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Proteolytic Enzyme Sensors Using an Ion-Sensitive Field Effect Transistor

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Two kinds of enzyme sensors were fabricated by chemically immobilizing an enzyme on the gate surface of an ion-sensitive field effect transistor (ISFET). One is a trypsin-membrane/ISFET sensor, which can measure concentrations of *N*-benzoyl-L-arginine ethyl ester in the range from 5×10^{-4} to 5×10^{-2} M. The other is an α-chymotrypsin-membrane/ISFET sensor, which can measure concentrations of *N*-acetyl-L-tyrosine ethyl ester solution in the range of 5×10^{-4}—5×10^{-3} M. The response of the sensors was affected markedly by pH and the concentration of the working buffer.

Keywords——enzyme sensor; proteolytic enzyme sensor; ion-sensitive field effect transistor; trypsin membrane; α-chymotrypsin membrane

An ion-sensitive field effect transistor (ISFET) can be made sensitive to some organic substrates by immobilizing a suitable enzyme on the gate surface. Several techniques of immobilizing enzymes have been developed recently. Because of the high selectivity originating from the highly specific enzymatic reaction, micro enzyme sensors based on the ISFET have proved to be useful in biochemical analysis for a variety of substrates.[1-9] In this connection, we have already reported urea sensors based on the ISFET.[2-5] In the present paper we describe some performance characteristics of trypsin-membrane/ISFET and α-chymotrypsin-membrane/ISFET sensors, which were prepared by directly covering the surface of the ISFET gate with a gel membrane composed of the enzyme, bovine serum albumin, and glutaraldehyde. Such sensors should be useful for the analysis of protein concentration in biological fluids.

Experimental

Chemicals——Trypsin, α-chymotrypsin and bovine serum albumin were purchased from Sigma Chemical Co. *N*-Benzoyl-L-arginine ethyl ester hydrochloride (BAEE) and *N*-acetyl-L-tyrosine ethyl ester H_2O (ATEE) were obtained from Research Organics Inc. and glutaraldehyde (25%) from Nakarai Chemical Ltd. The other chemicals were of reagent grade. Distilled water was used throughout.

Fabrication of the ISFET——The pH-sensitive field effect transistors used in the present study were fabricated on a p-silicon wafer, 0.5 mm wide, 6.5 mm long and 0.2 mm thick. The properties of the ISFET thus prepared were described previously.[9]

Fabrication of Enzyme Sensor——A mixture of equal volumes of the following three solutions was prepared; *ca.* 10% trypsin (a) and *ca.* 10% bovine serum albumin (b) in 10 mM phosphate buffer (pH 7.0) and *ca.* 8% glutaraldehyde (c). The ISFET gate was covered with a gel membrane composed of the above mixture, by a dip-coating method. The α-chymotrypsin-membrane/ISFET sensor was constructed similarly, using 10% α-chymotrypsin.

Apparatus and Measurements——Potentiometric measurements were carried out with a constant current device, as was reported previously,[5] and with a saturated calomel electrode as a reference electrode at room temperature (17—20 °C). The enzyme sensor is illustrated schematically in Fig. 1.

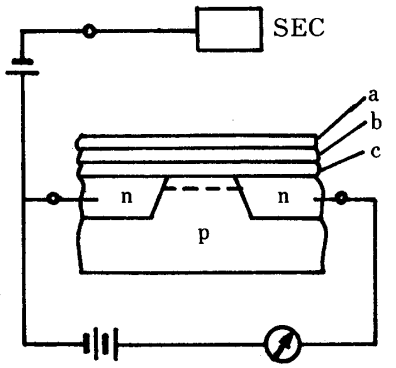


Fig. 1. Schematic Representation of the Enzyme-Membrane/ISFET Sensor

a, Enzyme membrane; b, Si_3N_4 layer; c, SiO_2 layer.

