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Enzyme electrode based on gold-plated polyester cloth

I. Preparation of glucose oxidase electrode which exchanges electron with electrode

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Summary

Glucose oxidase is immobilized on gold-plated polyester cloth by entrapment method and coupled with an electron mediator, ferrocene derivative. Cyclic voltammogram depends on the existence of the substrate glucose and the flavo-enzyme was shown to donate electron(s) to the cloth electrode with mediation of the ferrocene derivative even in the immobilized state. The glucose-concentration dependence of the peak current was linear upto several tens of 35 mM, depending on the concentration of the mediator. Co-immobilization of polymer-anchored electron mediator is also studied.

Many efforts have been made to utilize specific recognition and selective catalysis performed by enzymes in analytical or productive purposes; biosensors and bioreactors. One of the most important problems to obtain much more effective system for these purposes is how to communicate the direct exchange or transfer between the biology-origin materials and the energy supplying artificial systems.

Taking the most popular enzyme electrode, glucose oxidase (GOD)-electrode, as an example, conventional GOD electrodes measure the concentration of oxygen, consumed by GOD reaction with glucose, or of hydrogen peroxide, a product of the same reaction, with the electrolytic process on the platinum electrode. In such a system the high performance of the enzyme is utilized only indirectly and the principal detecting system is more or less non-specific and general. Therefore attempts of multi-functionalization by combining other bio-systems is found difficult and the supply of oxygen caused several problems in attempts of bioreactors.

It has been getting understood that by immobilizing enzymes directly on proper electrode and coupling with some electron mediators the electron transfer on the electrode surface is controlled by the electric potential given on it and hence the reaction of oxido-reductase is controlled.(1) Mediators are required since the direct electron exchange between the electrode and the enzyme is hard to occur in most of the cases.

Another important problem for the utilization of enzyme electrode in various purposes is in the materials of the electrode. Most usually platinum or gold is used but they are expensive and inflexible. Carbon fibers are better in these senses but they are still inconvenient to treat. In this connection we developed enzyme electrodes which use gold-plated polyester cloth.(2) It is advantageous in many points such as flexibility, wide-surface area, processability after enzyme immobilization and cheapness. In this study GOD was chosen since it is considered as a standard example in such a research of enzyme immobilization. The oxido-reductase

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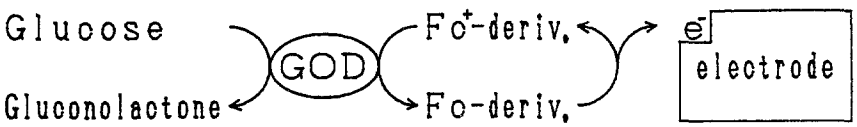
was immobilized by entrapment method and coupled with an electron mediator, ferrocene derivative, of which essential processes are as illustrated below. Co-immobilization of polymer-anchored electron mediator is also studied.



Experimental

Gold-plated polyester cloth (Au-plat) was prepared by chemical plating (Atomex, Nippon Engelhard) of Ni-plated cloth (Seiren Ni-plat). A direct plating of gold on polyester cloth was very difficult and the use of Ni-(or Cu-) pre-plated one was found to be essential for the preparation of gold-plated cloth in our case. Besides low-molecular weight electron mediator such as ferrocene carboxylic acid (FcCOOH) and ferrocene carboxaldehyde (FcCHO), we use a polymer-anchored mediator, bovine serum albumin treated with ferrocene carboxaldehyde (3).

Enzyme or polymer-anchored mediator was immobilized on the electrode by using self-emulsifying polyurethane (Aizelax; Hodogaya Chemical Ind.) (4) or by urethane-acrylate prepolymers (donated by Prof. Tanaka, Kyoto Univ.)(5).

After preliminary trials of immobilization on various golden cloth, one weaving pattern was selected (50 denier, mesh ca.500) for its final conductivity and hold of the enzyme layer.

