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Highly Sensitive Immunosensor with a Surface Photovoltage Technique

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INTRODUCTION

There has been increasing attention to the solid state biosensor in various processes, such as fermentation or biomedical. For example, several enzyme sensors employing microelectronic technology have been reported and some of them are already used in practical applications. Much attention has also been paid to solid state immunosensors. These types of sensing methods provide simpler detection than the conventional methods based on a radio immuno-assay. For practical applications, however, there still remains a crucial problem with sensitivity. In a potentiometric detection type sensor, it is essential to reduce the ion screening effect of sensitivity enhancement and to attain a low-noise, high-amplification system. To reduce the ion screening effect, the ion density in the electrolyte should be kept as low as possible. However, circuit impedance increases in the low ion density system resulting in the increase of circuit noise. Recently, we reported (*1*) that a semi-solid electrolyte successfully reduced the ion screening effect by suppressing noise owing to fluctuation of the solution near the electrode surface, which made it possible to enhance the sensitivity of IgG detection about one order of magnitude compared to the solution system. In the continuation of this study, we investigated another approach to get higher sensitivity using a surface photovoltage (SPV) technique. This is an ac measurement technique of a semiconductor surface potential that realizes the low noise amplication system (2). The surface photovoltage technique was first applied in an ion sensor by Hafman et al. (3). Their sensor was based on a low im-

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pedance system with an external reference electrode. We extended it to the high impedance system using a capacitive coupling circuit to minimize the ion screening effect. This report deals with an application of the surface photovoltage technique to a highly sensitive immuno sensor. The technique was also compared with the semi-solid electrolyte system.

EXPERIMENTAL

Protein Immobilization

The sample water was a *p* type silicon of 5–10 Ω cm covered with SiO_2 and Si_3N_4 films. After the surface of the electrode (Si_3N_4) was silanized, IgG antigen was immobilized by the LB method. Immobilization of immunogloblin-G(IgG) on the electrode was carried out according to the following procedure: 100 μL of 0.04 wt% solution of stearic acid in chloroform was spread on the surface of phosphate buffer solution containing $3\times10^{-5}M$ of $BaCl_2$ and $4\times10^{-4}M$ of $KHCO_3$. The volume of the subphase was fixed to be 50 mL and the area of the water surface 50 cm^2. Subsequently, 150 μL of 1 wt% of IgG aqueous solution was injected under the stearic acid layer which was then allowed to stand for 1 h in order to attain adsorption or incorporation of the IgG in it. The layer was compressed at 20 mN/m and transfered on the gate insulator by dipping at the rate of 8 mm/min to immobilize the IgG.

Measurement of Surface Photovoltage (SPV)

Fig. 1 shows a simplified illustration of the sensor structure and measurement principle. The sensor consists of a "coupling capacitor/transparent electrode/low-ion density electrolyte/IgG immobilized film/insulator/semiconductor/metal electrode." The detection principle was based on a measurement of surface potential change of the semiconductor owing to the change of surface charge density yielded by antigen-antibody binding. The semiconductor surface potential was measured by the ac photo-voltage technique using a semiconductor in the depletion condition. An alternatively modulated photon beam (PB) was irradiated on the silicon surface and the photo voltage generated in the surface depletion layer was picked up through a capacitance coupling circuit and measured by a lock-in amplifier.

Surface Charge Measurement

Fig. 2 shows time response of surface photo-voltage after injection of IgG antibody of final concentration of $1\times10^{-8}M$ in a diluted phosphate buffer solution of 1.0 m*M*. The response voltage represented was the difference of the photovoltages before and after the immuno reaction. The noise level was less than 0.1 μV and the signal to noise ratio (S/N) was

