Detection of inhibitory compounds of acetylcholine esterase with a novel ion responding impedance sensor (IRIS)

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Abstract

This paper describes the detection of an inhibitor of acetylcholine esterase with a new device. Detection of conductivity changes in a microcavity by the action of the enzyme was measured with the ion responding impedance sensor (IRIS). A model pesticide, paraoxone, was studied with the IRIS. The detection limit for paraoxone with the device was 10^{-8} M (3 ppb).

Introduction

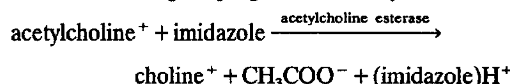
Detection and control of serious pollution incidents in surface waters used for domestic supply requires the development of broad-spectrum sensors capable of rapid and sensitive monitoring. Pollutants such as pesticides are mostly analysed with chromatographic methods that enable the identification of single components. Detection limits required for environmental monitoring must be very low. Time-consuming sample pretreatment is necessary and on-line analysis does not seem to be feasible [1, 2].

Many enzymes and microbial cells release ionic products as a result of a specific conversion. Furthermore, enzymes and cells are vulnerable when exposed to inhibitors such as pesticides, and exposure strongly affects their activity [3].

Recently the ion responding impedance sensor (IRIS) [4] was developed to detect charge density changes in a membrane as a result of an immunochemical reaction. The potentiometric detection was carried out by an 'ion-step'. A transient potential difference over a membrane was detected as a result of this actuation or disturbance of equilibrium. Here, the IRIS was transformed to a new biosensor design (ion responding impedance instead of immunosensor) which exploits conductivity changes as a result of the catalytic activity of an enzyme in a minute volume or microcavity. The internal intrinsic release of ions is detected in a membrane instead of the external actuation of the membrane by an ion step.

The specific reaction of the enzyme acetylcholine esterase, catalysing the conversion of acetylcholine to choline and acetate, was applied as selector for the biosensor. Acetylcholine esterase, which is very sensitive to numerous organophosphorus and carbamate insecticides, was immobilized onto a recently described latex/agarose membrane [4]. This membrane was deposited in disposable wells which created a microcavity when the device was placed on top of the membrane. The monitoring of conductivity changes in a microcavity as a result of enzyme activity was selected because the linear dependence of conductivity with ion concentration renders measurements simple and reliable. Further, the use of a microcavity in the IRIS makes the detection of conductivity changes fast, accurate and sensitive.

The conductivity change in the microcavity can be explained on the basis of the difference in ionic strength before and during the progress of the enzymatic reaction:



$$\text{acetylcholine}^+ + \text{imidazole} \xrightarrow{\text{acetylcholine esterase}} \text{choline}^+ + CH_3COO^- + (\text{imidazole})H^+$$

The hydrolysis of acetylcholine yields acetate ions as well as protonated imidazole, thus increasing the overall conductivity during the enzymatic reaction. If the molar conductivities of acetylcholine$^+$, choline$^+$, CH_3COO^- and imidazoleH^+ are the same then the change in conductivity as a result of the enzymatic reaction can only change by a maximum factor of 2, if initially unprotonated imidazole is used.

Materials and methods

The following materials were used in the experiments: acetylcholine esterase (EC 3.1.1.7, AcChE, Sigma, St. Louis, USA); acetylcholine chloride (Sigma, St. Louis,

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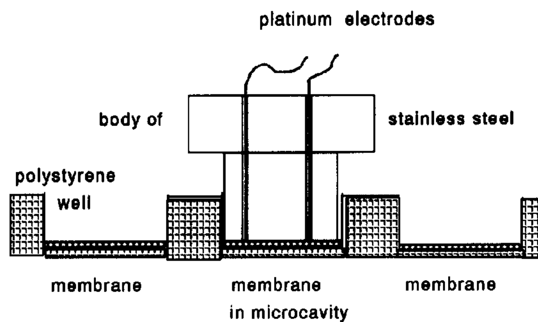

Fig. 1. The IRIS device with microcavity.

USA); Unisphere Polystyrene (PS) latex 11 (0.13 μm, 10%, Serva, Heidelberg, Germany); agarose (type VIII EEO < 0.02, Sigma, St. Louis, USA); 24 wells, cell culture plates (Nunc); imidazole and paraoxone (Fluka, Buchs, CH). The measurements were carried out with battery powered conductivity meters (CDM 80 Radiometer, Copenhagen).

The preparation of the latex/agarose membranes followed the method of Schasfoort *et al.* [4]. The membrane thickness was 5–10 μm, the bottom surface of the well was 200 mm^2 and the volume of the membrane was 1–2 μl.

The procedure was as follows. Five units of the esterase were incubated with the freshly prepared membrane overnight. The membrane was washed with washing buffer (either phosphate buffer 0.1 M or imidazole 2 mM pH 8.5). Paraoxone was incubated for 20 min. After washing of the membrane the experiments were carried out in working buffer; (imidazole, 2 mM, pH. 8.5; acetylcholine 1 mM (=10-fold Km). This concentration and the substrate/buffer ratio were optimized to the greatest signal/background ratio. The microcavity was created and the change in conductivity was followed for at least 2 min.