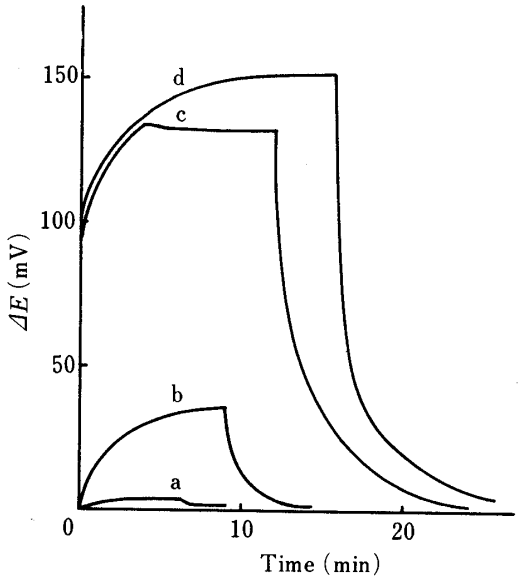


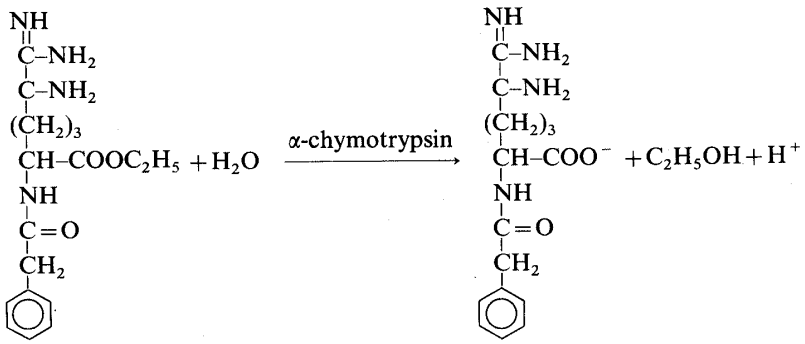
Fig. 2. Response Curves of the Trypsin-Membrane/ISFET Sensor in 10 mM Phosphate Buffer (pH 6.0)

The BAEE concentrations were a, 10^{-4}; b, 10^{-3}; c, 3×10^{-3}, d, 10^{-2} M.

Sample solutions were not stirred during measurement. Before measuring the standard solution of a substrate, the sensor was first equilibrated in the buffer for *ca.* 0.5—1 h to establish a base-line potential.

Results and Discussion

Figure 2 shows the response curves of the trypsin membrane sensor for 10^{-4}—10^{-2} M BAEE solutions. The output potentials of the sensor shifted in the positive direction from the buffer potential when the probe was dipped into the sample solutions. This implies that trypsin hydrolyzed BAEE according to the following reaction, producing H^+. Thus, near the



surface of the ISFET gate located below the enzyme membrane, the pH decreases, *i.e.*, the electrode potential shifts positively. This sensor has a high response to BAEE at substrate concentrations of more than 10^{-4} M. The response time to reach the steady-state potential as well as the time required for returning to the base-line potential turns out to be longer with increasing sample concentration in the buffer concentration. This is presumably bacause the trypsin membrane is thick enough to limit the diffusion of substrate and products through it. Such response times, however, can be considered permissible in practical use.

The potential differences between the sample solution and buffer at the steady-state, ΔE, were plotted against the logarithm of the substrate concentration in order to obtain the

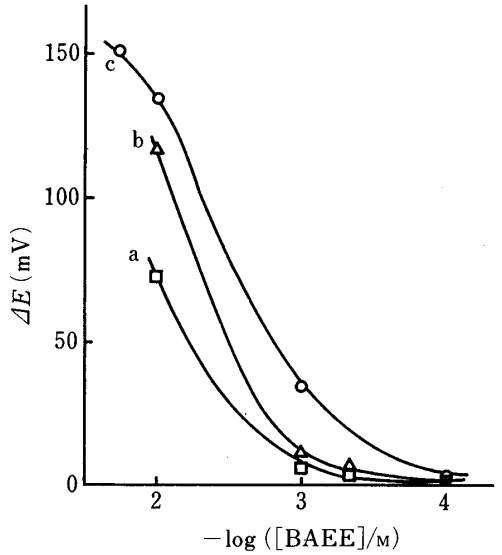


Fig. 3. Effects of Buffer Concentration on the Response of the Trypsin-Membrane/ISFET Sensor

The concentrations of phosphate (pH 7.0) were a, 10; b, 5; c, 2 mM.

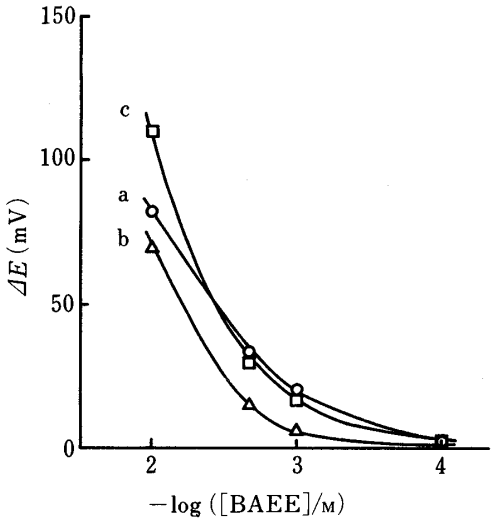


Fig. 4. Effects of Buffer pH on the Response of the Trypsin-Membrane/ISFET Sensor

The pH values of the buffer (10 mM phosphate buffer) were a, 6.0; b, 7.0; c, 8.0.