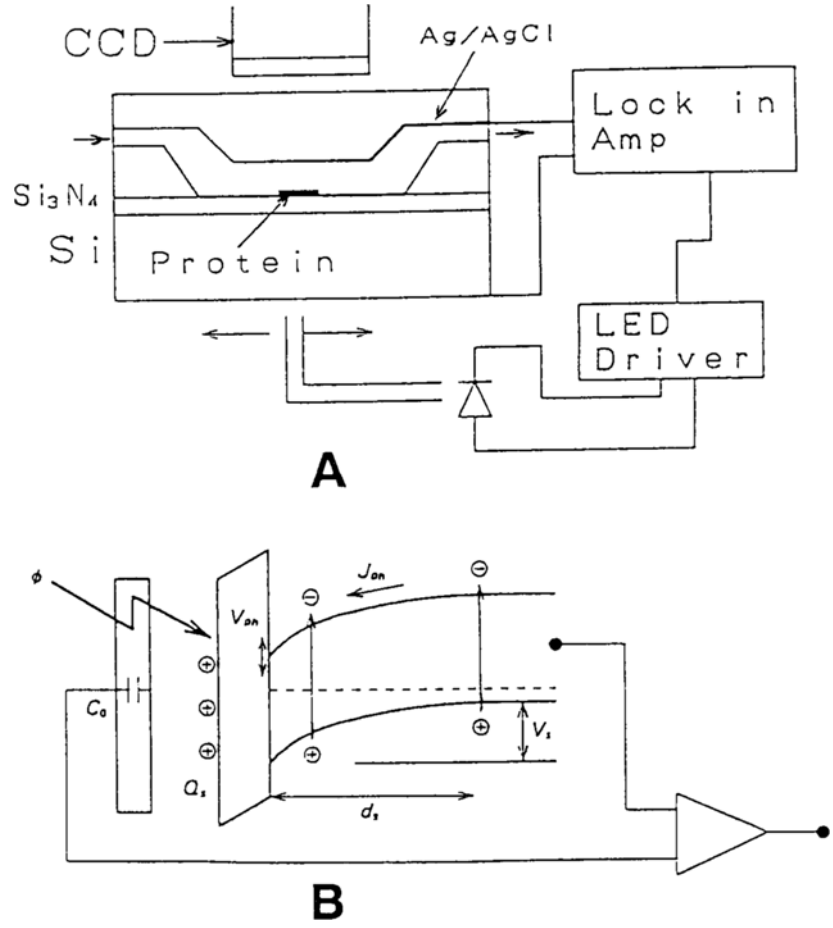

Fig. 1. Structure and principle of the surface photo voltage measurement system. (a) Cross-sectional structure of the sensor. (b) Detection principle of the semiconductor surface potential.

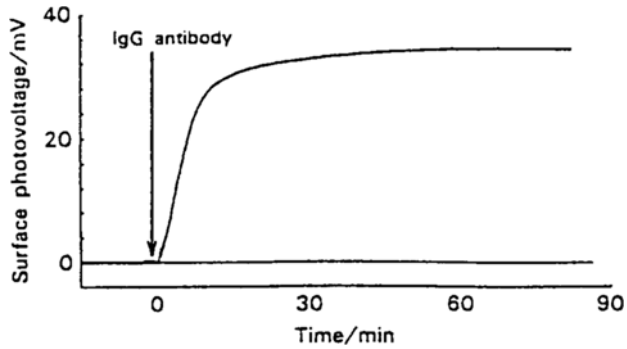

Fig. 2. Time response of a surface photovoltage of IgG sensor after IgG of 10^{-8}M was injected in the solution.

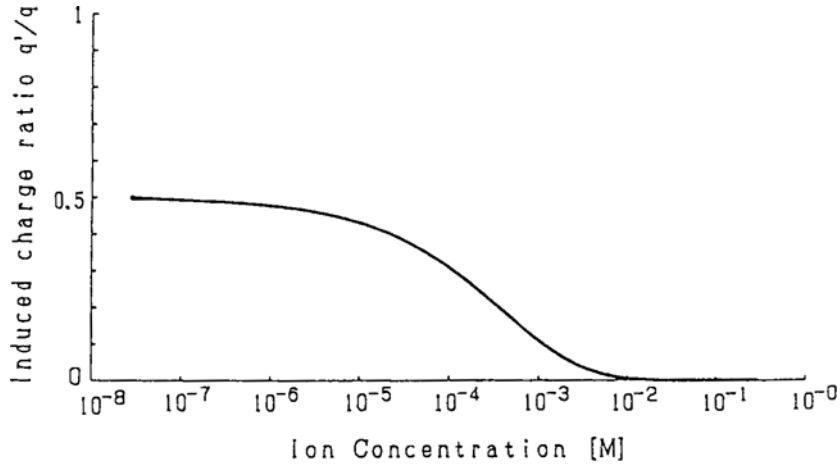

Fig. 3. Observed surface photovoltage as a function of the ion concentration of the electrolyte.

higher than 10^4. It should be noted that a low noise level was attained although a high impedance electrolyte was used in the system. Fig. 3 shows response voltage as a function of electrolyte concentration. The higher response voltage in the lower concentration electrolyte indicates that the ion screening effect had a significant effect on sensitivity. For comparison, output signals of a dc potentiometric sensor with Pt electrodes are shown in Fig. 4. Fig. 4(a) is the signal measured in a solution electrolyte of 25 m*M* and Fig. 4(b) is the response of a semisolid electrolyte (10 m*M*) sensor. The added IgG antibody concentration was $1\times10^{-8}M$ in both measurements. In a solution electrolyte, a large spike-like noise appeared in the signal immediately after the addition of sample solution. In the semisolid electrolyte measurement, however, such noise did not appear and the output voltage was one order of magnitude larger than that of a solution electrolyte, which may come from the low ion density of the semisolid electrolyte. Compared with these potentiometric measurements, it is obvious that the SPV response is considerably higher, as shown in Fig. 2.

