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A novel biosensor using electrochemical surface plasmon resonance measurements

Satoshi Koide,[a] Yuzuru Iwasaki,*[b] Tsutomu Horiuchi,[b] Osamu Niwa,[b] Eiichi Tamiya[a] and Kenji Yokoyama[a]

[a] *School of Materials Science, Japan Advanced Institute of Science and Technology Tatsunokuchi, Ishikawa 923-1292, Japan. E-mail: iwasaki@with.brl.ntt.co.jp*

[b] *NTT Lifestyle and Environmental Technology Laboratories, 3-1 Morinosato Wakamiya Atsugi-shi Kanagawa, 243-0198 Japan*

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Surface plasmon resonance measurements have been conducted to construct a unique electrochemical enzyme sensor which detected the reversible change in the refractive index of a redox film containing an enzyme.

A number of electrochemical biosensors have been constructed based on the enzymatic consumption of substrates and the electrochemical regeneration of an active enzyme by electron mediators. These electrochemical biosensors convert the analyte concentration to faradic current and this current is proportional to the concentration gradient of the electrochemically active molecules on the electrode surface. Meanwhile, surface plasmon resonance (SPR) has been used to measure biological reactions in real time. For instance, DNA elongation by polymerase has been measured in real time using SPR.[1] We have shown that SPR measurements can be used to detect certain electrochemical reactions.[2,3] The combination of SPR and electrochemical measurements will make it possible to detect the refractive index of an electrochemically activated enzyme-mediator film. Here we describe our SPR-based analysis of immobilized enzyme films for electrochemical biosensor applications, and propose a novel transducer element.

Thin gold film electrodes were coated with a horseradish peroxidase (HRP)–osmium redox polymer solution and dried for immobilization.[4] It is expected that reduced and oxidized HRP–osmium films in an electrolyte will have different refractive indices, and that the refractive index will change with the electrode potential and can be used to indicate an enzymatic reaction. However, the refractive index may also depend on the redox reaction of HRP, osmium ions and their ionic environment, the substrate/product concentration, and the packing of the film. When the redox reaction chain is working, all these values change and this affects the minimum reflection angle in SPR measurements (θ_{SPR}). We investigated the effect of catalytic electrochemical reactions on θ_{SPR} values. First, we measured θ_{SPR} for the HRP–osmium redox polymer immobilized electrode simultaneously with cyclic voltammetry without the HRP substrate. The cyclic voltammogram (CV) showed a narrow peak separation and symmetric waves which are characteristics of reactions of an immobilized species.[5] In the absence of hydrogen peroxide (substrate of HRP), Os^{3+} ions are not regenerated by chemical reaction. Therefore, the θ_{SPR} change in the CV was the result of the redox reaction of the osmium film. The θ_{SPR} response showed a bimodal change with potential (similar to that in Fig. 1). We also observed similar θ_{SPR} changes in a solution containing 1 M $NaClO_4$. The value of θ_{SPR} increased at lower potentials, and the potential at which the θ_{SPR} value showed the steepest change was the same as the CV peak potential. This is because of the change in the concentration of ions in the film to compensate the charge neutrality which depends on the valence state of the osmium ions.[6]

In the presence of the HRP substrate the CV shown in Fig. 1 was obtained. In this CV, the hydrogen peroxide was reduced by HRP and a catalytic current was observed. The dependence of θ_{SPR} on the potential was flat in the potential region far from the formal potential, indicating that θ_{SPR} changes from other factors is small. In the potential range in which the catalytic current was observed, oxidized osmium ions (Os^{3+}) were regenerated by the enzymatic reaction. However, the curve of θ_{SPR} *vs.* potential showed a bimodal change, and the graph was almost the same as that obtained without hydrogen peroxide. Therefore, the effect of the redox state of the enzyme and hydrogen peroxide on the θ_{SPR} was far less than that of the mediator film. In the above experiments, the maximum concentration gradient in the film was at the electrode surface when a catalytic current was observed. When the electrode potential is not controlled, the mediator will be oxidized chemically by the enzyme until it is fully oxidized. The concentration ratio of the reduced and oxidized mediator at the electrode surface can be monitored in terms of the electrode potential by means of a potentiometric experiment.

