (Figure S1 in the Supporting Information).

To demonstrate the applicability of this system, free enzyme in a sample solution was first determined according to protocol A. After current-driven release of the substrate, traditional zero-current potentials were used for enzyme quantification (pulse 3 in Figure 1). Figure 2A shows a rapid decrease of potential due to the diffusion of Buch cations released from the membrane surface into

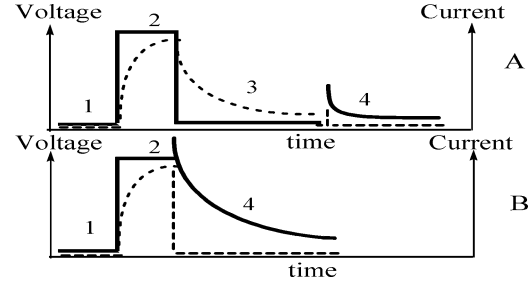

Figure 1. Typical current−time (solid line) and potential−time (dashed line) traces during macro-command-controlled procedures for switching between the potentiostatic and galvanostatic steps. Protocols A and B were used for measuring free enzyme and its inhibitor with immobilized enzyme, respectively. Pulses 1 and 3 are measurement pulses under zero-current conditions. An anodic current (5 μA) was applied for substrate release (pulse 2). Potentiostatic voltage control at the open-circuit potential of the electrode in the absence of analyte was used for membrane restoration (pulse 4).

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the sample bulk, while the presence of BuchE in the sample may further decrease the measured potential by enzyme-catalyzed hydrolysis of Buch. It was found that BuchE concentrations as low as 0.02 unit mL^{-1} could be detected (Figure S2). More interestingly, the potential response was very reproducible from pulse to pulse, with a short recovery time of 120 s. In contrast, the recovery time was more than 20 min for the zero-current release system (Figure 2B).

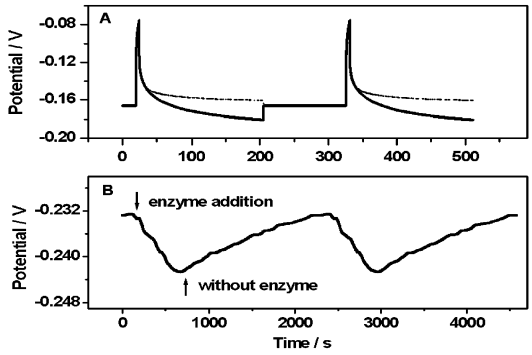

Figure 2. Recovery times of the ISE membranes after consumption of the substrate under (A) the current-driven strategy and (B) zero-current conditions. The enzyme concentrations were (A) 0.47 and (B) 0.08 unit mL^{-1}. For the current-driven strategy, the membrane was renewed at the open-circuit potential for 120 s before the next current pulse in PBS with (solid line) and without (dotted line) BuchE.

We further explored the reagentless detection of pesticides using BuchE, which was immobilized on a carboxylated PVC matrix at the outer layer of the ISE membrane via 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling chemistry (Figure S3). Dichlorvos was measured as an example of the organophosphate pesticide. In protocol B in Figure 1, the applied current pulse drives the substrate ions and generates the reproducible membrane potential. The presence of the target pesticide can inhibit BuchE activity, thus increasing the measured potential. Figure 3 shows that subsequent pulses produce highly repeatable and rapid responses. It shoud be noted that it was possible to develop a reagentless biosensor with immobilized enzyme for inhibitor determination under near-zero-current conditions. However, the recovery time was found to be rather long (data not shown). We also examined the electrode response by using protocol A. Unlike the free-enzyme detection, in which a longer response time of 180 s was needed under zero-current conditions for efficient enzyme-catalyzed hydrolysis of Buch, a potential increase after the dichlorvos inhibition can be observed at both the current-dependent potential (pulse 2) and the zero-current potential (pulse 3), as shown in Figure S4. This is probably due to the fact that a relatively large amount of enzyme is immobilized on the membrane surface, thus causing a rapid potential response even during the applied current pulse of 1 s. Figure S4 also shows that a much larger potential increase can be obtained with the current-dependent potential than with the zero-current potential, indicating that protocol B is suitable for rapid determination of the inhibitor using immobilized enzyme on the ISE membrane surface. Indeed, with protocol B, the inhibition of dichlorvos on BuchE was found to be proportional to its concentration ($R = 0.996$) in the range 0.1−100 ng mL^{-1} with a lower detection limit (3σ) of 0.05 ng mL^{-1}, which is 1 order of magnitude lower than those reported by other researchers.[12] The electrodes exposed to the pesticides may not spontaneously regain their enzyme activities upon removal from the pesticide solution. However, the inhibited BuchE can be completely reactivated through the use of nucleophilic compounds such as pralidoxime iodide (Figure S5). With the reactivation procedure, such a biosensor could be repeatedly used for at least 10 cycles.

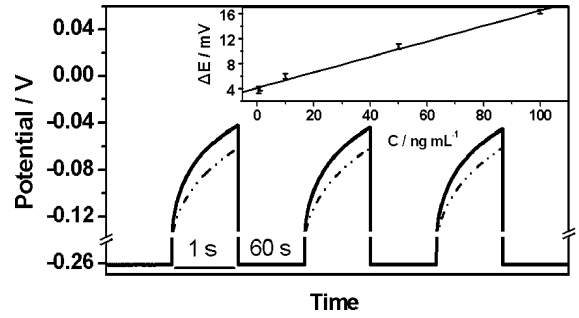

Figure 3. Potentiometric response of the ISE in 0.02 M PBS before (dotted line) and after (solid line) 100 ng mL^{-1} dichlorvos inhibition for 5 min. The inset shows the calibration curve of the ISE for detection of dichlorvos.

In summary, we have reported here a polymeric membrane ion-selective electrode for potentiometric biosensing. This system has the flexibility of trapping substrate ions of enzymatic systems in the inner filling solution. The substrate ions at the membrane−sample interface can be renewed by current-controlled reagent delivery, offering a rapid, reproducible, and continuous biosensing format.

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Supporting Information Available: ISE construction, enzyme immobilization and activation, SEM images, and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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