DISCUSSION

In the surface photo-voltage measurement, the surface depletion layer potential was directly modulated with photon beam and was detected with a lock-in amplifier. The phase sensitive amplification led to the reduction of random noise, such as fluctuation and spike-like noise, which made it possible to use a low-ion concentration electrolyte, resulting in the reduction of the ion screening effect. This kind of fluctuation noise was partly suppressed by the use of a semisolid electrolyte. However, it is

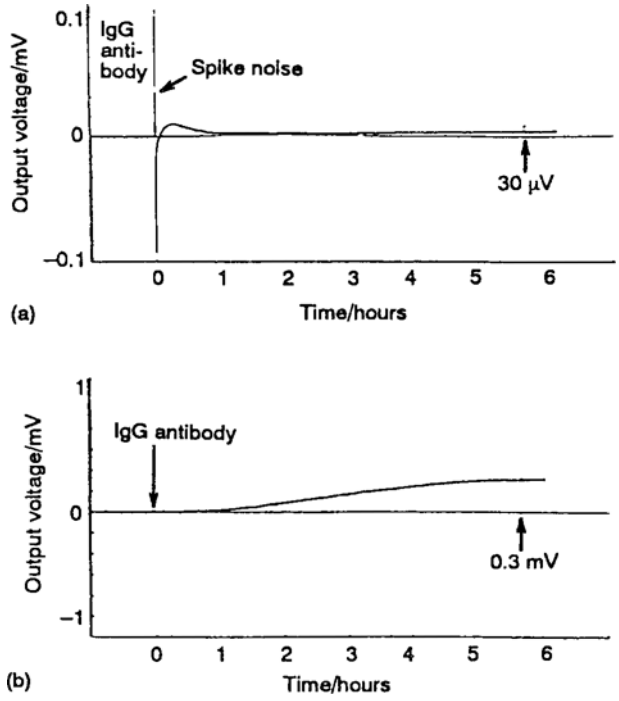

Fig. 4. Output responses of IgG sensors measured in (a) buffered solution and (b) semisolid electrolyte.

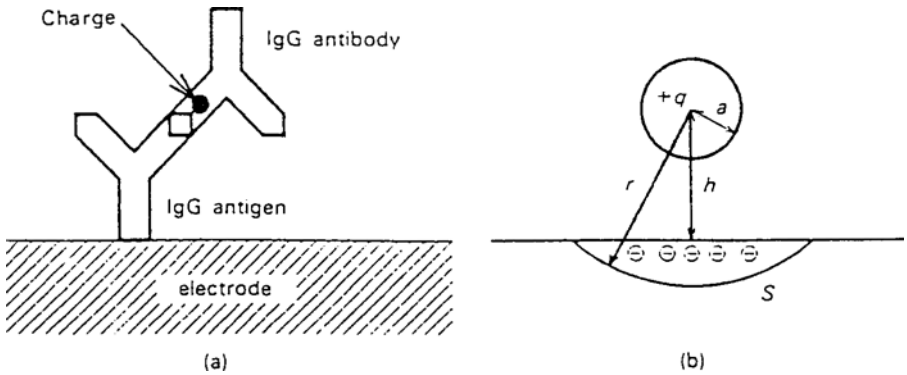

Fig. 5. A postulated model for the calculation of the induced surface charge density owing to the antigen–antibody reaction.

noted that the improvement of the signal-to-noise ratio was very effective in SPV measurement, as mentioned in the previous section.

