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Preparation of Resistive-Type Glucose Sensor by Layer-by-Layer Technique and Their Properties

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Introduction

Biosensors are functional analogs that are based on the direct coupling of an immobilized biologically active compound with a signal transducer and an electronic amplifier. The main function of a transducer is to convert the physicochemical change in the biologically active material resulting from the interaction with the analyte into an output signal.[1,2]

Due to their high sensitivity and potential selectivity as well as low cost and possibility of miniaturization, biosensors containing enzymes have widely applied in chemistry and biology. Among enzymatic biosensors, glucose sensors have been the most studied owing to their usefulness in the diagnostic analysis of diabetes.[3,4] Self-assembled monolayer (SAMs) have become a popular, simple and reliable producer to immobilize enzymes and molecules on various metal and oxide surfaces mostly owing to their simplicity, versatility and the establishment of a high level of order on a molecular scale as a means of preparing modified surface. In this strategy the strong electrostatic interactions between polyanion and polycation are the driving force for multilayer self-assembly, and uniform molecular films of well-defined thickness are formed.[5-13]

Diabetes mellitus is one of the leading causes of death and disability in the world. It is estimated that world-wide, more than about 30 million patients are diabetic. Therefore, the determination of blood glucose levels is an indispensable test for the diagnosis and management of diabetes mellitus. Although innumerable methods have been developed, glucose biosensors for diabetic patients become the most popular. Besides diabetes control, such devices offer great promise for other important applications, ranging from food analysis to bioprocess monitoring. In the present work, the glucose sensing characteristics of layer-by-layer (LbL) self assembled films of polyelectrolyte, which are formed with ferrocene and glucose oxidase on the ITO electrode, are investigated.

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Experimental

Reagents and Instrument. The glucose oxidase (10,000 unit; Aspergillium niger type) was purchased from Sigma-Aldrich. Sodium ferrocene carboxylate (SFC) was prepared by reacting ferrocene carboxylic acid and sodium hydroxide. Polyallylamine hydrochloride (PAA, M_w=15,000, Aldrich Chem. Co.) and poly(sodium 4-styrenesulfonate) (PSS, M_w= 70,000, Aldrich Chem. Co.) were used as received. Glucose (JIN Chem. Co.) solution was freshly prepared by dissolving glucose in the phosphate buffer. The stock glucose solution was stored at 4 °C for more than 24 h prior to use. Resistances of glucose sensors were recorded using LCR meter (ED-Lab Korea, Model EDC 1630, 0.1~80 MΩ) and pH meter (Thermon Orion) was used to adjust the pH of solutions. The comb-type ITO electrode was prepared according to the method previously reported.[14]

Preparation of Layer Glucose Sensor by LbL Technique. The Anionic solution was prepared by mixing equivalent weight of SFC and PSS. The PSS (1 mg/mL) and SFC (1 mg/mL) were dissolved in deionized water (1 mL), and the solution was adjusted to pH 7 by adding NaOH. The cationic solution was prepared by dissolving PAA (1 mg/mL) and GOD (10,000 unit, 1 mg/mL). The cationic solution was adjusted to pH 7.0 with 0.01 M of NaOH for the LbL film fabrication.

(1) The positively charged substrate was dipped into PSS/SFC solution for 20 min to obtain a negatively charged surface. (2) Next, the PSS/SFC coated substrate was washed in deionized water and dried by blowing with nitrogen gas. (3) The PSS/SFC coated substrate was immersed in the solution of PAA/GOD and then (4) washed in deionized water. Steps (1)-(4) were repeated until 1~5 pairs of polycation/polyanion layers were absorbed. The immersion time in the individual solution was 20 min. The membrane was dipped in the solutions by using a dipping apparatus. Other content of SFC/GOD (1/2, 2/1) and the glucose sensors with 2-5 layers were prepared according to the procedures described above.

Measurements of Resistance for the Glucose Sensors. The resistance of glucose sensors was measured by dropping 1 drop of each glucose solution on electrode of sensor after at 25 °C and 10 kHz. The sensors were connected to a resistance measurement system while 1 V was applied across it. The temperature dependence was measured at 15, 25 and 30 °C at 1 V and 10 kHz. Frequency dependence was obtained by changing frequencies at 1 and 10 kHz at 1 V and 25 °C, respectively.

