Preparation of L-lactate Biosensor that uses Polyion Complex Membrane Containing Peroxidase and Ferrocene

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In order to detect lactate, high sensitivity lactate sensor was completed by attaching lactate oxidase (LOD) to the hydrogen peroxide sensor. The hydrogen peroxide sensor was prepared as follows; poly(4-styrenesulfonate), peroxidase (POD), ferrocene and poly-l-lysine solutions were dropped on a glassy carbon electrode. After drying this electrode, LOD and glutaraldehyde solutions were dropped on the membrane to immobilize the enzyme. The electrode was immersed into a buffer solution (pH 6.5), and a potential of –0.2 V vs. Ag/AgCl was applied to detect the reduction current. Immediately after the addition of l-lactate (10 μM) to the test solution, the reductive current increased and reached a plateau within 20 s. The lower detection limit was 1 μM (S/N = 3), and the ratio of the response for the interferent to that for l-lactate on the electrode was 0.18.

Key Words : Biosensor, Peroxide, L-lactate, Polyion Complex Membrane

1 Introduction

Easy preparation, quick response and high sensitivity in the measurement of lactate are required for the sensor in the area of the medical treatment and the food industry.1) The general analysis methods of lactate detection are either HPLC with UV spectrophotometer or refractive index detection. But those methods require a pre-treatment with a complicated procedure.2) Electrochemical biosensors have been developed to achieve such simple measuring methods. Boujtilta et al.3) constructed an amperometric l-lactate biosensor by confining lactate oxidase (LOD) and hydroxymethyl ferrocene (as a redox mediator) in a carbon paste electrode. Patel et al.4) used a mixture of LOD with polyethyleneimine (PEI) and poly(carbamoyl)sulphonate (PCS) hydrogel for enzyme immobilization onto the platinum disk of the transducers. Palmisano et al.5) operated biosensor in flow injection analysis, which was obtained by glutaraldehyde co-crosslinking of LOD with bovine serum albumin cast on an underlying electropolymerized layer of oxidized polypyrrole. Halliwell et al.6) immobilized lactate dehydrogenase on poly(aniline)-poly(acrylate) and poly(aniline)-poly-(vinyl sulphonate) films. Wang et al.7) has reported on the formation of colloidal biocomposite between LOD and nanoscaled cobalt phthalocyanine (NanoCoPc) colloid through electrostatic interactions. Sung et al.8) synthesized LOD enzyme electrode based on polypyrrole with a polyanion/PEG/enzyme conjugate dopant. Parra et al.9) developed a lactate biosensor based on immobilized LOD onto gold surfaces (substrates). Suman et al.10) made an amperometric enzyme electrode by immobilizing commercial LOD onto polyaniline-co-fluoroaniline film deposited on an Indium tin oxide (ITO) coated glass plate. Mizutani et al.11) made amperometric l-lactate-sensing electrode using an enzyme ultra-thin layer produced through the co-adsorption of poly-l-lysine and LOD onto mercaptoalkanoic acid-modified gold surface. In the previous work,12,13) LOD immobilized in the polyion complex (PIC) layer was used as an electrode of lactate sensor.

It is known for the enzyme sensor that the measurement is affected by the impurities in the sample. To suppress the effect by them, there are some kinds of method such as; 1) using more sensitive sensors that combine with the hydrogen peroxide sensor,14,15) 2) using perm-selective membrane to prevent penetration of those foreign substances. The PIC membrane obtained by mixing polyanion and polycation is available to perm-selective film.16)

This paper, an L-lactate sensor using an electrode with LOD over PIC layer was newly designed to suppress interfering current. LOD was chemically conjugated using glutaraldehyde onto peroxidase- and ferrocene-immobilized on PIC membrane. The current response of the L-lactate sensor was evaluated under differing L-lactate concentrations. Furthermore, effect of L-ascorbate as an interfering substance on current response was examined.
2 Experimental

2.1 Equipment and reagent
A potentiostat (HA-501; Hokuto Denko) was used for current detection. Glassy carbon electrode (disc, 3.0 mm in diameter; Bioanalytical Systems) was used as an base electrode. Pen recorder (type 3056; Yokogawa Electric Works) printed out the results. Poly(4-styrenesulfonate) (average MW 70,000, Aldrich), POD (EC 1.11.1.7, from horseradish, 131 U/mg, Toyobo), aqueous dispersion of ferrocene (Kanto Chemical), and poly-l-lysine (average MW 83,300, Sigma Chemicals) were used to make PIC membrane. LOD (EC 1.13.12.4, from Pedicoccus sp., 47 U/mg Sigma Chemicals), glutaraldehyde (8%; Polysciences), L-ascorbic acid (Kanto Chemical), distilled water (WA73; Yamato Scientific) were used to create the L-lactate sensor.