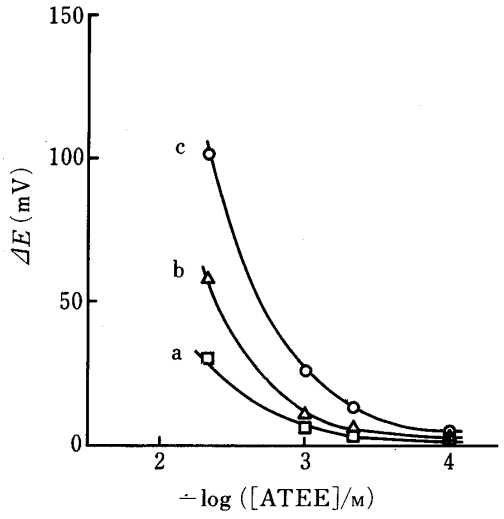


Fig. 5. Effects of Buffer Concentration on the Response of α-Chymotrypsin-Membrane/ISFET Sensor

The concentrations of phosphate (pH 6.0) were a, 20; b, 10; c, 5 mM.

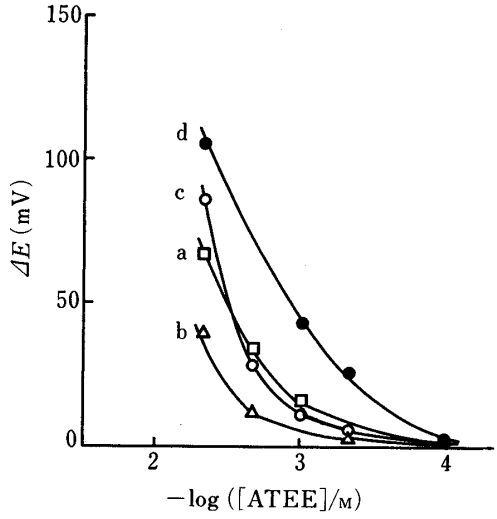


Fig. 6. Effects of Buffer pH on the Response of the α-Chymotrypsin-Membrane/ISFET Sensor

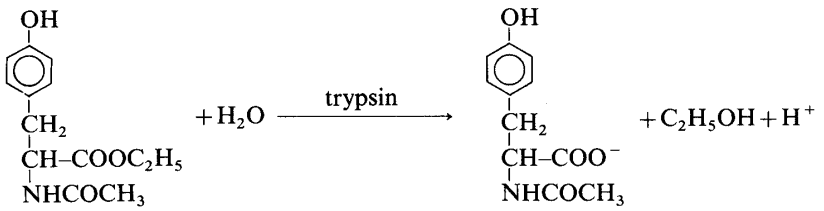
Phosphate buffer concentration; 10 mM. a, pH 6.0; b, pH 7.0; c, pH 8.0; d, pH 9.0.

calibration graph for BAEE in 2×10^{-3}, 5×10^{-3}, and 10^{-2} M buffer solutions (Fig. 3). The calibration of the sensor, is clearly affected by buffer concentration. The response was markedly reduced with increasing buffer concentration. This is presumably bacause a higher concentration of buffer has a stronger buffer capacity which can cancel the pH change originating from hydrolysis of BAEE near the surface of the ISFET gate. The experiments indicate that use of a low buffer concentration, for example 2 mM, is of benefit to determine substrate concentrations lower than 10^{-3} M.

The pH-dependence of the potentiometric response of the sensor was checked in 10 mM

phosphate buffer of pH 6.0, 7.0 and 8.0. Figure 4 shows that the effect of buffer pH on the response characteristics of the sensor is significant. This sensor can be used in phosphate buffer of pH 6—8, though the performance at pH 7.0 is lower than at pH 6.0 and 8.0. The reason for this is not clear at present.

The experiments showed that the performance of the α-chymotrypsin membrane/ISFET sensor is very similar to that of the trypsin-membrane sensor. The calibration graphs of this



sensor for $10^{-4}-5\times10^{-3}$ M ATEE solutions at various buffer concentrations and pH values are depicted in Figs. 5 and 6, respectively. Determination of ATEE is limited to concentrations of up to 5×10^{-3} M at room temperature because of its low solubility.

In summary, trypsin or α-chymotrypsin can be chemically immobilized by mixing with bovine serum albumin and glutaraldehyde on the surface of an ISFET gate. The proteolytic enzyme-membrane sensors thus prepared can be used to determine BAEE and ATEE, although the durability of the sensors is still limited (within one week).

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