REGULATION OF OUTPUT CURRENT OF L-LACTATE SENSORS BASED ON ALTERNATE DEPOSITION OF AVIDIN AND BIOTINYLATED LACTATE OXIDASE ON ELECTRODE SURFACE THROUGH AVIDIN/BIOTIN COMPLEXATION

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An alternate and repeated deposition of avidin and biotinylated lactate oxidase (LOx) gave a thin film on the surface of platinum electrode. Amperometric response of L-lactate sensors thus prepared was enhanced stepwise by increasing the number of LOx layers. The enhanced response contributed to the extension of the lower detection limit of the sensor. The response time of the sensors was satisfactorily fast (ca. 10 s), irrespective of the number of LOx layers.

KEY WORDS avidin/biotin system; lactate oxidase; L-lactate sensor; protein multilayer

We[1] and other groups[2,3] have reported a novel technique to immobilize enzymes on electrode surface using an avidin/biotin system, in which biotinylated enzymes are anchored to the avidin-modified electrodes through a strong affinity between avidin and biotin (binding constant:1×10^{15} M^{-1}).[4] In this procedure, enzymes are deposited on the electrodes as a monomolecular layer, resulting in rapid response biosensors. However, such monolayer-modified biosensors often suffer from low response arising from insufficient enzyme activity (or lack of enzyme load), because the output current of enzyme sensors depends basically on the total activity of the enzyme on the electrode.[3,5] The present communication reports a facile method for the regulation of the magnitude of the output current of L-lactate sensors based on repeated deposition of avidin and biotinylated LOx on a platinum (Pt) electrode (Fig.1). We can expect that an amperometric response of L-lactate sensors thus prepared depends stepwise on the number of LOx layers.

Lactate oxidase (E.C.1.1.3.2) was biotinylated with an excess amount of succinimidyl 6-(biotinamide)hexanoate, according to the standard procedure.[6] By this treatment, several biotin residues would be introduced to a single molecule of LOx. For the construction of L-

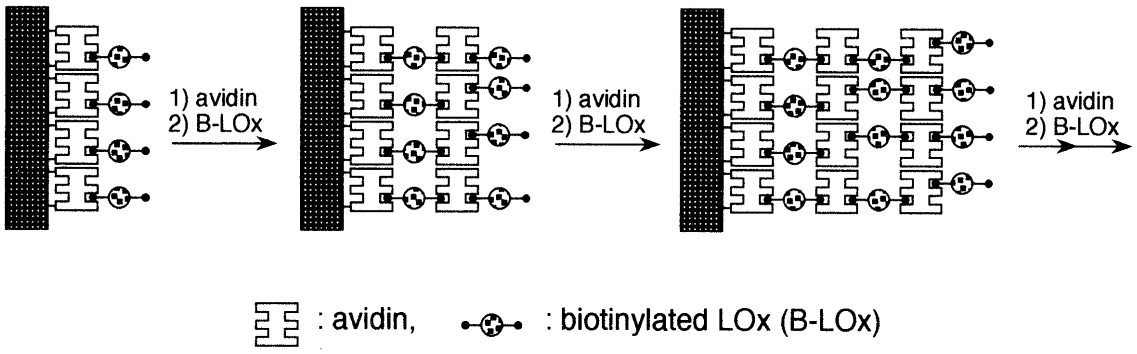

Fig. 1. Schematic Illustration of the Deposition of Avidin/LOx Multilayer

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lactate sensors, a Pt disk electrode (3 mm diameter) was used throughout. The first avidin layer was deposited on the Pt electrode by immersing the electrode in an avidin solution (10 mg/ml phosphate buffered saline, PBS) for 30 min and then in PBS for 12 h to wash out any weakly adsorbed avidin. This treatment would give a monolayer film of avidin on the electrode surface.[7] Subsequently the avidin-modified electrode was immersed in a biotinylated LOx solution (50 μg/ml PBS) for 30 min to immobilize LOx on the avidin film through avidin/biotin complexation. For the deposition of the second layer of avidin and LOx, the LOx-modified electrode was immersed in the avidin solution for 60 min, rinsed with PBS for 30 min and then immersed in the biotinylated LOx solution for 60 min. The deposition of avidin and LOx was performed at room temperature. The same procedure was repeated 10 times to deposit further layers.

The electrochemical response of the LOx-modified electrode was measured with a conventional three-electrode system at 0.6 V vs. a Ag/AgCl reference electrode.[8] The LOx catalyzes the oxidation reaction of L-lactate to produce pyruvate and H_2O_2 (Equation **1**), the latter of which can be oxidized at the Pt surface at this potential. All measurements were carried out at ca. 20°C.

$$\text{L-lactate} + O_2 \xrightarrow{\text{LOx}} \text{pyruvate} + H_2O_2 \text{ --- (1)}$$

Figure 2 plots the output current (ΔI) of the lactate sensors to 0.3 and 1.0 mM L-lactate as a function of the number of LOx layers on the electrode. The ΔI values increased with increasing the LOx layers, showing that the LOx was accumulated successively on the electrode surface through avidin/biotin complexation. The slope of the plots (e.g., Δ(ΔI) per deposition) is 200 and 400 nA for 0.3 and 1.0 mM L-lactate samples, respectively. These results strongly suggest the formation of multiple layers of avidin and LOx on the Pt electrode, an idealized structure of which is illustrated in Fig. 1. Such a relationship between the ΔI values and the number of depositions could not be observed when native LOx was used instead of the biotinylated LOx.

