Fabrication of Novel Electrochemical Reduction Systems Using Alcohol Dehydrogenase as a Bifunctional Electrocatalyst

Ruo Yuan, Susumu Kuwabata, and Hiroshi Yoneyama*
Department of Applied Chemistry, Faculty of Engineering, Osaka University, Yamada-oka 2-1, Suita, Osaka 565

(Received October 25, 1995)

Electrochemical reduction of $NADP^+$ to NADPH and of NAD^+ to NADH with current efficiencies of more than 97% has been achieved at alcohol dehydrogenase (ALDH) in the presence of acetophenone as an electron mediator. Addition of acetone or acetaldehyde as a substrate to the above electrolytic system allowed reduction of the substrate to the corresponding alcohol at ALDH accompanied by oxidation of the resulting NAD(P)H.

The use of enzymes as electrocatalysts is of interest for the construction of electrochemical systems having high reactivities and high selectivities associated with the enzymes. We have previously reported results of electrochemical fixation of CO_2 in organic molecules,[1,2] reduction of CO_2 to methanol,[3] and reduction of ketones and aldehydes to the corresponding alcohols[4] using enzymes that catalyze *in-vivo* oxidation reactions, which are reverse to our electrochemical reduction reactions. A reaction scheme using $NADP^+$-dependent alcohol dehydrogenase, ALDH (EC 1.1.1.2), as an electrocatalyst to induce reduction of acetone to 2-propanol is shown in Figure 1 (A), as an example. The reduction of acetone takes place at $NADP^+$-dependent ALDH with the assistance of the reduced form of nicotinamide adenine dinucleotide phosphate, *i.e.* NADPH. Since NADPH is oxidized to $NADP^+$ on the reduction of acetone, it is necessary to regenerate NADPH. The most efficient way for the regeneration is to use another enzyme, ferredoxin-NADP-reductase (FNR),[5] as shown in the figure. The use of FNR needs an electron mediator of high reversibility such as methylviologen (MV^{2+}). NAD^+-dependent alcohol dehydrogenase, ALDH (EC 1.1.1.1), is a good electrocatalyst for producing primary alcohols from the corresponding aldehydes but needs the reduced form of nicotinamide adenine dinucleotide (NADH) in the reduction of aldehyde. To regenerate NADH, another enzyme of diaphorase is required in the electrolytic systems.[6]

In this communication, we report the achievement of reduction reactions at ALDH without using any other enzymes. Novel reaction systems in which reduction of acetone or acetaldehyde and regeneration of NADPH or NADH took place efficiently were fabricated by using only the enzyme ALDH as a bifunctional electrocatalyst. The reaction scheme for such reactions is shown in Figure 1 (B). Since there is no problem in the reduction of substrate(2) to product(2) at ALDH with the assistance of NAD(P)H,[4] a key in constructing the new reduction scheme lies in finding out a redox couple which works well as substrate(1) and product(1). It has been found that methylviologen does not work as such a redox agent. In case of using $NADP^+$-dependent ALDH, the enzyme catalyzes oxidation of secondary alcohols to the corresponding ketones in the presence of $NADP^+$. If the produced ketone can be directly reduced to the original alcohol at the cathode, the ketone/alcohol couple would work as the product(1)/substrate(1) couple shown in Figure 1 (B). Then, electrochemical reduction of several compounds having a carbonyl

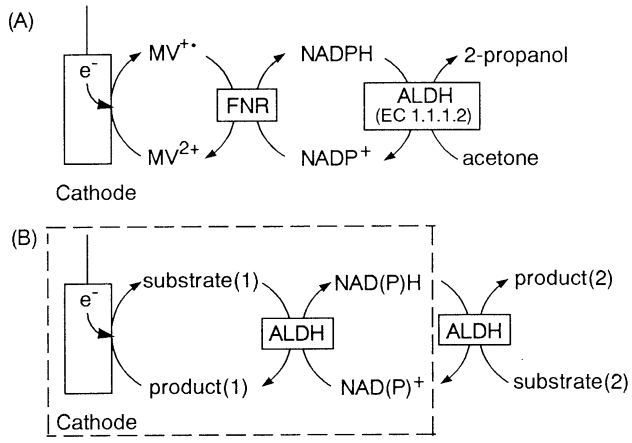

Figure 1. Electrochemical reduction system using ALDH and FNR (A) and that using ALDH alone as a bifunctional electrocatalyst (B).