Results and Discussion

Fabrication of LbL Films. PSS and PAA were chosen as cationic and anionic polyion building blocks. The polyelectrolyte membranes were prepared through alternate dipping of quaternized ammonium-coated ITO electrode into aqueous solutions of an anionic PSS/SFC and a cationic PAA/GOD. In the each dipping step, PSS and SFC layer is adsorbed under the reversal of the surface charge so that in the next dipping step a PAA and GOD layer of opposite charge can be adsorbed as shown in Figure 1 (I and II). Using polyelectrolyte such as PAA, comparing with small molecule, it has a good adhesion to the substrate and a good ionic interaction with films. The film-mass of polyelectrolyte layer was very important and was controlled by the frequency shift dipper. Multilayer membranes consisting of 1~5 polycation/polyanion layer pairs were prepared as illustrated in Figure 1. Since the membranes were coated on the twenty sensor chips at a time, their resistance characteristics are in a close agreement with each other. The accuracy of the response curve is better than ±1%RH.

Optimization of Working Electrode. The experimental parameters such as pH, temperature and frequency were investigated to optimize analytical performance. The effect of pH was evaluated at different pH values in phosphate buffer containing various concentrations of glucose. Earlier studies had described a shift in the pH optimum displayed due to the electron mediator utilized.[15] Hence the best response at pH 7.5 was chosen for all studies, since the excellent response had been at this pH as shown in Figure 2.

The effect of temperature was reported to be very important on the functioning of the enzyme. With the temperature varying from 15 to 30 °C, the activity of the glucose oxidase increased. Taking this into consideration, a temperature of 25 °C has been chosen for this work. The data were plotted as the measured resistance of glucose sensor with 5 polycation/polyanion layer pairs at 15, 25 and 30 °C at an operating frequency of 10 kHz as shown in Figure 3. Thus the current response increased with increasing temperature, which means that the compensation of temperature is necessary for the application of a glucose sensor.

The electrical characteristics of the polymeric film glucose sensor were measured in a.c. fields, as the sensor became unstable under d.c. fields due to the electrolysis of the polyelectrolyte and enzyme. The resistance of the sensor is affected by the frequency of the applied a.c. voltage. Direct current operation of the sensor must be avoided because degradation is caused by electrolysis of the film. The resistance of the glucose sensor with polycation/polyanion layer pairs were measured in the frequency range from 1 to 10 kHz as shown in Figure 4. The large difference was observed in the lower concentration range. The linearity of the sensor

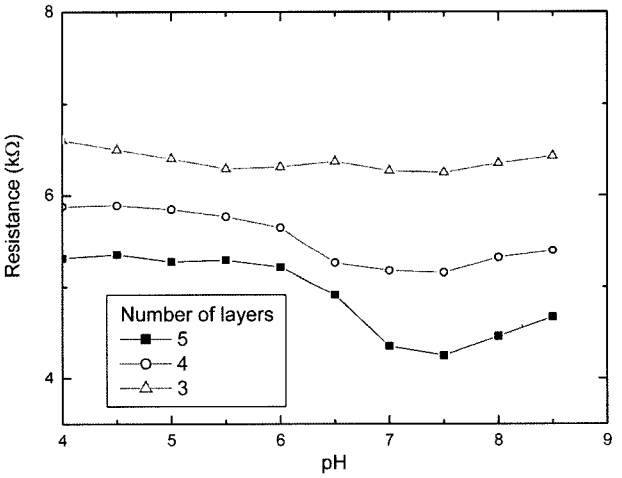

Figure 2. The influence of pH on the response of the sensor with SFC/PSS-PAA/GOD (1/1, w/w) to 0.001 M glucose solution.

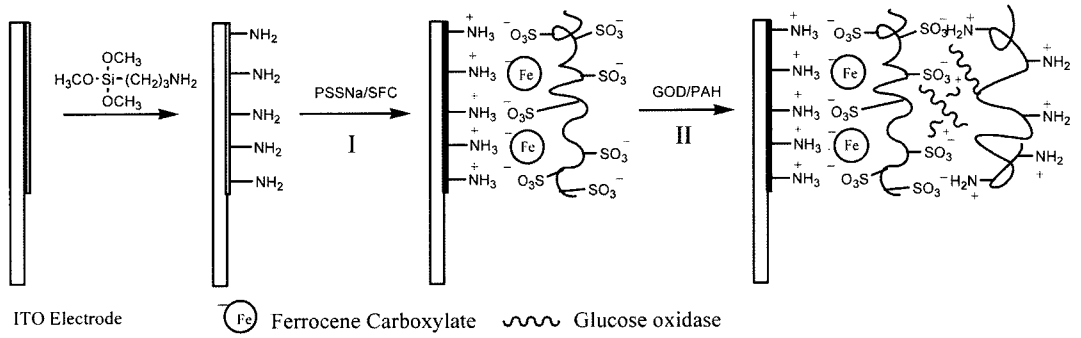

Figure 1. Layer by layer construction of PSS/SFC-PAA/GOD multilayer films on the ITO electrode surface.