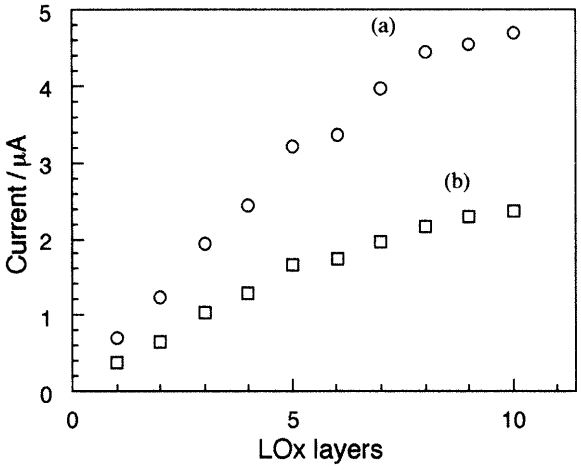

Fig. 2. Relationship between the Output Current and Number of LOx Layers of the Sensors Sample:1.0 (a) and 0.3 mM (b) L-lactate

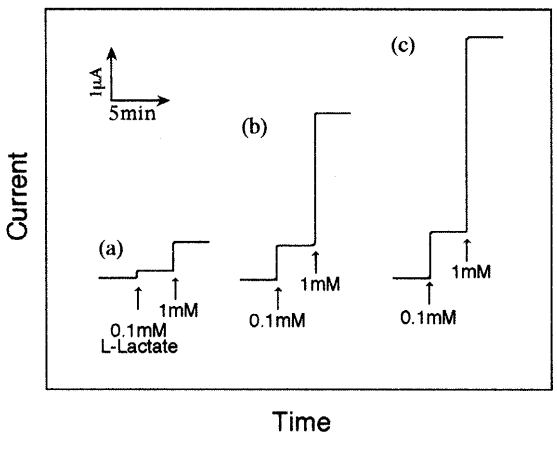

Fig. 3. Typical Response of the Sensors Modified with Monolayer (a), 5-layer (b), and 10-layer avidin/LOx (c)

It is often the case that the response time of enzyme sensors depends significantly on the thickness of the enzyme layer. Figure 3 shows the amperometric response of the sensors modified with mono-, 5-, and 10-layers of LOx to 0.1 and 1.0 mM L-lactate. These sensors responded rapidly to L-lactate, and the response time was virtually independent of the number of LOx layers. These results suggest that the analyte and reaction products of the enzymatic reaction are transported smoothly in the avidin/LOx layer. The response time of the sensors may be determined by the rate of the enzyme reaction.

Table I. Detection Limits, Maximum Current (I_{max}), and Apparent Michaelis-Menten Constant (K_m^{app}) of the L-Lactate Sensors

Number of LOx layers	Lower detection limit (μM)[a]	Higher detection limit (mM)	I_{max} (μA)[b]	K_m^{app} (mM)[b]
Monolayer	10	1.0	1.1	0.63
5 layers	2.0	1.0	5.5	0.69
10 layers	0.5	1.0	8.3	0.79

a) Defined as a L-lactate concentration to which the sensor showed a current response more than 10 nA.

b) Estimated by a curve-fitting based on the electrochemical expression of Michaelis-Menten equation ($I=I_{max}\cdot C/(K_m^{app}+C)$, where I and C denote the steady-state current and substrate concentration, respectively.

Table I summarizes detection limits and kinetic parameters of the sensors. The lower detection limit was extended to some extent by the multiple deposition of LOx layers, resulting in a wider dynamic range of the sensors. The maximum response current (I_{max}) of the sensors depended clearly on the number of LOx layers. Thus the multiple deposition of the LOx layer is effective to enhance the magnitude of output current of the sensors. This is reasonable because the I_{max} should reflect the total activity of the enzyme on the electrode. On the other hand, the multiple deposition affected very slightly the K_m^{app} values of the sensors. The observed K_m^{app} values are well consistent with the reported K_m value (0.7 mM) for lactate oxidase.[9]

In order to check the stability of the sensor modified with 20-layer LOx, the response of the sensor to 1 mM L-lactate was measured once a day. The ΔI value decreased gradually, and ca. 50% response of the original ΔI value was maintained after two weeks.

In conclusion, the multiple deposition of avidin and bioitinylated LOx is a useful technique for the enhancement of the output current of L-lactate sensors.

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