group was investigated to find out candidates for the product(1). Cyclic voltammograms of acetophenone, benzophenone, and 2,3-butanedione taken in 0.2 mol dm^{-3} phosphate buffer (pH 7) gave definite reduction waves at around -0.8 V *vs.* Ag/AgCl, suggesting that these serve as promising candidates for the product(1). Then, electrolysis experiments were carried out to examine whether or not the reduction of product(1) to substrate(1) truly occurs in the presence of $NADP^+$ and $NADP^+$-dependent ALDH with the scheme given in a dotted frame in Figure 1 (B). The electrolysis was carried out using a two-compartment cell divided by a cation exchange membrane (Nafion 417, Aldrich). A glassy carbon plate having an exposed area of 1 cm^2 and a Pt foil of 3 cm^2 were used as a test and a counter electrode, respectively. An Ag/AgCl in KCl-saturated solution served as a reference electrode. $NADP^+$-dependent ALDH obtained from *Thermoanaerobium Brockii* was commercially available from SIGMA. The electrolyte solution used was 10 cm^3 of 0.2 mol dm^{-3} phosphate buffer (pH 7) containing 1 mmol dm^{-3} $NADP^+$, 50 units ALDH, and 4 mmol dm^{-3} of a product(1) tested. Since the product(1) used here has low solubility in aqueous solution, 2 (v/v)% of Triton X-100 was dissolved in the electrolyte solution as a solubilizing agent. The amount of NADPH produced was determined by measuring absorbance of the electrolyte solution at 360 nm where NADPH shows intense absorption but product(1), substrate(1) and $NADP^+$ do not.

It was found that NADPH was linearly produced with electrolysis time at -0.85 V *vs.* Ag/AgCl, as shown by curve (a) of Figure 2, indicating that acetophenone and its reduced form, *i.e.*, 1-phenylethanol, worked as the product(1) and the substrate(1), respectively. The current efficiency obtained by the electrolysis

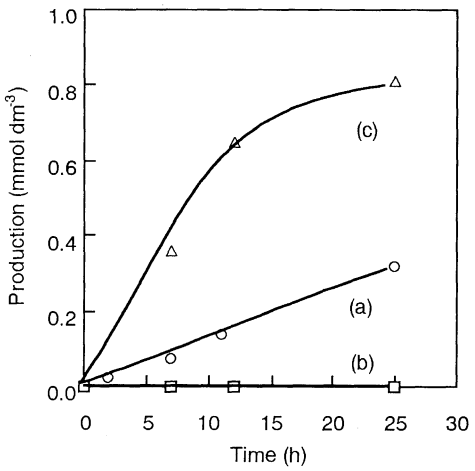

Figure 2. Electrochemical reduction of $NADP^+$ to NADPH at $NADP^+$-dependent ALDH (a), and that of NAD^+ to NADH at NAD^+-dependent ALDH obtained from *Bakers Yeast* (b) and obtained from *Equine Liver* (c). E = -0.85 V *vs.* Ag/AgCl.

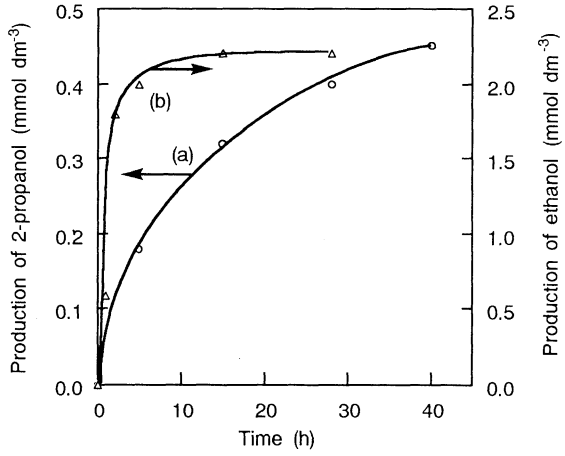

Figure 3. Electrochemical reduction of acetone to 2-propanol (a) and that of acetaldehyde to ethanol (b) using $NADP^+$-dependent ALDH and NAD^+-dependent ALDH as a bifunctional electrocatalyst, respectively, together with acetophenone as the product(1). E = -0.85 V *vs.* Ag/AgCl.

for 25 h was as high as 97%. However, if benzophenone or 2,3-butanedione was used, the amount by NADPH produced was less than one tenth of that obtained for the use of acetophenone. It was revealed by chemical oxidation of 1-phenylethanol, benzhydrol, and 3-hydroxy-2-butanone using $NADP^+$-dependent ALDH and $NADP^+$ that the differences in the reaction rate of the electrolysis was attributable to that of oxidation rate of substrate(1) at the enzyme.

