CAPSULES notes and applications from BASi

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Determination of Lactate Concentrations Using a Lactate Dehydrogenase-Based Biosensor

Purpose

Although a large proportion of biosensor studies have been devoted to the determination of glucose, a number of other physiologically important molecules have also been studied, including lactate. Two enzymes have been used for lactate biosensors—lactate oxidase and lactate dehydrogenase. The principles of the operation of lactate oxidase biosensors is identical to that of glucose oxidase biosensors (1,2). This Capsule discusses the operation of lactate dehydrogenase biosensors.

Reference

Evaluation of a New Disposable Screen-Printed Sensor Strip for the Measurement of NADH and Its Modification to Produce a Lactate Biosensor Employing Microliter Volumes, S.D. Sprules, J.P. Hart, R. Pittson, and S.A. Wring, Electroanalysis 8 (1996) 539-543.

Method

A schematic diagram for the operation of a lactate dehydrogenase biosensor is shown in ***F1***. The enzyme reaction involves the oxidation of lactate to pyruvate using the NAD^+ cofactor of lactate dehydrogenase. In order for the catalytic reaction to continue, the reduced NADH that is generated by the enzyme reaction must be reoxidized. Ideally, NADH would be directly reoxidized at the electrode surface. However, NADH does not show such ideal behavior, as a large overpotential is typically required for this oxidation (in addition, oxidation products may include radicals that can dimerize and passivate the electrode surface). Therefore, indirect electron transfer based on Meldola Blue as the mediator was used in this study.

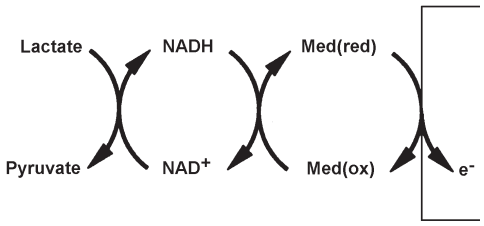

Figure 1. Scheme for the mediated enzymatic oxidation of lactate using lactate dehydrogenase.

There were two parts to this study. In the first, the indirect oxidation of NADH using the Meldola Blue mediator was characterized using a Meldola Blue-modified screen-printed carbon electrode. In the second part, this electrode was further modified by the addition of NAD^+ and lactate dehydrogenase, and the ability of this system to act as a biosensor for lactate was investigated.

Results

A crucial component of any biosensor based on a dehydrogenase enzyme is the method used to lower the overpotential observed for the NAD^+/NADH couple at bare electrode. In this study, the Meldola Blue mediator was combined with the carbon ink used for the screen-printed working electrode. The hydrodynamic voltammograms for NADH oxidation are shown in ***F2*** for unmodified carbon electrodes

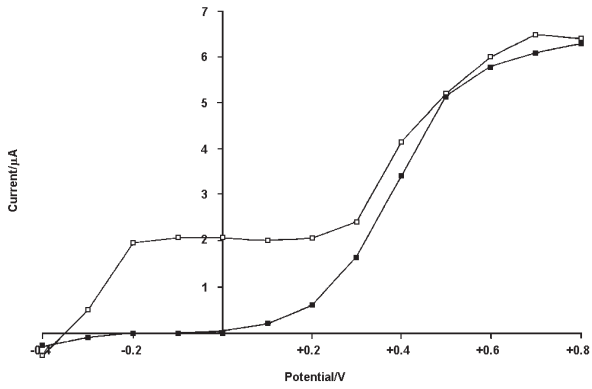

Figure 2. Hydrodynamic voltammograms for NADH (1 mM) in phosphate buffer at an unmodified carbon electrode (shaded squares) and a Meldola Blue modified carbon electrode (open squares). All potentials measured with reference to Ag/AgCl. Adapted with permission from primary reference. Copyright 1996 VCH.

(shaded squares) and for Meldola Blue modifed carbon electrodes (open squares). The current plateau for the modified electrode is attained at about 0.2 V (vs. Ag/AgCl), whereas for the unmodified electrode, there is no significant current at potentials more negative than zero; that is, there is a significant decrease in the overpotential required for the oxidation of NADH when Meldola blue is used as a mediator.

The effect of varying the concentration of NADH on the current response was measured using chrono-amperometry (i.e., the current response to a potential step was recorded). The current response to the potential step for a number of concentrations are shown in ***F3***, and the resulting calibration curve using currents measured 60 seconds after the potential step is shown in ***F4***.

