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Urease-Based Heavy Metal Ion Sensing Using a Silicon Transducer

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A heavy metal ion sensor based on urease inhibition and a semiconductor transducer were fabricated and characterized. Urease (EC 3.5.1.5) was reacted with copper (II) ion and mercury (II) ion, and the activity of the enzyme was measured. The pH change resulting from the enzyme reaction was monitored using a light-addressable potentiometric sensor (LAPS). This device detected the alkalization of the solution due to the enzymatic reaction, and the alkalization depended on the heavy metal ion concentration. The alkalization rates were approximately linear from 10 μM to 30 μM and from 1 μM to10 μM for copper and mercury, respectively. In addition, urease was immobilized on the electrode surface. The sensor responded to the addition of copper, and the enzyme membrane was restored by treatment with EDTA.

1. Introduction

The detection of toxic metals such as copper and mercury is important because they are environmental pollutants in soil, water and air. Furthermore, when bioaccumulation of these metals in living organisms takes place, serious issues concerning the pollution of food such as fish and meats arise. In particular, mercury has a very high toxicity. Therefore, sensitive and convenient assay methods for heavy metals are required.

It is well known that heavy metals inhibit the activity of enzymes.[1] Many enzymes have been used to detect heavy metals, and urease is the most frequently used because it is relatively inexpensive and readily available.[2–5] In the urease reaction, production of ammonium ion due to the enzymatic reaction causes the alkalization of the solution. This reaction has been applied not only to the detection of urea but also to the detection of these

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hazardous toxic metals. Many types of urea sensors have been reported previously.[3–6] Direct urea detection has been performed using urease immobilized on the surface of pH-sensitive devices. In urease-based biosensors, alkalization at the electrode surface caused by urease was detected using pH-sensitive transducers such as a glass electrode and ion-sensitive field effect transistors (ISFET).[7]

On the other hand, since LAPS was reported by Hafeman *et al.*,[8] many chemical sensors and biosensors based on LAPS have been reported.[9] LAPS is in the ISFET family, and it detects variations in surface potential. LAPS has been coupled to enzymes, micro-organisms and ionophores to measure many types of biomaterials.[10,11,12] In this paper, a LAPS-based biosensor coupled to the urease reaction for heavy metals is described.

2. Materials and Methods

The n-Si/SiO_2/Si_3N_4 electrode used in this experiment was kindly donated by Horiba, Ltd. (Kyoto, Japan). Urease (EC 3.5.1.5) (Jack bean, Type IX) and bovine serum albumin were purchased from Sigma. Mercury (II) nitrate solution was purchased from Kanto Kagaku (Tokyo, Japan). Copper ion solution was prepared by dissolving copper sulphate in 5 mM Tris-HNO_3 solution (pH 7.0). All other chemicals were of analytical grade.

The measurement system used in this experiment is illustrated in Fig. 1: it consists of a glass vessel directly connected to the surface of the Si_3N_4 layer of the sensor chip, an Ag/AgCl electrode, a light source, a current amplifier, an oscilloscope and a personal computer with a GP-IB interface and LabVIEW. The backsurface of the silicon electrode was

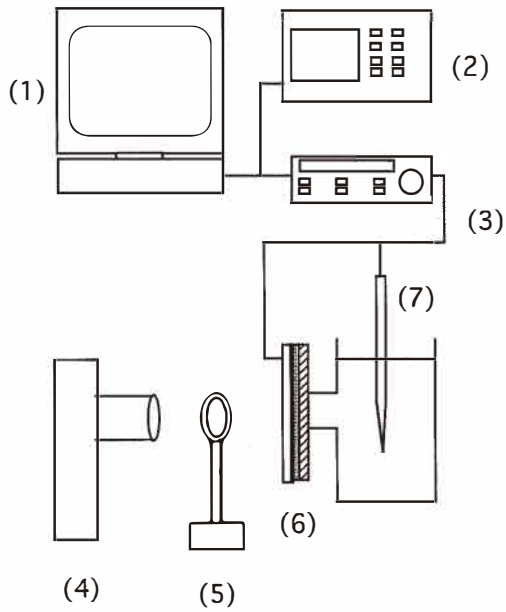

Fig. 1. Schematic diagram of measurement system: (1) Personal computer, (2) Oscilloscope, (3) Current amplifier, (4) White light source, (5) Mechanical shutter, (6) LAPS electrode and (7) Ag/AgCl electrode.

illuminated by light, and the light-induced transient current was measured as a function of applied bias voltage against the Ag/AgCl electrode. To detect the pH change, V-I characteristics were measured at intervals and the time course of the pH change was estimated.