Next, we carried out chronopotentiometry for the catalytic electrochemical reaction as shown in Fig. 2A. Before the chronopotentiometry was started (E = 0 mV), θ_{SPR} increased because of reduction of the film. At zero time, the working electrode was set in the potentiometer mode and θ_{SPR} remained unchanged in the absence of hydrogen peroxide (0 M). In the presence of hydrogen peroxide, θ_{SPR} began to decrease with this being more marked with an increase in hydrogen peroxide concentration. This indicates that the ratio of the reduced form of the film (Os^{2+}) near the electrode surface decreased through the enzymatic consumption of hydrogen peroxide by HRP. The rate of change of θ_{SPR} can thus be used to determine the substrate concentration. Moreover, this method does not require electrochemical instrumentation; we can simply employ the chemical reduction of a mediator using a flow system. Fig. 2B shows the potential change measured by chronopotentiometry. As with the SPR measurement, a potential change was observed in the presence of hydrogen peroxide owing to a decrease in the reduced form of the film. At low hydrogen peroxide concentra-

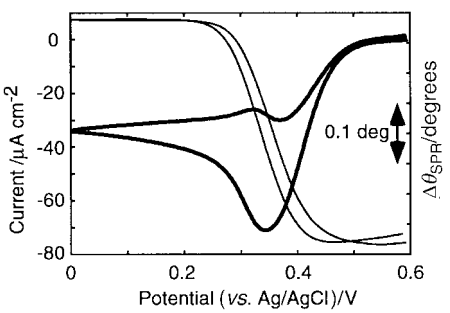

Fig. 1 θ_{SPR} (thin line) dependence of the electrode potential measured simultaneously with cyclic voltammetry (thick line) for an HRP–osmium redox polymer immobilized electrode in 0.1 M phosphate buffer (pH 7) with 10 mM hydrogen peroxide: scan rate 10 mV s^{-1}, geometric surface area = 0.16 cm^2.

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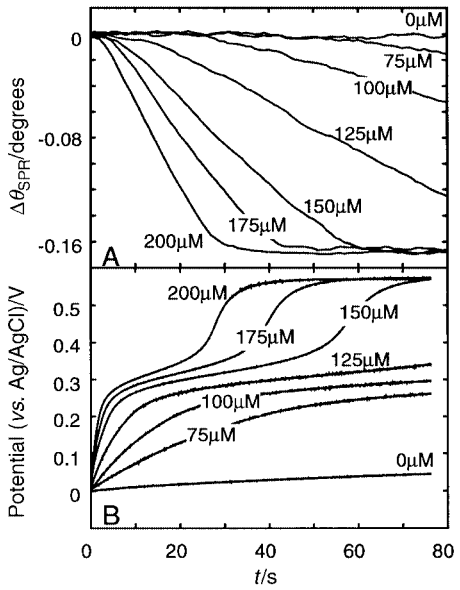

Fig. 2 Time course of θ_{SPR} (A) and equilibrium potential (B) in the chronopotentiometry of an HRP–osmium film with various substrate concentrations. The SPR measurement was initially held a potential at 0 mV for 1 min and then the working electrode was set in the potentiometer mode (t = 0) and simultaneous chronopotentiometry was started. The formal potential ($E^{0\prime}$) was evaluated from the peak potentials of the CV in the absence of the substrate, F is the Faraday constant, R is the gas constant and T is the measuring temperature.

tions, the potential change was also smaller than at higher hydrogen peroxide concentrations. As the concentration ratio of reduced Os ions (Os^{2+}) approached 0.5, the electrode potential change became slower, and a potential plateau (Fig. 2B) was observed.

Fig. 3 shows the relation between θ_{SPR} and the electrode potential of the experiment in Fig. 2. The fraction of reduced film was calculated from the measured potential (Fig. 2B) based on the Nernst equation, and θ_{SPR} was plotted. Despite the

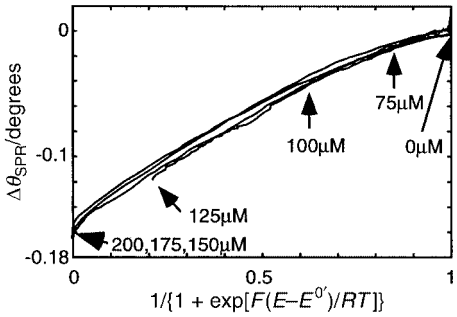

Fig. 3 Relation between θ_{SPR} and surface concentration ratio in the experiment shown in Fig. 2. The arrows indicate the position at 80 s for each substrate concentration.

different substrate concentrations, the trajectories traced the same curve. This implies that the film reacted uniformly and θ_{SPR} represented the redox state of the mediator film in real time. The SPR detection of an enzyme containing redox film can provide a new method of enzyme sensor detection. The physical sensing space can be greatly reduced because of the nature of SPR, and a small dead volume sensor can be constructed. The time response of this method can be improved using a higher sampling rate for the SPR signal. In addition, kinetic information on the multistage enzyme reaction can be obtained. We are currently constructing enzyme sensors using glucose oxidase.

This work was completed while S. K. was staying at NTT.

Notes and references

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