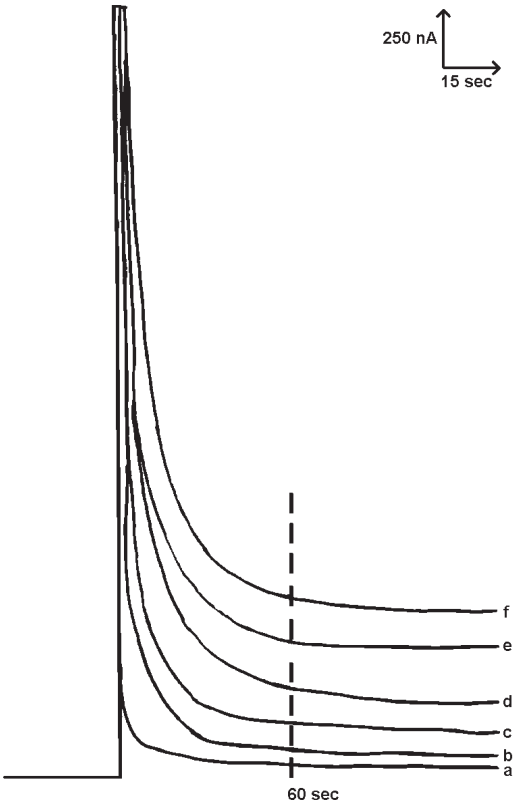

Figure 3. Current-time plots for chronoamperometric responses of NADH solutions in phosphate buffer. Concentration of NADH = 0 (a), 0.1 mM (b), 0.2 mM (c), 0.3 mM (d), 0.4 mM (e), and 0.5 mM (f). Adapted with permission from primary reference. Copyright 1996 VCH.

Since NADH is the product of enzymatic oxidation of lactate by lactate dehydrogenase and NAD^+, the Meldola Blue modifed carbon electrode can be used as a lactate biosensor once the materials required for the enzyme reaction (e.g., lactate dehydrogenase and

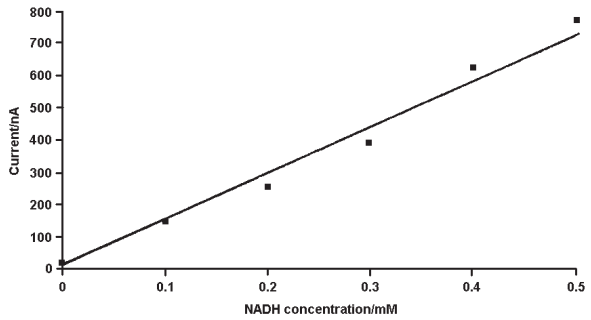

Figure 4. Calibration plot for the data shown in F3. Adapted with permission from primary reference. Copyright 1996 VCH.

NAD^+ cofactor) are included in the modified electrode. An incubation period of two minutes following the addition of lactate was allowed to allow the accumulation of NADH at the modified electrode, the concentration of which was then again measured using chronoamperometry. The calibration curves for bovine serum (open squares) and phosphate buffer (shaded squares) spiked with lactate are shown in ***F5***. The responses were approximately linear over the range 0 - 20 mM.

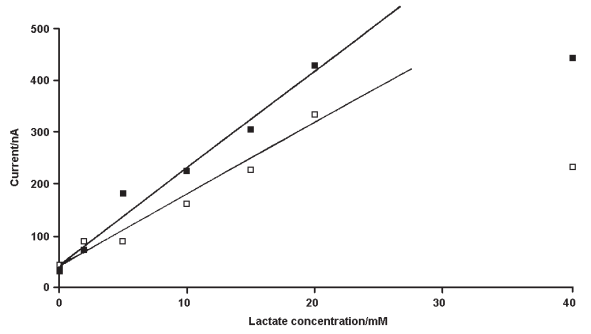

Figure 5. Calibration plots for lactate spiked buffer solution (shaded squares) and bovine serum (open squares) using the lactate dehydrogenase-based biosensor. Adapted with permission from primary reference. Copyright 1996 VCH.

References

1. A.W. Bott, Curr. Seps. 17 (1998) 25-31 and references therein.
2. Capsule 361