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Electrochemical Preparation of Active Avidin Films for Enzyme Sensor Applications

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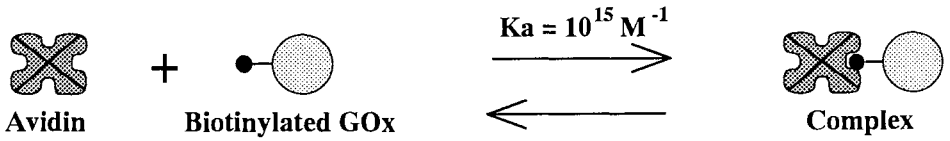
A thin film of avidin was prepared on the surface of platinum electrode by applying an alternating potential of a triangular-wave form (38.5 Hz) from -0.5 to +2.1 V vs. Ag/AgCl to the electrode immersed in the avidin solution. The electro-deposited avidin film exhibited a strong ability to bind biotin-labeled glucose oxidase. The optimum conditions for the deposition of avidin film were studied in relation to the application of the film to enzyme sensors.

Avidin is a glyco-protein which has an exceptionally high affinity to biotin, the binding constant being ca. 10^{15} M^{-1} ($M=mol/dm^3$) (Scheme 1). Owing to the specific and strong complexation, the avidin/biotin system has been used as a useful tool in affinity chromatography, histopathology, immunological diagnostic assay, etc.[1] Very recently the avidin/biotin system has been employed for the immobilization of enzymes on the electrode surface, with the intention of constructing enzyme sensors.[2-5] Kuhr et al. have immobilized biotin-labeled glutamate dehydrogenase and horseradish peroxidase on the biotin-modified carbon-fiber microelectrodes by using avidin as a binder.[2,3] On the other hand, metal electrodes coated with Langmuir-Blodgett membranes and self-assembled lipid membranes have been used for immobilizing the complex of avidin and biotin-labeled glucose oxidase (GOx) by us[4] and Snejdarkova et al.,[5] respectively. These studies have established the usefulness



Scheme 1. Complexation between avidin and biotin-labeled GOx.

of the avidin/biotin system in the biosensor fabrications. To ascertain their maximum performance, however, the further improvement is still needed in the procedure of the avidin/biotin-based immobilization of enzymes. We report here a facile technique to prepare active avidin films on the electrode surface for the construction of highly responsive and durable sensors.

The avidin films were deposited on the surface of a platinum (Pt) wire electrode (diameter;0.5 mm, effective length;5 mm) by applying an alternating potential of a triangular-wave form from -0.5 to +2.1 V vs. Ag/AgCl (frequency;38.5 Hz) in the phosphate buffer solution (pH 7.4) of avidin (Calzyme Lab.) for 1-10 min. The concentration of avidin was varied from 0.1 to 10 mg/cm^3. After the electrolysis the avidin-deposited electrode was rinsed with the working buffer for 10 min, and then immersed in the 50 μg/cm^3 biotin-labeled GOx (Sigma Co.) solution (phosphate buffer, pH 7.4) for 30 min at room temperature. The GOx-modified electrode thus prepared was rinsed thoroughly with the buffer before use. The electrochemical response of the GOx-modified electrode to glucose was evaluated by a conventional three electrode system with counter (Pt) and reference (Ag/AgCl) electrodes.

Figure 1-a) depicts a typical response curve of the GOx-modified electrode prepared according to the above-mentioned procedure, in which the electro-deposition of the avidin film was carried out in 10 mg/cm^3 avidin solution for 5 min. The electrode exhibited an amperometric response to 10^{-4} and 10^{-3} M glucose. The electrochemical response should originate from the oxidation current of H_2O_2 produced by the GOx-catalyzed reaction of glucose on the electrode, which, in turn, suggests that the biotin-labeled GOx is immobilized on the avidin film through avidin/biotin complexation. These results mean that the present method gives a thin film of avidin without loss of the binding ability to biotin. This view is confirmed by the fact that the response is very small when a native GOx (biotin-free) is used in place of the biotin-labeled GOx (Fig. 1-b)). The small response in Fig. 1-b) may come from the oxidation reaction of glucose catalyzed by the non-specifically adsorbed GOx.