The device is schematized in Fig. 1. The platinum electrodes were placed in capillaries perpendicular to the membrane. The IRIS device was made heavier with a steel body for a reproducible pressure of the sensor head on the membrane.

Results

Figure 2 shows the inhibitory effect of paraoxone at different concentrations. A better detection limit was obtained when paraoxone in phosphate buffer (pH 7.0) was used instead of the working buffer imidazole. The detection limit was found to be better than 10^{-8} M (3 ppb).

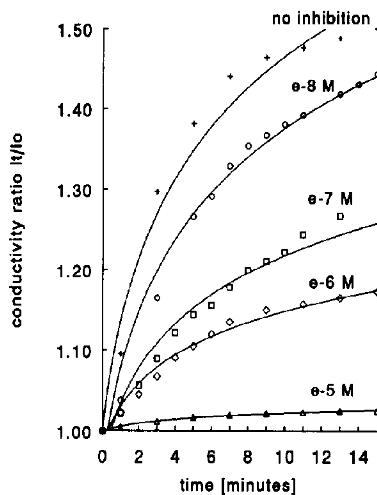

Fig. 2. Detection of the change in conductivity as a function of time (ratio of conductivity at time t (I_t) and initial conductivity (I_0)). Paraoxone inhibition of the response at 0, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M.

Discussion

Since the initial conductivity also influences the change in signal it is important to select the buffer system for the IRIS very carefully. Parameters such as buffer capacity and pH can influence the response to a great extent. This implies that the buffer of choice should possess the highest buffer capacity in the range of the enzyme activity. In all experiments reported the imidazole buffer has been employed because of its pK value (6.93) and its low degree of dissociation at the beginning of a measurement (pH 8.5). This ensures a considerable increase of conductivity as a result of AcChE activity and a simultaneous conversion of imidazole into (imidazole)H$^+$ which amplifies the total response.

Besides the buffer for detection, we found that the choice of buffer for incubation was also of great importance. The buffer has to ensure a suitable pH and ionic strength for a stable paraoxone at low concentrations including an unaffected reaction (inhibition) of paraoxone with AcChE. A 0.1 M phosphate buffer was used for that purpose.

The porous polystyrene latex/agarose membrane deposited on the bottom of disposable plates enabled the creation of well defined microcavities exhibiting similar conductivity values. The observed differences found nonetheless can be attributed to:

(i) discrepancies among individually deposited membranes (non-uniform thickness and porosity),

(ii) a non-ideal loading,

(iii) an irreproducible washing procedure.

The amount of AcChE deposited on the membrane had been evaluated according to procedures described elsewhere [4]. The activity of the enzyme adsorbed to the membrane was about 2% of the activity in solution. It appeared the enzyme loaded onto the membrane had already largely been inactivated. The inactivated part reacts with irreversible inhibitors too, thus decreasing the sensitivity of the IRIS.

The logarithmic curve depicting the change in conductivity as a function of time had two important features: the response was almost linear over the first 2 min of the measurement with a successive decline until saturation was reached after 15–20 min. The reasons why no further increase in conductivity was recorded are:

(i) a slow pH decrease resulting in an non-optimal pH for AcChE lying beyond the enzyme activity range,

(ii) too low a substrate concentration and depletion of the substrate in the microvolume.

A measuring time of 2 min was sufficient for detecting AcChE inhibition with higher inhibitor concentrations. However, incubation times of 20 min are required for the detection of paraoxone concentrations as low as 10^{-8} M (3 ppb).

Conclusions

This paper describes a novel technique that exploits variation in conductivity in a minute volume applied for the detection of pesticides. The change in conductivity in the microcavity as a result of enzyme activity was measured. The ion responding impedance sensor (IRIS) has some advantages over other AcChE electrode systems used for the detection of pesticides. First, conductivity measurements are uncomplicated, reliable and cheap. Second, the measurements are fast in a two-step procedure. Third, the preparation of the plates covered with the latex/agarose membranes and AcChE immobilization is simple and plates are fully disposable. Fourth, the designed sensor head makes the system easy to use and automation seems to be feasible.

The monitoring of AcChE activity in microvolume is suitable for on-line pesticide analysis. However, intensive studies will have to be done to find out the system sensitivity for the broad range of organophosphorus and carbamate pesticides. With respect to low detection limits the creation of an optimized microcavity has to be investigated. The implementation of this device in a flow-through system is of particular interest, resulting in automatic biosensor detection of inhibitory compounds as early warning signals for operation of waste water treatment plants.

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Biography

Richard B.M. Schasfoort (1959) completed his studies in chemistry, especially biotechnology, at the University of Groningen, Netherlands (1984). After a stage at the Institute of Public Health and Environmental Protection (RIVM, Bilthoven) he started a Ph.D. study at the University of Twente (1985). His thesis (1989) entitled 'A new approach to ImmunoFET operation', described the development of an immunochemical biosensor. He is now project manager at the Netherlands Organization for Applied Scientific Research (TNO) on novel (bio)chemical sensor subjects such as ion responding immuno and impedance sensors and surface plasmon resonance immunosensors, operated with flow injection analysis systems.