2.2 Preparation of PIC membrane containing POD and ferrocene.
Poly(4-styrenesulfonate) solution (20 μL; 25 mM as monomer unit), aqueous dispersion of ferrocene (20 μL; 20 wt%), poly-l-lysine solution (20 μL; 20 mM as monomer unit), and POD solution (20 μL; 2 wt%) were dropped successively on the glassy carbon electrode. After drying the electrode at room temperature for 4 h, LOD was immobilized on the electrode.

2.3 Preparation of LOD-attached PIC membrane containing POD and ferrocene
LOD was attached by glutaraldehyde on to the PIC membrane containing POD and ferrocene; LOD (10 μL; 1 wt%) solution and glutaraldehyde (10 μL; 0.1 wt%) solution were successively placed on the PIC membrane. The membrane was dried at room temperature for 4 h. Then the electrode was stored in a 20 mM phosphate buffer (pH 6.5) solution at 4°C.

2.4 Measurement of current response to hydrogen peroxide and L-lactate
A potentiostat was used in a three-electrode configuration for amperometric measurements; enzyme electrode, platinum wire (0.5 mm in diameter) and Ag/AgCl electrode were used as working, auxiliary and reference electrodes, respectively. The test solution containing 0.2 M phosphate buffer (pH 6.5, 15 ml) was used for the measurement of the current response to H₂O₂ or L-lactate. A potential of −0.2 V Ag/AgCl was applied on the working electrode to detect the reductive current response.

3 Results and Discussion

3.1 Current response on LOD immobilized-electrode to L-lactate and interfering current by L-ascorbate

Figure 1(a) shows current response on the L-lactate sensor that uses PIC-based electrode with LOD-attached (A) and without LOD (B) to 10 μM L-lactate. When the L-lactate was added, the current immediately increased toward reduction and became a plateau within 20 s.

Increment of reduction current was not observed in the case of PIC membrane without LOD. Therefore, it is considered that such a reduction current showed the LOD- and POD-catalyzed reactions would be proceed in the PIC membrane.

The effect from the electrochemistry obstruction material was examined. The L-ascorbate was chosen as an electrochemistry obstruction material for the experiment. Figure 1(b) shows response current to 10 μM L-ascorbate after adding the same concentration of L-lactate. When L-ascorbate was added, reduction current was decreased. It is considered that the L-ascorbate penetrated into the membrane and reduced the oxidation form of the mediator (ferrocene) generated from POD. Details of this mechanism are under consideration. Response current ratio between L-lactate and L-ascorbate at the same concentration was determined to be 0.18. Usually, significant current is observed when L-ascorbate was oxidized on the base electrode. However, the response current to L-ascorbate on this electrode sug-
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suggests that there is small penetration of L-ascorbate through the PIC membrane. The current response ratio of L-ascorbate/l-lactate for the electrode on which LOD was immobilized into the PIC membrane used in the former report was 0.25. The response of the electrode used in this report was improved by 0.18 than that of the former report. It is thought that the influence of l-lactate was decreased by the existence of POD on the electrode. From the result, it was proved to be able to measure the concentration of the l-lactate more accurately.

3.2 Detection sensitivity of l-lactate

Figure 2 is this reduction current against the l-lactate concentration. That is, for PIC-based enzyme electrode with LOD-attached, the reduction current increased with increasing l-lactate concentration. Especially, the response current was proportional to the l-lactate concentration from 1 µM (signal-to-noise ratio = 3) to 30 µM. Minimum concentration detected is 1 µM (S/N = 3). The slope of this calibration curve was 3.93 nA/µM and has almost the same sensitivity as the l-lactate sensor of the former report. From this result, this electrode can be used as an l-lactate sensor.

References