The ion screening effect mentioned above was further investigated theoretically by using the simple model described in Fig. 5. Here, the antiIgG molecule reacting with the immobilized IgG molecule was assumed to contain a spherical charge with a radius a and a charge q_0, which exists

at the central part of the Fab of antiIgG molecules. The charge is surrounded by the counter ion species, giving rise to a charge screening effect. According to the Debye-Huckel theory, the charge density ρ is described as a function of the distance r from the charge center.

$$\rho = \varkappa^2 q_0 \exp(-\varkappa r) \exp(\varkappa a) / 4\pi r(1 + \varkappa a) \quad (1)$$

$$q_s = \int_h^\infty S\rho \, dr, \; S = 2\pi r(r - h) \quad (2)$$

where S is the surface area of the hemispherical endcap with radial r, and h is the distance between the central charge and the electrode. From Eqs. (1) and (2), the charge density can be calculated as:

$$q_s = -q_0/2 \exp(\varkappa a) \exp(-\varkappa h)/(1 + \varkappa a) \quad (3)$$

Then the surface charge density ΔQ_s is expressed as

$$\Delta Q_s = N q_s \quad (4)$$

Here N is the number of the antibody combined with the immobilized antigen in an equilibrated state, which was calculated from the following equation

$$A_b + A_g \overset{K}{\rightleftharpoons} N \quad (5)$$

where A_b and A_g are the concentration of antibody in the solution and concentration of antigen on the electrode, respectively. K is the equilibrium constant and was measured to be 10^{-6} L/mol from another experiment. The surface voltage of the semiconductor electrode, ΔV, is obtained from Eq. (4) as

$$\Delta V_s = (2V_s/(\epsilon_s e N a))^{1/2} \Delta Q_s \quad (6)$$

where Na is the doping density and e_s is the dielectric constant of the semiconductor. The debye length $1/\varkappa$ depends on the ionic strength I which is given by

$$(1/\varkappa)^2 = \epsilon kT / e^2 2LI \times 10^3 \quad (7)$$

where ϵ is the dielectric constant of the solution, k is the Boltzmann's constant, T is temperature, and L is the Avogadro's constant. Assuming a one-dimensional structure of the antiIgG molecule, q_0 is estimated as 10 e(coulomb). From a higher-order structure, a and h are estimated to be 30 A and 80 A, respectively. The ionic strength I is estimated from the following equation:

$$I = (2^2[HPO_4^{2-}] + [H_2PO_4^-] + [Na^+])/2 \quad (8)$$

Fig. 6 shows calculated results of the surface photovoltage as a function of ion concentration for various IgG concentrations. The surface voltage increases monotonically with the increase of debye length $(1/\varkappa)$ and gradually is saturated for the sufficiently large debye length. Points

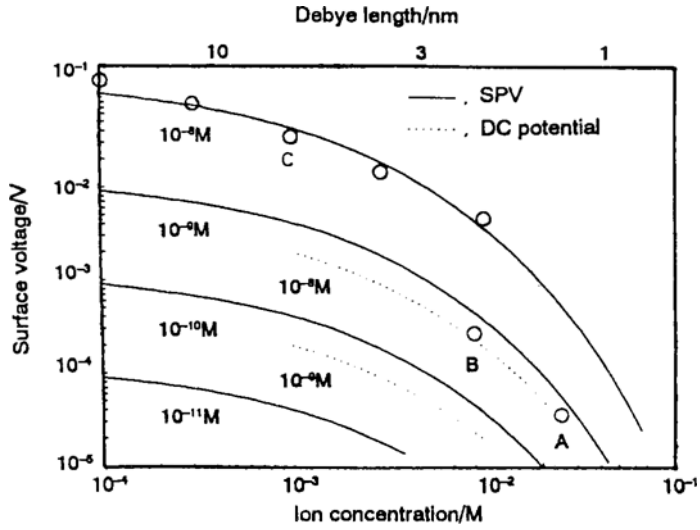

Fig. 6. Calculated and observed response voltage for various IgG concentrations as a function of electrolyte ion concentration (Solid line: ac surface photovoltage; broken line: dc surface voltage measured in a solution electrolyte (A) and with a semisolid electrolyte (B)).

A, B, and C are the experimental results of Figs. 4(a) and (b) and Fig. 2, respectively. It is noted that there is fairly good agreement of the theoretical calculation with these experimental results. These results strongly suggest that higher sensitivity is attained with lower ion density electrolyte.

Another possibility for the sensitivity improvement is the shortage of the distance between surface induced charge and electrode (h in Fig. 5). In order to get smaller h, we proposed a usage of electrically conductive polymer networks to surround the sample protein. If we assume the small value of h less than 40 A and the system can detect the signal as low as 10 μV, the detectable IgG concentration will reach $< 10^{-12}M$.

CONCLUSION

An ac surface photo voltage technique was successfully applied to construct a highly sensitive immuno-sensor by reducing the ion screening effect. A low noise amplification was attained by using a phase sensitive amplification technique, which made it possible to use low-ion density electrolyte leading to suppress the ion screening effect. The response voltage attained was as high as 7 mV for the detection of $10^{-8}M$ IgG antigen with a signal-to-noise ratio of 10^4.

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