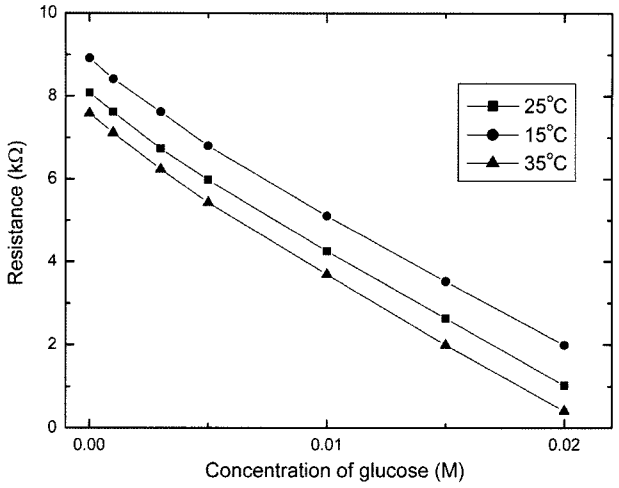

Figure 3. The resistance dependence of the sensor with 5 polycation/polyanion layer pairs on concentration of glucose at (●) 15 °C, (■) 25 °C and (▲) 30 °C at 1 kHz and 1 V.

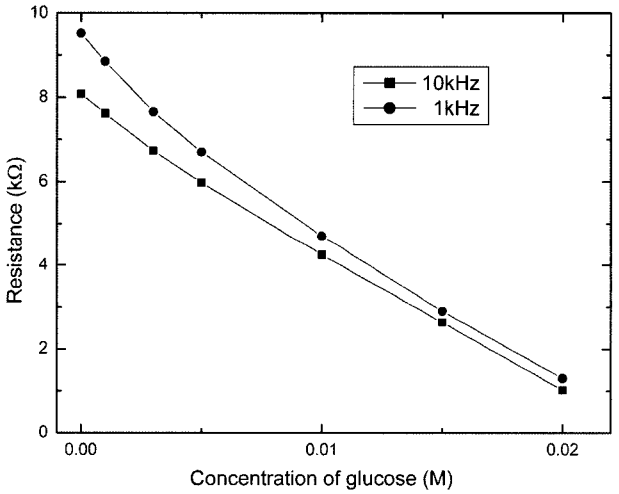

Figure 4. The resistance dependence on the applied frequency of (●) 1 kHz and (■) 10 kHz for the glucose sensor with 5 polycation/polyanion layer pairs at 25 °C and 1 V.

was superior at higher frequency.

The Response of the Working Electrode to the Glucose. The device fabricated with 3 layers showed resistance between 8.65 and 2.75 kΩ with increasing glucose concentration from 0 to 0.02 M. The resistance of the glucose sensor with 5 polycation/polyanion layer pairs was varied in the range of 8.35 and 1.05 kΩ when the concentration of glucose was between 0 and 0.02 M, which is the applicable resistance range for the glucose sensor. It may be seen that that the sensor resistance showed around 8 kΩ value without glucose by an electrolytic conducting mechanism of polyelectrolyte in water. Since the resistance responses decrease with increasing the number of assembled SFC/PSS-GOD/PAA layers