2.1 *Urease activity measurement using LAPS*

A urease solution (200 μL, 33.8 μM) and 10 μl of copper and/or mercury solution were mixed for 15 min, after which the mixture was added to 30 ml of Tris-HNO_3 buffer solution (5 mM, pH 7.0) containing 30 mM of urea in the glass cell attached to the silicon electrode at 37°C. The V-I characteristics were measured at intervals and pH changes were estimated as the heavy metal ion concentration was altered.

Immobilization of urease was performed as follows: 15 mg of urease and 15 mg of bovine serum albumin were mixed with 200 μl of Tris-HNO_3 buffer solution (5 mM, pH 7.0), after which 5 μl of glultaraldehyde was added. Then, the mixture was cast on the silicon surface, and subsequently dried at 4°C overnight. The electrode was attached to the measuring cell, and V-I characteristics were measured at intervals. In this experiment, after inhibition by copper ion, the enzyme membrane was immersed in 100 mM EDTA solution for 10 min to recover enzymatic activity.

3. Results and Discussion

Figure 2 shows the pH dependence of the V-I characteristics of the LAPS system used in this experiment. At the lower voltages, the semiconductor was in the depletion mode, and light illumination caused the transient current. As the applied voltage increased,

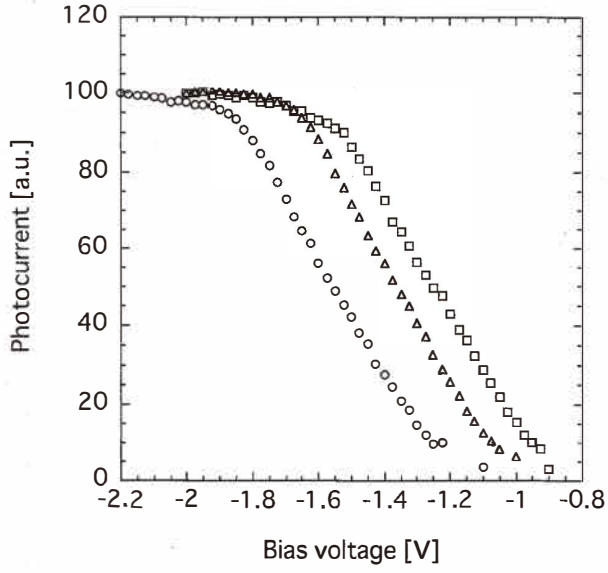

Fig. 2. V-I characteristics of the LAPS at various values of pH. (○) pH 4.01, (△) pH 6.86, (□) pH 9.18.

photocurrent decreased from the maximum level to nearly zero. The photocurrent depended on the applied voltage and the pH of the solution because the sensor surface has amino groups and silanol groups. In an acid solution, protons bind to the groups on the sensor surface, and the width of the depletion layer in the silicon substrate is varied by the field effect generated by the electric charge of the bound protons. Therefore, the V-I curve shifts along the voltage axis according to the variation in pH.[13] As a result, the changes in pH could be measured using the shift of the V-I curve. In this study, bias potential, at which the photocurrent is half its maximum value, was used as the index of pH.[7]

In this detecting system, pH changes caused by urease are measured. Therefore, the concentration of the buffer solution is very important in measuring pH change. Therefore, a low concentration (5 mM) of Tris-HNO_3 buffer was used in this experiment. In addition, optimal substrate concentration is important. Urea concentration must be much higher than the Michaelis constant, Km. In this experiment therefore, urea concentration is 30 mM.[4,5]

Figures 3(a) and 3(b) show the time course of urease-catalysed reaction with/without heavy metal (copper). The shift to the right indicates the alkalization of the solution. Without heavy metal (Fig. 3(a)), the V-I curve shifted to the right as soon as urea was added to the glass cell. On the other hand, the shift of V-I curve to the right was inhibited by the addition of copper ion (Fig. 3(b)). Furthermore, with increasing copper ion concentration in the solution, the shift of the V-I curves in the direction of alkalization of the solution was inhibited. This phenomenon indicates that the urease activity is inhibited by adding heavy metal ions. Inhibition by mercury was also observed.

