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Urea Sensor Based on an Ion-Sensitive Field Effect Transistor. III.[1] Effects of Enzyme Load and Ionic Strength on the Potentiometric Response

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The effects of ionic strength of the sample solution and enzyme load on the potentiometric response of a urea sensor based on an ion-sensitive field effect transistor (ISFET) were examined. The output voltage of the sensor for urea solutions shifted slightly when NaCl was added to the solutions. The enzyme load on the ISFET gate affected the potentiometric response significantly. The sensor showed a higher response when the ISFET gate was coated with an enzyme membrane with a higher content of urease.

Keywords——urea sensor; ion-sensitive field effect transistor; urease; enzyme sensor

Recently, the development of enzyme sensors based on an ion-sensitive field effect transistor (ISFET) has been attracting much attention.[1-8] In this connection, we[1,4] and Miyahara *et al.*[3] have reported that urea sensors can be fabricated by covering the ISFET gate with a urease membrane. The urea sensor is based on the urease-catalyzed reaction (1), in which urea is decomposed to ammonia and carbon dioxide with consumption of H^+. The urease-bearing ISFET can detect the amount of H^+ consumed through the reaction (1) as a pH change around the gate surface. The effects of some operating variables such as membrane thickness and concentration and pH of the working buffer on the potentiometric response of the sensor have been described in the previous papers.[1,4] The purpose of this paper is to describe the effects of the amount of urease immobilized on the ISFET gate and the ionic strength of the sample solution.

$$(NH_2)_2CO + 2H_2O + H^+ \xrightarrow{\text{urease}} 2NH_4^+ + HCO_3^- \quad (1)$$

Experimental

Fabrication of ISFET——The ISFET used in the present study was fabricated on a p^--silicon wafer, which was 0.5 mm wide, 6.5 mm long and 0.2 mm thick. The silicon nitride gate (1000 Å) was grown on an SiO_2 layer by the chemical vapor deposition method using SiH_4 and NH_3. The procedure for making the ISFET and its properties were reported previously.[9]

Reagents——Urease (EC 3.5.1.5, Type III) and bovine serum albumin were purchased from Sigma Chemical Co. as lyophilized powders. Glutaraldehyde, urea and NaCl were of reagent grade.

Preparation of Urease Membrane on the ISFET——*Ca.* 10—20% urease solution and *ca.* 10% albumin solution were prepared using Tris–HCl or phosphate buffer. Aqueous glutaraldehyde solution of *ca.* 8% was also prepared. Appropriate amounts of these three solutions were mixed thoroughly and the mixture was applied to the ISFET gate before gelation began. The probe was air-dried for *ca.* 30 min, and was immersed in the working buffer for *ca.* 30 min before use. A schematic representation of the urease membrane-coated ISFET is illustrated in Fig. 1.

Measurements——All measurements were carried out at 23 °C using an ISFET driving circuit of a drain-source

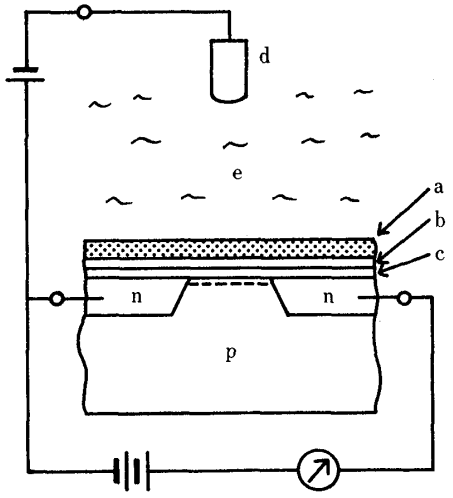

Fig. 1. Cross Section of Urease Membrane-Coated ISFET

a, urease membrane; b, Si_3N_4 layer; c, SiO_2 layer; d, SCE; e, sample solution.

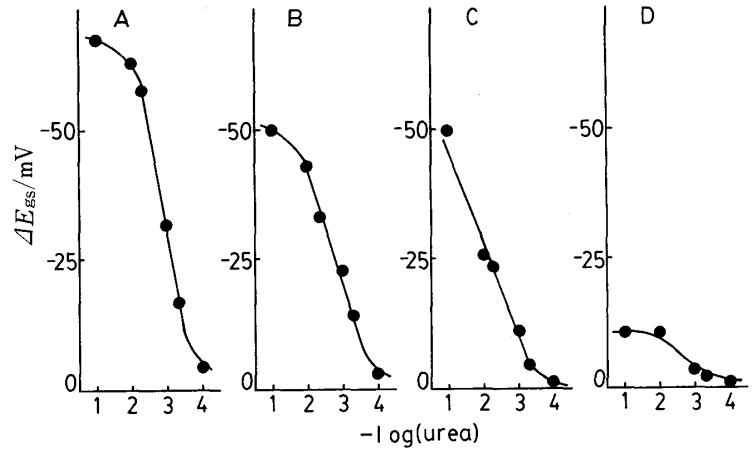

Fig. 2. Typical Calibration Graphs Obtained by Using Sensors with Various Amounts of Urease

Membrane compositions are urease : albumin = 8 : 1 (A), 6 : 1 (B), 4 : 1 (C), and 2 : 1 (D) by weight. A 5 mM phosphate buffer (pH 7.50) was used. The thickness of the enzyme membrane was about 10 μm in the dry state.

follower type with a saturated calomel electrode (SCE) as a reference electrode. Sample solutions were not stirred during the measurement, and the probe was rinsed with the buffer solution after each measurement.

