CHEMISTRY LETTERS, pp. 199 - 202, 1984.

CATALYTIC ASSAY OF L-LACTATE OR PYRUVATE WITH AN ENZYME ELECTRODE BASED ON IMMOBILIZED LACTATE OXIDASE AND LACTATE DEHYDROGENASE

Fumio MIZUTANI,* Yukio SHIMURA, and Keishiro TSUDA
Research Institute for Polymers and Textiles,
1-1-4 Yatabe-Higashi, Tsukuba, Ibaraki 305

An enzyme electrode using immobilized lactate oxidase and lactate dehydrogenase is prepared for the catalytic assay of L-lactate or pyruvate: the rate of the circulating enzymatic reactions between L-lactate and pyruvate is monitored by an oxygen electrode. Either of the substrates is determined in the concentration range 0.08 - 8 μM.

For the enzymatic determination of small quantities of substrate, the method of catalytic assay[1] is very useful. The concentration of the substrate is kept constant by the use of coupled enzymes, i.e., the one consuming the substrate and the other regenerating it, so that the substrate behaves as an intermediate catalyst (this is why it is referred to as "catalytic" assay[1]). The rate of the above process is monitored spectrophotometrically.

When the coupled enzymes are immobilized and when the spectrophotometric monitoring is replaced by electrochemical one, the resulting "catalytic enzyme electrode" will provide simple, rapid, and in particular, highly-sensitive measuring methods. Such an enzyme electrode has not yet been reported, although two or more enzymes are reported to be utilized in a single enzyme electrode in various manners.[2-8]

We have developed an enzyme electrode for the catalytic assay of L-lactate or pyruvate by the use of lactate oxidase (LOD) and lactate dehydrogenase (LDH) as a couple. Some of the preliminary results are reported here.

The schematic diagram of the enzyme electrode and the reactions with LOD and

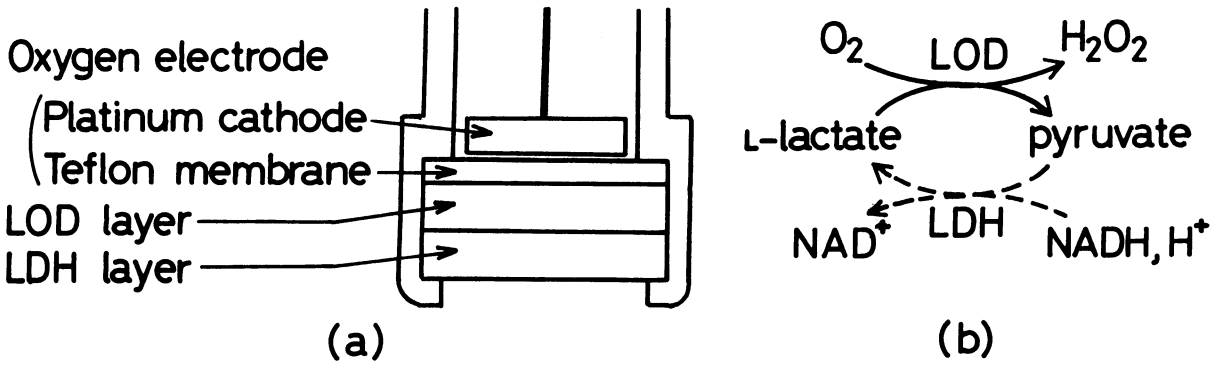

Fig. 1. Schematic diagram of the enzyme electrode (a) and the enzymatic reactions at the electrode (b).

LDH are shown in Fig. 1. LOD[9] (from Pediococcus sp., 17 IU mg^{-1}, Toyo Jozo) and LDH (EC 1.1.1.27, from bovine heart, 400 IU mg^{-1}, Sigma Chemical) were separately entrapped in a layer of photocrosslinked polymer[10] by the procedure similar to that described previously.[11] These layers were attached on a Teflon membrane of a Clark oxygen electrode (Ishikawa Manufacturing, battery type with a platinum cathode of 3-mm diameter). The 10-cm^3 test solutions were 0.1 M (1 M = 1 mol dm^{-3} in this paper) phosphate buffer at 30 °C at pH 7.4. The pH of the solution was very close to the optimal pH for LOD[12] and LDH.[13] The other experimental conditions and apparatus used have been described elsewhere.[12]

Figure 2 shows the response curve of the enzyme electrode in the solution containing 1 mM NADH for a successive addition of L-lactate (4 μM) and pyruvate (4 μM). The addition of each substrate caused a significant decrease in the electrode current (0.50 μA at the steady state). On the other hand, when the similar experiment was carried out in the solution without NADH, the electrode current showed a vanishingly small decrease for L-lactate (less than 8 nA) and no response for pyruvate. The above results can be interpreted as follows. In the absence of NADH, only the LOD reaction (the solid line of the reaction scheme in Fig. 1) can proceed, and the enzyme electrode responded only to L-lactate, although its concentration was too low to give an appreciable response. In the presence of NADH, in contrast, the entire reactions shown in Fig. 1 can proceed after the addition of L-lactate or pyruvate, each of which acts as the "catalyst". The rate of the reactions, i.e., the rate of oxygen consumption in the LOD layer, is high enough to give the appreciable response to the very low concentration of L-lactate or pyruvate (Fig. 2). The electrode response for L-lactate in the NADH-containing solution was amplified more than 60-fold of the value obtained in the solution without NADH.