Figure 4 shows the concentration dependence of urease inhibition by copper and mercury. The alkalization rate, which is the rate of the V-I shift, without heavy metal ions was 7.8 mV/min, and the reaction rate in the figure was normalized using the alkalization

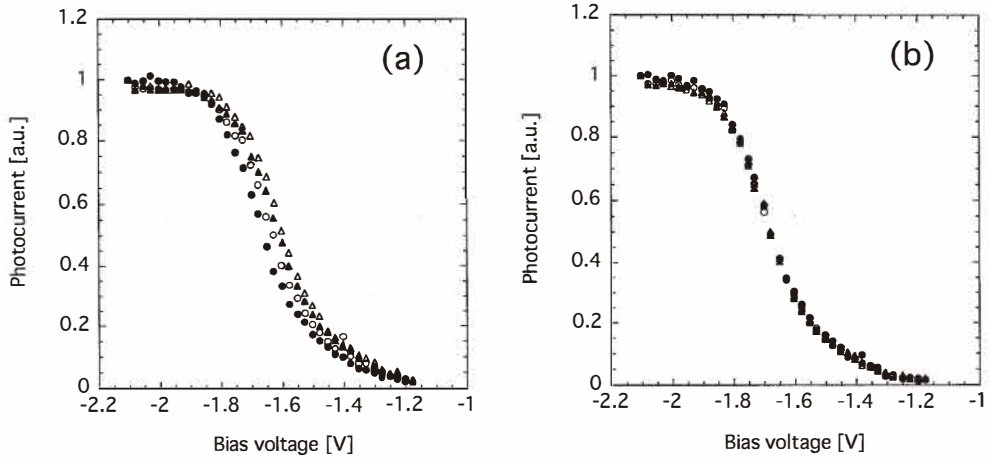

Fig. 3. Time course of V-I characteristics ; (a)without copper, (●) 0 min, (○) 5 min, (▲) 10 min, (△) 20 min; (b)with copper (50 μM), (●) 0 min, (○) 5 min, (▲) 10 min, (△) 20 min.

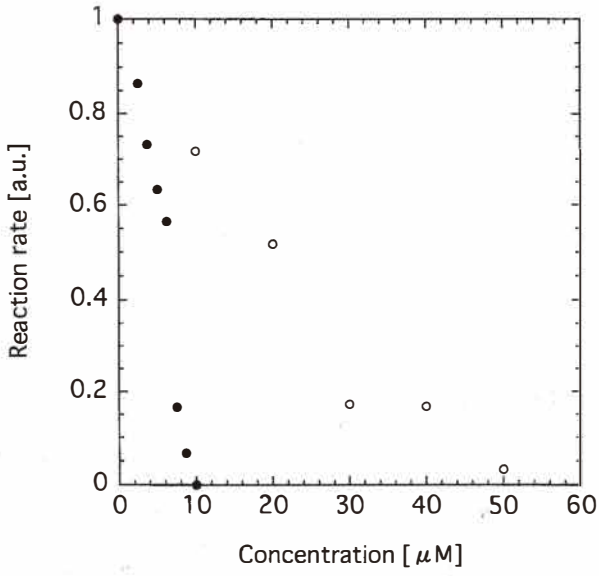

Fig. 4. The relationship between metal ion concentration and bias potential change; (●) mercury, (○) copper.

rate without heavy metal ions as 1. With increasing concentration of both copper and mercury in the solution, the alkalization rate was decreased. The linear ranges of copper and mercury were 10–30 μM and 1–10 μM, respectively. Furthermore, urease was immobilized on the surface of the silicon electrode, and V-I characteristics were recorded. Addition of urea caused a shift of the V-I curve to the right. However, after the membrane was immersed in copper and/or mercury solution, a shift of the V-I curve was not observed. It is well known that urease inhibition by copper is restored by EDTA, and the inhibition by mercury is restored with NaI.[3] In this experiment, the addition of EDTA (33 mM) and NaI (100 mM) to the enzyme solution suppressed urease inhibition by copper and mercury, respectively. Moreover, the urease membrane inhibited by copper was restored by immersing the membrane in EDTA solution (100 mM for 10 min.).

4. Conclusions

In this report, the application of LAPS to a heavy metal ion assay based on the urease inhibition reaction was presented. It was shown that the alkalization of the solution by urease could be detected, as could the inhibition of the urease reaction by copper (II) and mercury (II). This type of assay is interfered with other ions, and total inhibition by heavy metal ions was estimated. However, discriminatory addition of NaI or EDTA to the reaction solution will make the assay system selective.

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