Results and Discussion

Performance characteristics of enzyme sensors depend significantly upon the activity of the enzyme loaded on the electrode, because the enzyme-catalyzed reaction in the sensitive layer (*i.e.*, the enzyme-immobilized membrane) produces or consumes the electrode-active substance. In the case of the ISFET-based urea sensor, the urease-catalyzed decomposition reaction of urea consumes H^+, which changes the gate voltage (E_{gs}) of the sensor. Therefore, the urease content in the membrane is an important factor in determining the performance of the sensor. Typical calibration graphs of sensors with various amounts of urease for 0.1—100 mM urea solutions are given in Fig. 2. The enzyme load on the electrode was regulated by changing the urease: albumin ratio of the membrane. When the content of urease in the membrane was higher, the potentiometric response of the sensor was satisfactory (Fig. 2-A). The dynamic range of the sensor was *ca.* 0.1—10 mM urea concentration, the slope being −40 mV/decade. This calibration graph can be used for determining the urea in human blood in

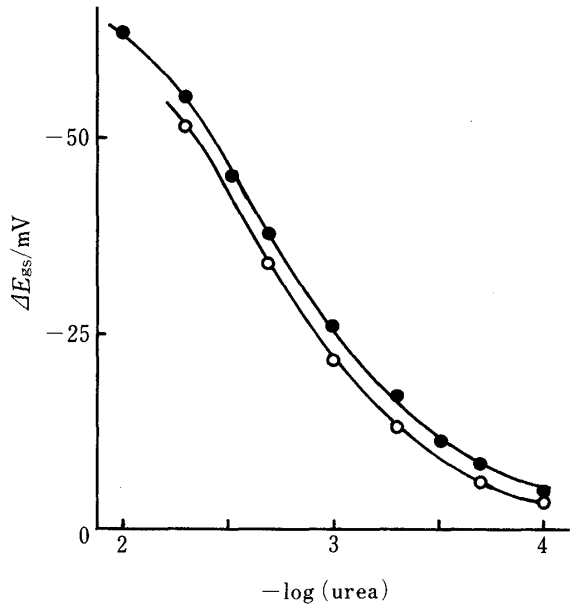

Fig. 3. Effects of Ionic Strength of the Solution on the Potentiometric Response of the Sensor

Calibration graphs were obtained in the presence (—●—) and in the absence (—○—) of 160 mM NaCl. A 10 mM Tris–HCl buffer (pH 7.35) was used. The thickness of the enzyme membrane was about 3 μm in the dry state.

view of the fact that the urea concentration in blood is normally at the mM level. The potentiometric response was suppressed slightly when the ISFET was covered with a membrane composed of urease: albumin = 6 : 1. The slope of the calibration graph was *ca.* −20 mV/decade (Fig. 2-B). In contrast to Figs. 2-A and 2-B, the response characteristics of the sensors equipped with a membrane of lower urease content were relatively poor (Figs. 2-C and 2-D). The ΔE_{gs} values[10] for all urea solutions were small and the reproducibility of the calibration curves was rather poor.

The dependence of the ΔE_{gs} value on the enzyme content in the membrane was significant in the samples of higher urea concentration such as 10 and 100 mM solutions, though this was not so clear for the lower concentration samples. This tendency of the response of the sensors may arise from the fact that, in general, the rate of an enzyme-catalyzed reaction depends upon the concentration of the enzyme when excess substrate is present.[11] From the viewpoint of practical application of the sensor, it is preferable to cover the probe with an enzyme membrane of higher urease content to obtain maximum performance of the sensor.[12]

In order to apply the sensor for the determination of urea in human blood, which is our goal, it is necessary to clarify the effects of ionic strength of the sample solutions on the potentiometric response of the sensor, because the blood level of inorganic salts is rather high. Figure 3 shows the calibration curves obtained by using urea solutions in the presence or absence of 160 mM NaCl. Over the range of 0.1—5 mM urea concentration, ΔE_{gs} values shifted by about 5 mV or less upon addition of 160 mM NaCl. This means that, when preparing a calibration graph for determining the urea in blood, one should add inorganic salts such as NaCl to the urea solution so as to compensate for the difference in ionic strength between the urea solutions and the blood sample. We are now trying to determine urea in human blood with this sensor, and the results will be reported soon.

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10) ΔE_{gs} value means the difference between the E_{gs} value for sample solution and that for the working buffer.
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12) It should be noted here that a thicker membrane, even if it has a higher enzyme composition, is not desirable due to the slow response. A detailed discussion on this point is given in ref. 4.