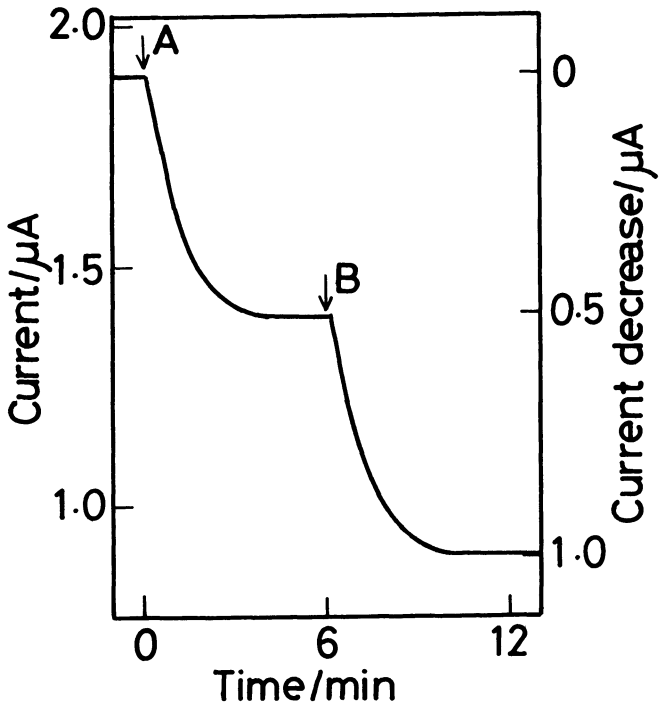

Fig. 2. Response curve of the enzyme electrode for a successive addition of L-lactate (4 μM) and pyruvate (4 μM) into the solution containing 1 mM NADH. (A) and (B) are the times when L-lactate and pyruvate are added, respectively.

Figure 3 shows the calibration curves for L-lactate or pyruvate at different NADH concentrations. In the solution containing 1 mM NADH, the highest sensitivity was obtained; the detection limit was as low as 0.08 μM (signal to noise, 5). The calibration curve showed a linear region up to 8 μM in this

condition. The further increment of the NADH concentration brought about an expansion of the linear range, while the sensitivity lowered a little. The reducing of the sensitivity with increase in the NADH concentration is attributable to the substrate inhibition of LDH and/or to the inhibition of the enzyme by some impurities in NADH.[14] The relative standard deviation was ca. 2% for 20 successive measurements of the electrode response (decrease in the electrode current at the steady state) at 1 μM L-lactate (NADH concentration, 1 mM).

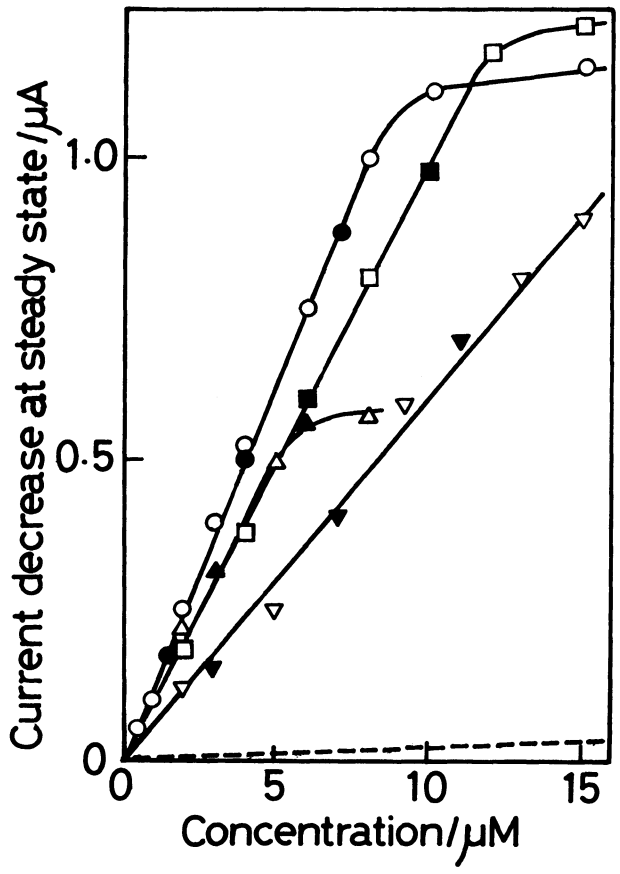

Fig. 3. Calibration curves for L-lactate (△, ○, □, ▽) and pyruvate (▲, ●, ■, ▼). NADH concentrations are 0.5 mM (△, ▲), 1 mM (○, ●), 2 mM (□, ■), and 6 mM (▽, ▼). Dashed line is the calibration graph for L-lactate in the solution without NADH.

