Amperometric Enzyme Electrode for L-Lactate with the Use of Lipid-Modified Lactate Oxidase

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Received February 10, 1993 ; Accepted March 29, 1993

1. INTRODUCTION

In conventional amperometric enzyme electrodes, at least two membranes are placed on the surface of base electrodes: one is an immobilized enzyme membrane and the other is a dialysis membrane for preventing electrochemical interferences and electrode fouling. Cellulose acetate is commonly used as a semipermeable and protective coating on enzyme electrodes^{1,2}. The application of a cellulose acetate membrane to the matrix for enzyme immobilization is an interesting approach to realizing enzyme electrodes with simple membrane structure. In order to prepare such a cellulose acetate-based system, the use of lipid-modified enzyme³, instead of native enzyme, is considered to be suitable⁴: the water-insoluble, modified enzyme molecules can be far more stably immobilized in the polymer matrix.

In this paper, we describe the preparation and the use of an amperometric L-lactate-sensing electrode with a lipid-modified lactate oxidase and cellulose acetate coating. The assay of L-lactate is necessary in clinical and industrial food laboratories.

2. EXPERIMENTAL

Lactate oxidase (LOD, Asahi Chemical Industry) modified with an amphiphile [N-(α -trimethylammonioacetyl)-didodecyl-L-glutamate chloride, Sogo Pharmaceutical] was prepared according to the procedure of Okahata et al³): an aqueous solution of LOD was mixed with an aqueous dispersion of the amphiphile, and the precipitate formed after incubation of the mixture at 4°C for 24 h was lyophilized. The cataionic amphiphile was useful to modify the surface of anionically-charged LOD molecule (isoelectric point, pH 4.6). The LOD content in the modified enzyme was determined by

measuring the absorption by flavin adenine dinucleotide (FAD) in a benzene solution of the product at 450 nm. The enzyme activities of the modified and unmodified LOD were measured by using a peroxidase-phenol-4-aminoantipyrine chromogenic system. The solution (or dispersed medium in the case of the modified enzyme) used for measuring LOD activity was a 0.1 M potassium phosphate buffer solution (pH 7, 25°C).

A benzene solution containing the modified enzyme was placed on the surface of a glassy carbon electrode (Bioanalytical Systems, 3 mm in diameter) and the solvent was allowed to evaporate. The surface density of the modified enzyme on the electrode was 1.4 mg cm⁻². Then a cellulose acetate coating was made by dip coating the electrode in an acetone solution of cellulose acetate [1%(w/v)]. The thickness of the modified LOD-cellulose acetate layer was several microns. The enzyme electrode thus prepared, an Ag/AgCl electrode, and a platinum wire served as working, reference, and auxiliary electrodes, respectively. These electrodes were immersed in a test solution (0.1 M potassium phosphate buffer, pH 7, 10 ml, 25°C). The solution was saturated with air and stirred by using a magnetic bar. The enzyme electrode was stored in the solution at 4°C when not in use.

3. RESULTS AND DISCUSSION

The LOD content in the lyophilized powder of the modified enzyme was calculated to be 30%. The LOD activity of the modified enzyme was determined to be 9.1 U mg⁻¹ solid, which corresponded to 30 U mg⁻¹ LOD. The LOD activity of the unmodified enzyme was determined to be 32 U mg⁻¹. These results show that enzyme activity does not decrease during the modification process.

Figure 1 shows response curves on the enzyme electrode. The potential of the electrode was set at 0.9 V vs. Ag/AgCl. The current increased immediately after the addition of L-lactate and

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key words: Amperometry, Enzyme electrode, Modified enzyme, L-Lactate.

Fig. 1. Electrode response to (A) 0.05 mM, (B) 0.1 mM, and (C) 0.2 mM L-lactate, and (D) 0.1 mM L-ascorbate.

reached a steady state within 5 s. The response on the present electrode was about ten times as quick as that on previous L-lactate-sensing electrodes using immobilized native-LOD⁵⁻⁷). The L-lactate added is expected to diffuse quickly through the thin cellulose acetate/amphiphile layer and be available to be oxidized by the enzyme. Hydrogen peroxide generated by the LOD reaction near the electrode surface is immediately oxidized. These rapid processes result in a fast response on the enzyme electrode.

L-Ascorbate is a typical interferent: its oxidation on the electrode disturbs the anionic indication of hydrogen peroxide. Therefore the electrode response to L-ascorbate was examined. The ratio of response for 0.1 mM L-ascorbate to that for the same concentration of L-lactate was 1.6, as shown in Fig. 1. The approximate concentrations of L-ascorbate and L-lactate in normal sera are 0.05 mM and 1.5 mM, respectively. For determining L-lactate in such samples with the enzyme electrode, the level of electrochemical interference by L-ascorbate is expected to be ca. 5% of the signal output. The electrode would be useful for the rapid estimation of the L-lactate concentration in sera.

Figure 2 shows a calibration graph obtained with the enzyme electrode. The enzyme electrode gave a linear current response up to 0.3 mM, and a significant increase in the current was still observed with L-lactate concentration between 0.3–1.0 mM. The detection limit was as low as 0.1 μ M. This unusually high sensitivity (the detection limit with conventional, native LOD-based electrodes has been reported to be 5–10 μ M^{5,6}) is considered to be obtained by the use of a thin layer containing LOD

Fig. 2. Calibration graph for L-lactate on the enzyme electrode.

with a high activity (0.2 U cm⁻²).

The long-term stability of the enzyme electrode was tested by determining 0.1 mM L-lactate five times a day each day for 4 weeks. The average value of the electrode response did not decrease for 10 days. The electrode responses gradually decreased after 10 days, but they are measurable until the end of 4 weeks. The detection limit was lower than 0.3 μ M on the 28th day. The lifetime, 4 weeks, was comparable to that of previous L-lactate sensors using covalently immobilized LOD^{6,7}).

The combination of the modified LOD with a thin polymer overcoat provided an L-lactate-sensing electrode with high performance characteristics such as rapid response, high sensitivity, and high stability.

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