It was independently checked the possibility of simple adsorption of avidin on the electrode surface. For this purpose a Pt elctrode was simply immersed in the 10 mg/cm^3 avidin solution for 10 min and then treated with biotin-labeled GOx. The electrode thus prepared showed a very low and non-reproducible response to glucose (100 nA or less to 10 mM glucose), confirming the effectiveness of the electrochemical treatment for the preparation of functionally active avidin films. We also

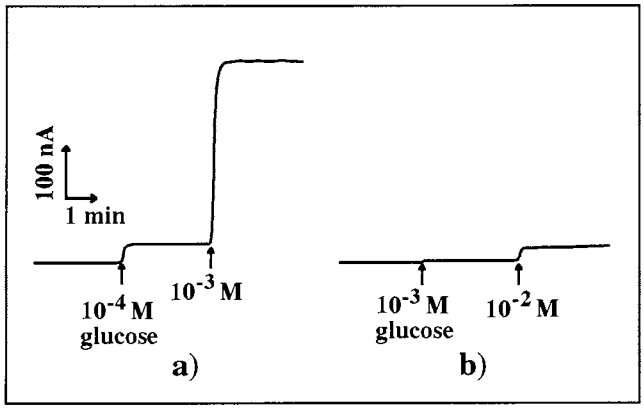


Fig. 1. Typical response of the glucose sensors prepared based on the electro-deposited avidin film coupled with a biotin-labeled GOx (a) and a native GOx (b).
Electrode potential; +0.6 V vs. Ag/AgCl

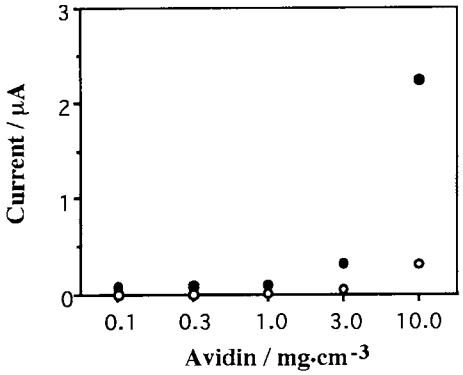


Fig. 2. Effects of the concentration of avidin solution on the sensor response to 1 (o) and 10 mM (●) glucose.
Electrolysis; 5 min.

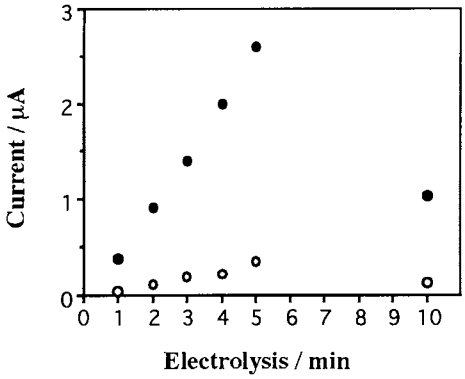


Fig. 3. Effects of the electrolysis time on the sensor response to 1 (o) and 10 mM (●) glucose.
Avidin solution; 10 mg/cm^3.

tried to deposit GOx film directly on the Pt electrode by applying the alternating potential in a GOx solution but, unfortunately, the response of the electrode was very small.

In order to elucidate the optimum conditions in the electrodeposition of the avidin film, the highest and lowest potentials and the frequency of the alternating potential were varied in the range of -1.0 – +2.6 V vs. Ag/AgCl and 38.5 – 70.0 Hz, respectively. Among them the 38.5 Hz alternating potential from -0.5 to +2.1 V gave the best result. We also checked the effects of the concentration of avidin solution (Fig. 2) and the electrolysis time (Fig. 3). Figure 2 shows that the higher concentration solutions of avidin should be used to prepare the avidin film. On the other hand, Fig. 3 shows that the electro-deposition should be continued for ca. 5 min to obtain the highest response. The thickness of the avidin film may increase with increasing the electrolysis time up to 5 min judging from the linear dependence of the response on the electrolysis time. In other words, the magnitude of the response may be determined by the thickness of the avidin film, or by the loading of GOx. However the prolonged electrolysis lowered the response, which might arise from some morphological changes of the avidin film. In fact, we observed visually a rather rugged surface of the avidin film which was prepared by 10-min electrolysis, as compared with relatively smooth films deposited in shorter time. The quantitative estimation of the loading of avidin and GOx on the electrode as well as the deposition mechanism of the film are now in progress.

The glucose sensors prepared by the present technique have a high stability in the buffer solution at 4 °C for long time. Typically, the slope of the calibration graph for 0.1 – 10 mM glucose has kept practically unchanged for more than 3 months.

Thus, we have demonstrated that the active avidin film which strongly binds biotin-labeled enzyme can be deposited electrochemically on the Pt electrode.

References

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