Although the present catalytic enzyme electrode can determine neither L-lactate nor pyruvate separately, it is still useful for the estimation of L-lactate concentration in various samples such as food and serum because the concentration of pyruvate in such samples is usually very low compared with that of L-lactate. Here, the concentration of L-lactate in five kinds of soured milk were determined by the present method (NADH concentration, 1 mM) and by the conventional spectrophotometric method.[15] The results agreeded satisfactorily (correlation coefficient between the two methods, 0.97) for the 5 assays of L-lactate concentration over the range of 0.01 - 0.11 M. Only a sample volume less than a microliter was required in the present method. The separate determination of L-lactate and pyruvate can be performed, if necessary, by a simple pretreatment of a sample. For example, the preincubation of the sample with the enzyme LOD (decarboxylating, EC 1.13.12.4[9]) can eliminate L-lactate from the sample, and the measurements of the electrode response of the sample with or without the preincubation give both concentrations of L-lactate and pyruvate.

The long-term stability of the enzyme electrode was then examined; the determination of L-lactate (1 μM) was carried out 20 times a day and continued for two weeks (NADH concentration, 1 mM). The deviation of the average value for the electrode response in the 20 successive measurements was within 10% of the relative value for 10 days.

An entirely new method for getting a highly-sensitive enzyme electrode can be

proposed in this study. If the constitution of the catalytic enzyme electrode system were sophisticated, as has been carried out on the conventional enzyme electrodes,[16-18] higher sensitivity should be obtained. Such an investigation is now under progress.

We are grateful to Dr. Kunihiro Ichimura for providing the photosensitive polymer and to Dr. Kunio Matsumoto for providing LOD.

References

1) H. U. Bergmeyer, "Method of Enzymatic Analysis," ed by H. U. Bergmeyer, Verlag Chemie-Academic Press, New York and London (1974), Vol. 1, p. 131.
2) G. G. Guilbault and M. Nanjo, Anal. Chim. Acta, 78, 69 (1975).
3) I. Satoh, I. Karube, and S. Suzuki, Biotechnol. Bioeng., 18, 269 (1976).
4) I. Satoh, I. Karube, and S. Suzuki, Biotechnol. Bioeng., 19, 1095 (1977).
5) M. J. Jensen and G. A. Rechnitz, J. Membr. Sci., 5, 117 (1979).
6) F. Scheller and D. Pfeiffer, Anal. Chim. Acta, 117, 383 (1980).
7) R. Renneberg, D. Pfeiffer, and F. Scheller, Anal. Chim. Acta, 134, 359 (1982).
8) R. Renneberg and F. Scheller, "Chemical Sensors," ed by T. Seiyama, K. Fueki, J. Shiokawa, and S. Suzuki, Kodansha-Elsevier, Tokyo and Amsterdam (1983), p. 711.
9) The LOD from Pediococcus sp. without NADH oxidase activity was selected and used for the experiments. A commercially-available LOD (EC 1.13.12.4, Böhringer Manheim) cannot be used for the present enzyme electrode because it catalyzes the oxidative decarboxylation of L-lactate to produce acetate, CO_2, and H_2O.
10) K. Ichimura, U. S. Patent 4269941 (1981); K. Ichimura and S. Watanabe, J. Polym. Sci., Polym. Chem. Ed., 20, 1419 (1982).
11) F. Mizutani, K. Sasaki, Y. Shimura, and K. Tsuda, in Ref. 8, p. 644. The amount of entrapped LOD and LDH were 6 and 1 mg cm^{-2}, respectively.
12) F. Mizutani, K. Sasaki, and Y. Shimura, Anal. Chem., 55, 35 (1983).
13) R. J. Henry, N. Chiamori, O. J. Gloub, and S. Berkman, Am. J. Clin. Pathol., 34, 381 (1960).
14) H. Bergmeyer, in Ref. 1, p. 129.
15) F. Noll, Biochem. Z., 346, 41 (1966).
16) D. R. Thevenot, R. Sternberg, P. R. Coulet, J. Laurent, and G. Gautheron, Anal. Chem., 51, 96 (1979).
17) M. Koyama, Y. Sato, M. Aizawa, and S. Suzuki, Anal. Chim. Acta, 116, 307 (1980).
18) T. Yao, Anal. Chim. Acta, 148, 27 (1983).

(Received November 1, 1983)