Enzyme activity of the obtained electrode was first examined by usual colorimetric method of immobilized enzymes and then measured by a cyclic voltammetry (Ref., Ag/AgCl; Counter, Pt ; Working, Au-plat, 200 mm^2. Electrolytes, 0.1M citrate pH 5.7, 0.1M KCl, containing 0 - 20 % ethanol to solubilized FcCOOH. Scanning; -0.1-+0.5V, 20 mV/s). Reaction mixture was thoroughly deoxygenized by vigorously passing N_2 before each measurement.

Results and Discussion

Figure 1-a shows cyclic voltammogram (CV) of the reaction system

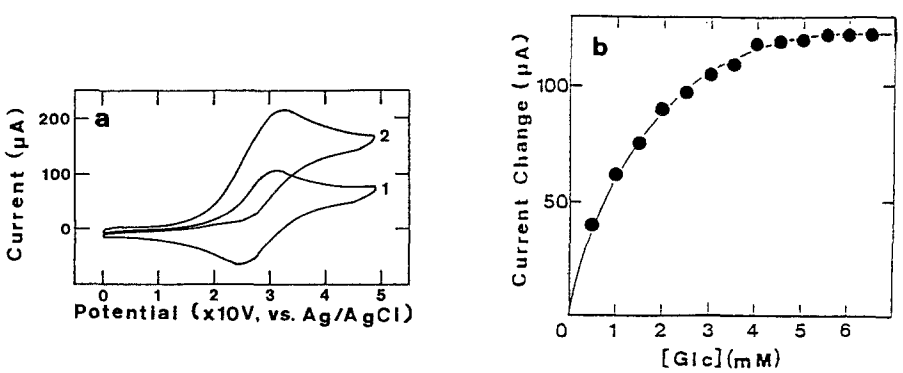


Fig.1 Cyclic voltammogram of the system composed of Gold-plated cloth electrode, free GOD and free FcCOOH (a) and the glucose concentration dependence of the current at 0.5V (b).
(a) curve 1, [glucose]=0mM, curve 2, [glucose]=10mM;(b)[FcCOOH]= 1mM. Other conditions are as explained in Experimentals.

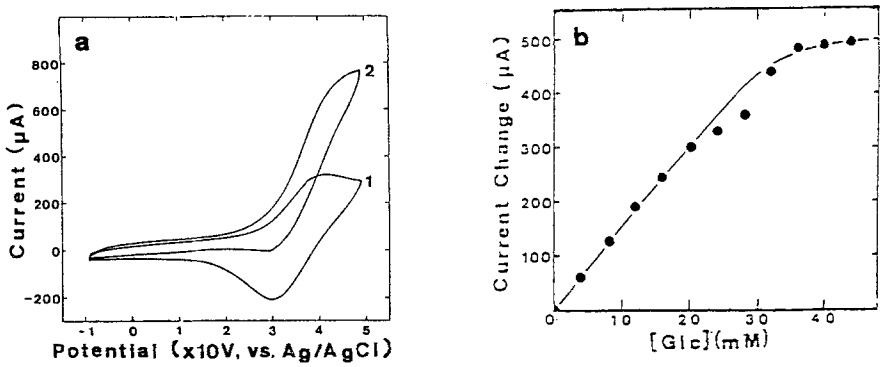


Fig.2. Cyclic voltammogram of the system composed of GOD immobilized gold-plated cloth electrode and free FcCOOH (a) and the glucose concentration dependence of the current at 0.5V (b). (a) curve 1, [glucose]=0mM, curve 2, [glucose]=35mM;(b)[FcCOOH]= 1mM. Other conditions are as explained in Experimentals.

containing Au-plat electrode, free GOD and free FcCOOH. It is clear that current depends on the existence of the substrate glucose and the flavo-enzyme GOD can donate electron(s) to the cloth with mediation of Fc. The glucose-concentration dependence of the peak current was shown in Fig.1-b.

When GOD is immobilized on gold-plated cloth by prepolymer method, CV was observed as in Fig.2-a and their substrate concentration dependence was as in Fig.2-b.

