Electrochemical Surface Plasmon Resonance (SPR) Method; New Technique for Opto-Electrochemistry and Electrochemical Imaging

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INTRODUCTION

Various optical measurement techniques have been developed for the characterization of surface states during electrochemical processes. Surface plasmon resonance (SPR) spectroscopy has been used in combination with electrochemistry to investigate the electronic structure of metal-electrolyte interfaces [1]. We reported the current efficiency of refractive index changes that occurred during the monitoring of oxide film formation and ion adsorption on gold electrodes by simultaneous voltammetry and SPR measurements [2]. We also applied this system to measure the electrochemical reaction of methylene blue (MB) and ferrocyanide [3-4]. We also fabricated an SPR-based electrochemical biosensor based on the detection of the reversible refractive index change that occurs in redox mediator film [5]. Here, we report on electrochemical-SPR measurement for biosensor application and multi-channel detection realized by the SPR imaging of bioelectrochemical reactions.

EXPERIMENTAL

We measured the SPR resonance angle using an instrument with a Kretschmann configuration. Os-poly (vinylpyridine)-wired horseradish peroxidase (Os-gel-HRP) film was formed by spotting diluted solution on the gold electrodes. Then, glucose oxidase was immobilized on the electrode. We performed the electrochemical experiments by placing counter (Pt) and reference electrodes in the instrument's cell. We obtained cyclic voltammograms and the SPR angle change of the Os-gel-HRP using a potentiostat and a PARC-175 universal programmer. We detected the chemical conversion of an enzymatic reaction to a dielectric property change in a modified layer on the electrode (Fig. 1).

To image the electrochemical reaction with SPR, we used an expanded laser beam to illuminate the internal surface of a prism to which we had attached an SPR chip. We monitored the reflected light using a CCD camera. We were able to detect refractive index changes on the SPR chip surface as reflection intensity by choosing an incident light angle near the SPR dip angle. This system allows us to image the refractive index change on the SPR chip caused by the redox reaction of the Os-gel-HRP film, which is oxidized by the enzymatic reaction chain.

RESULTS AND DISCUSSION

We observed that the difference between the reflection minimum angles (θ_{SPR}) of the Os-gel-HRP in the oxidized and reduced state was about 0.14 degrees. From this reversible SPR change, we can determine the redox state of the mediator film immobilized on the gold surface. The substrate concentration can be determined by measuring the rate of oxidation of pre-reduced mediator. When we used the potentiostat in the electrometer mode after reducing the Os-gel-HRP and adding glucose, the Os-gel in the fully reduced state was gradually oxidized by sequential reactions of glucose oxidase and HRP. The $\Delta\theta_{SPR}$ value is steeper for higher glucose concentrations. Therefore, we can detect the glucose concentration by measuring the θ_{SPR} slope. Our SPR based biosensor is suitable for imaging enzymatic reactions.

To image the whole of the enzyme sensor array, we used a 2-dimensional SPR optical configuration. The sensor array was incorporated in the microfluidic channel (Fig. 2).

The enzyme sensors consisted of a redox mediator layer and an enzyme layer formed by an automated spotting machine. With this sensor chip, we successfully detected individual sensor operation and the flow pattern in a microfluidic channel.

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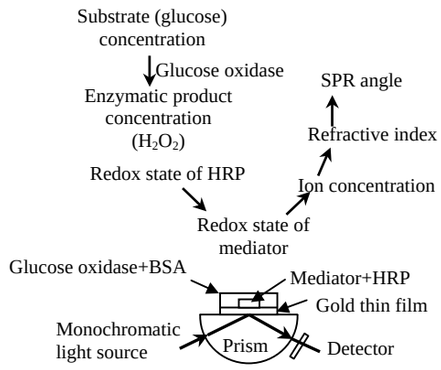
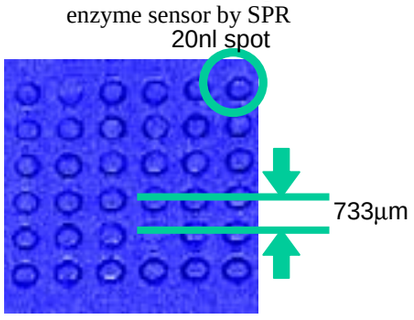
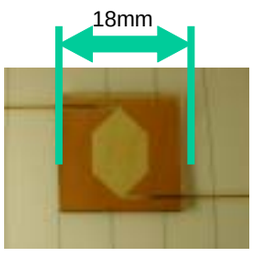


Figure 1 Detection scheme using the mediated enzyme sensor by SPR



SPR image of enzyme array



Enzyme array in thin layer channel

Figure 2 SPR imaging of enzymatic reaction