NAD^+-dependent ALDH physiologically catalyzes oxidation of primary alcohols to aldehydes with NAD^+. Reflecting such substrate selectivity, the use of this enzyme and NAD^+ in place of $NADP^+$-dependent ALDH and $NADP^+$ was not useful in regeneration of NADH if NAD^+-dependent ALDH obtained from *Bakers Yeast* (SIGMA) was used in the benzophenone solution, as shown by curve (b) of Figure 1. However, contrary to the prediction based on such substrate selectivity of NAD^+-dependent ALDH, a significant activity appeared in reduction of NAD^+ if NAD^+-dependent ALDH obtained from *Equine Liver* was used. As shown in Figure 2, the production rate of NADH given by curve (c) is greater than that of NADPH given by curve (a). The results obtained here clearly show that this enzyme possesses a lower substrate selectivity than NAD^+-dependent ALDH obtained from *Bakers Yeast* and shows a relatively high activity for oxidation of secondary alcohols.[7]

Since it has been found that the couple acetophenone/1-phenylethanol work well as the product(1)/substrate(1) in Figure 1 (B) to electrochemically regenerate NADH and NADPH, attempts were made to reduce substrate(2) in the presence of ALDH, $NAD(P)^+$ and acetophenone. Acetone and acetaldehyde were chosen as the substrate(2). When the former was used, $NADP^+$-dependent ALDH was used as the enzyme, whereas reduction of acetaldehyde was attempted using NAD^+-dependent ALDH obtained from *Equine Lever*. The concentration of the substrate(2) was 2.2 mmol dm^{-3}, and the concentrations of $NAD(P)^+$ and acetophenone were 0.03 mmol dm^{-3} and 0.1 mmol dm^{-3}, respectively, which was lower than that of substrate(2). Products were analyzed by gas chromatography. The time course of production of 2-propanol from acetone and of ethanol from acetaldehyde are shown in Figure 3 by curve (a) and (b), respectively. If the same electrolysis was carried out in the absence of $NAD(P)^+$, negligible production of 2-propanol and ethanol was obtained (<0.008 mmol dm^{-3}), as expected. It is evident from these findings that 2-propanol and ethanol were produced by reduction reactions at ALDH where simultaneous oxidation of 1-phenylethanol produced at the cathode took place. NAD(P)H was regenerated with the reaction scheme shown in Figure 1 (B) during the course of reduction of acetone and acetaldehyde. The current efficiency for the production of 2-propanol and ethanol was 96% for electrolysis for 30 h and 92% for 4 h, respectively. It is recognized from the results shown in Figure 3 that the rate of ethanol production was much higher than that of 2-propanol. In the former case, electrolysis for 15 h achieved 100% of conversion from acetaldehyde to ethanol. The high reaction rate obtained might be due to the high production rate of NADPH compared to that of NADH as shown in Figure. 2. Experiments to determine the kinetics as well as the detailed mechanism of the electrolyses are underway to find out the optimal conditions.

This research was supported by Grant-in Aid for Scientific Research on Priority Area, No. 07215249 from Ministry of Education, Science, Sports, and Culture.

References

1 K. Sugimura, S. Kuwabata, and H. Yoneyama, *J. Am. Chem. Soc.*, **111**, 2361 (1989).
2 K. Sugimura, S. Kuwabata, and H. Yoneyama, *Bioelectrochem. Bioenerg.*, **24**, 241 (1990).
3 S. Kuwabata, R. Tsuda, and H. Yoneyama, *J. Am. Chem. Soc.*, **116**, 5437 (1994).
4 S. Kuwabata, K. Nishida, and H. Yoneyama, *Chem. Lett.*, **1994**, 407.
5 H.-C. Chang, T. Matsue, I. Uchida, and T. Osa, *Chem. Lett.*, **1989**, 1119.
6 M. Ito and T. Kuwana, *J. Electroanal. Chem.*, **32**, 415 (1971).
7 K. Dalziel and F. M. Dickinson, *Biochem. J.*, **100**, 34 (1966).