These results show that the gold-plated cloth can soundly work as an electrode, electrons are transferred efficiently among GOD, Fc and electrode surface and the larger surface area of the cloth electrode gave high current.

The CV-current and its glucose concentration dependence are strongly dependent on the FcCOOH concentration in the system. Fig.3 shows thisdependency. The higher is the Fc concentration, the the higher is the limiting current and the bending point concentration. This will be explained the current is practically controlled, not by the enzyme reaction but by the electron mediation, ig. the effective concentration of Fc in the intermediary region between the electrode and the enzyme. In the concentration range studied here the limiting current is roughly a linear function of the Fc concentration (Fig.3,insert).

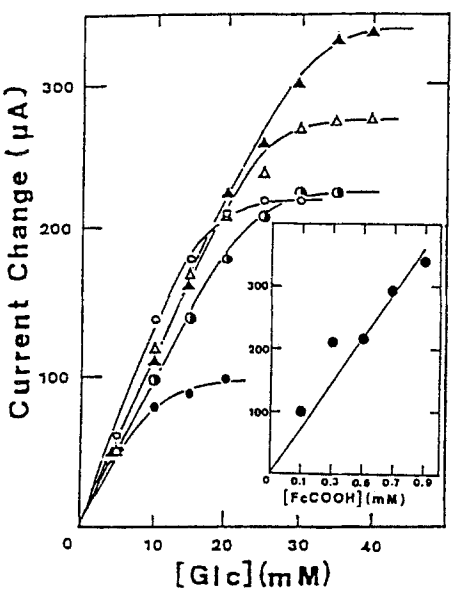


Fig.3. Mediator concentration dependence of glucose-response of GOD electrode.
[FcCOOH]=0.1(●), 0.3(○), 0.5(◑), 0.7 (△), and 0.9(▲) mM, 25°C.
Insert; glucose concentration dependence of current at plateau.

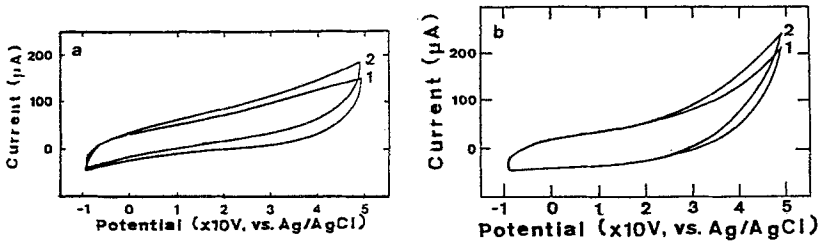


Fig.4. Cyclic voltammogram of GOD and Fc-BSA co-immobilized cloth electrode.
ca. 1mg of GOD and 1mg of BSA were coated on 200 mm^2 cloth electrode by prepolymer method (a) and by urethane emulsion method (b). curve 1. [glucose]=0 mM, curve 2, [glucose]= 15mM.

This consideration teaches us that the effective concentration of the mediator should be increased in the immobilized system. There will be several ways to challenge this problem; such as covalent or noncovalent modification of the electrode surface with mediator derivatives, and co-immobilization of mediator with the enzyme.

Here one attempt was tested with regard to the latter type; co-immobilization of a polymer anchored mediator with the enzyme, which is preferable to our entrapment method of enzyme immobilization.

For this purpose Fc anchored on BSA, which was shown to act successfully as a soluble mediator in the study of F.M.(3), was used. Figure 3 shows the CV curves of the system immobilizing polymer-anchored mediator with the enzyme. The system did certainly work but the current increase was not sufficient. We can mention that, though the concentration factor is solved, the immobilized mediator has smaller mobility in the matrix and the mediation (acceptance, diffusion and transfer) of the electron will be highly limited. The freed of the oxidized mediator away from the electrode is also slow, which can partly compensate the above negative factor.

As the next step selection of anchoring polymer substance and further search of the immobilizing method are to be required for the presentation of more successful system. Application for other oxido-reductase, especially reduction reaction, is also to be seeked.

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