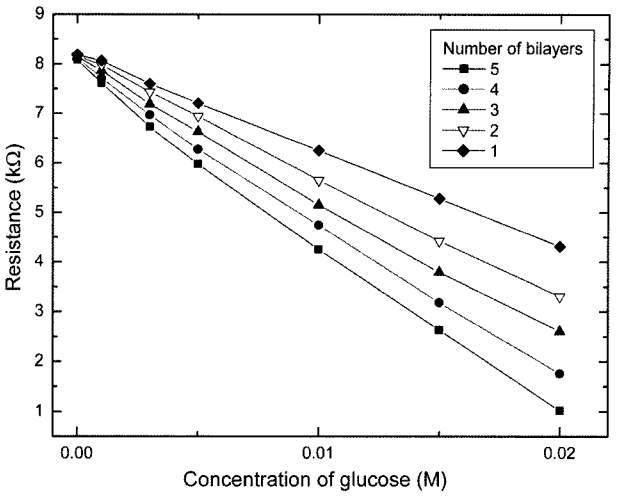

Figure 5. Relationship between the resistance and the number of bilayers for PSS/SFC-PAA/GOD (GOD/SFC=1/1) LbL film between 0.0 and 0.02 M of glucose concentration.

or more specifically the loading of active glucose oxidase, the density of the SFC/PSS-GOD/PAA multilayered electrode should be able to be tuned to a desired level by adjusting the number of attached bilayers.

Figure 5 shows the resistance for the electrode modified with different bilayers of SFC/PSS-GOD/PAA as a function of glucose concentration in solution. It can be seen that the resistance of the SFC/PSS-GOD/PAA multilayer electrodes are obviously amplified with multilayer growth between 0 and 0.02 M glucose concentration. The resistance decreased gradually between 1 and 5 polycation/polyanion layer pairs. This is typical enzyme-dependent catalytic process. When the reactions proceeded at the electrode surface, first, mediator PAA-Fe was oxidized to PAA-Fe^{+} and an electron generates. Second, GOD was reduced and then the reduced GOD returned to original form of GOD by oxidation of a redox mediator PAA-Fe. Third, the β-D-glucose was oxidized to glucono-D-lactone by GOD. These processes result in an oxidation resistance correlated with the concentration of enzyme when the mediator concentration was sufficient.

Effect of the Ratio of Catalyst and Mediator. Figure 6 shows the dependence of resistance on the ratio of enzyme to mediator. The biosensors composed of 5 layers of equimolar weight of SFC and GOD showed a good linearity.

The resistance values observed with SFC/GOD=1/2 was higher than those of SFC/GOD= 2/1 as shown in Figure 6. It can be seen that enzyme concentration had an effect on the electrochemical property and the stability of sensor. In the higher enzyme concentration, the more enzymes can be immobilized on the substrate. Since the polyelectrolyte covered the surface of enzyme, the spatial conformations were generated according to the enzyme concentration. Thus, the ability to oxidize the glucose was variable according to the

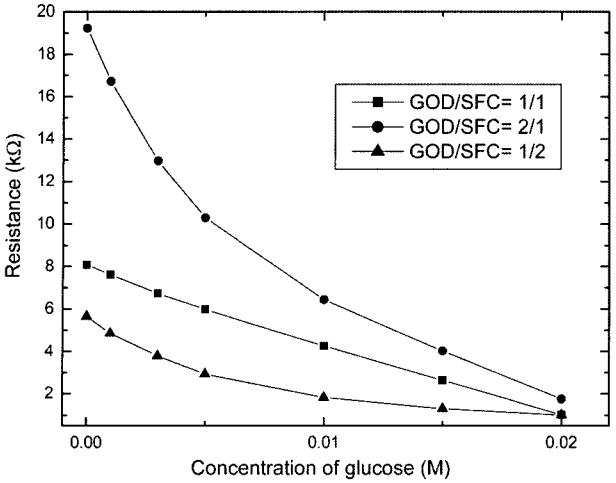

Figure 6. Relationship between the resistance and the concentration of glucose sensor with GOD/SFC=(●) 2/1, (■) 1/1, and (▲) 1/2) and 5 polycation/polyanion layer pairs.

enzyme concentration.

When the mediator SFC was increased, the resistances increased as the concentration of glucose increased. It can be seen that mediator concentration also had an effect on the sensing abilities. Mediated enzyme electrodes are known to be less susceptible to interfering substances due to lower electrode potentials.

In conclusion, the LbL method has been exploited to produce simple resistive-type glucose biosensors with the immobilization of GOD and SFC as mediators onto an ITO substrate. The biosensor exhibited a fast and sensitive response to changes in the glucose concentration which allowed convenient quantification of glucose between 0.0 and 0.01 M. Other experiments on the cyclic voltammetry characterization of self-assembly system including individual role of polyelectrolyte, enzyme and mediator are now in progress. In addition further study of the stability with prolonged exposure to air will be carried out.

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