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Electrochemical Reduction of Formaldehyde, Acetaldehyde, and Acetone to The Corresponding Alcohols Using Alcohol Dehydrogenase as an Electrocatalyst

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Electrochemical reduction of formaldehyde to methanol, acetaldehyde to ethanol, and acetone to 2-propanol has been achieved using either one of the two kinds of alcohol dehydrogenases, ADH(EC1.1.1.1) and ADH(EC1.1.1.2), as an electrocatalyst. ADH(EC1.1.1.1) was effective in reducing formaldehyde at a relatively high reaction rate, while ADH(EC1.1.1.2) worked well for the reduction of acetaldehyde and acetone. In all cases the current efficiency and the production yield attained to *ca*. 100%.

Enzymes possess high catalytic activities and high reaction selectivities *in-vivo* organic reactions. Using these properties as biocatalysts, numerous organic syntheses have been attempted.1) However, few investigations have been done by electroorganic syntheses using enzymes as electrocatalysts. In this paper, we would like to report the achievement of highly selective electrochemical reductions of formate, acetaldehyde, and acetone to the corresponding alcohols using enzymes as electrocatalysts.

We have already reported the utility of enzymes for the electrochemical fixation of carbon dioxide in organic substrates such as α-oxoglutaric acid2) and pyruvic acid,3) and the electrochemical reduction of carbon dioxide to formate, and of formate to methanol.4) In these reactions, isocitrate dehydrogenase, malic enzyme, and methanol dehydrogenase(EC1.1.99.8) worked well as the electrocatalysts in the respective reactions. These reactions are the reverse reactions to the spontaneous reactions that the enzymes catalyze.

In the present study, we have used enzymes of high reaction selectivities. It is well known that alcohol dehydrogenase, ADH(EC1.1.1.1) is the enzyme that catalyzes *in-vivo* oxidation of primary alcohols to the corresponding aldehyde with the assistance of nicotinamide adenine dinucleotide (NAD^+) as an electron acceptor.5,6) ADH(EC1.1.1.2) catalyzes oxidation of secondary alcohols to the corresponding ketones with nicotinamide adenine dinucleotide phosphate ($NADP^+$).7) It is expected that the reverse reactions of these spontaneous oxidation reactions occur as the equilibrium reactions as shown in Fig. 1. In fact, it has been reported that ADH(EC1.1.1.2) has catalytic activity for the reduction of acetone,6) and both enzymes can catalyze the reduction of acetaldehyde,6,7) though the reduction of formaldehyde using both enzymes has not yet been reported. Our approach was first to compare enzymatic activities of the above mentioned two kinds of ADHs for the three kinds of the reverse reactions under the same conditions, and then to construct electrochemical reaction systems that allow continuous production of the reduction products. To achieve the continuous production, NADH and NADPH must be regenerated. The generation of NADH from NAD^+ can be achieved electrochemically at diaphorase using methylviologen (MV^{2+}) as an electron mediator.[8] Similarly, NADPH is generated from $NADP^+$ at ferredoxin-$NADP^+$-reductase (FNR).[9] Then, if we have succeeded in obtaining the reduction products by chemical reactions as shown in Fig. 1, that is, methanol, ethanol, and 2-propanol, we can construct the reaction schemes that allow continuous production of these substances, as shown in Fig. 2.

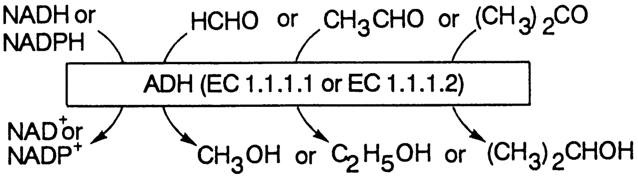

Fig. 1. Chemical reduction of formaldehyde, acetaldehyde, and acetone using ADH (EC 1.1.1.1 or EC 1.1.1.2).

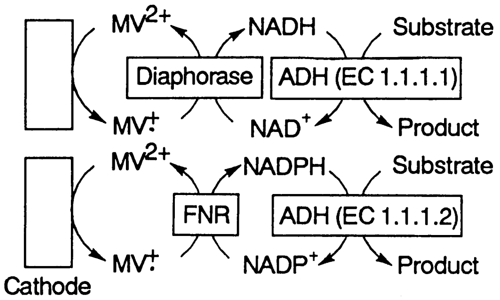

Fig. 2. Electrochemical reduction systems using ADH (EC 1.1.1.1) and ADH (EC 1.1.1.2) as electrocatalyst.

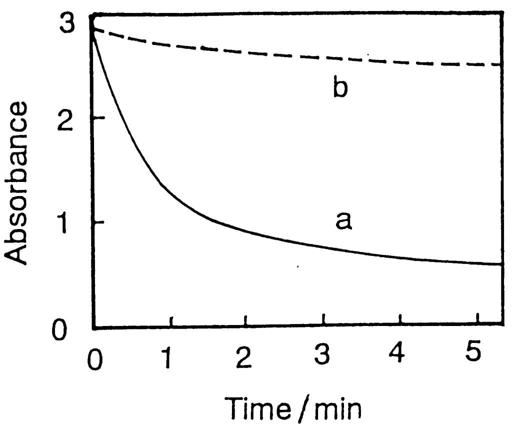

Fig. 3. Changes in absorbance at 360 nm of 5 cm^3 of 0.5 mol dm^{-3} phosphate buffer solution containing 10 mmol dm^{-3}formaldehyde and 1 mmol dm^{-3} NADH (a) or NADPH (b) caused by addition of 100 units of ADH(EC1.1.1.1) (a) or ADH(EC1.1.1.2) (b).

ADH(EC1.1.1.2) obtained from *thermoanaerobium brockii* and FNR were commercially available from SIGMA. ADH(EC1.1.1.1) obtained from *bakers yeast* and other biochemical reagents were purchased from Oriental Yeast Co., Ltd. The enzymatic activities of ADH(EC1.1.1.1) and ADH(EC1.1.1.2) for the reduction of formaldehyde, acetaldehyde, and acetone were examined by chemical reactions with the use of NADH for the former enzyme and NADPH for the latter as an reducing agent. 5 cm^3 of 0.5 mol dm^{-3} phosphate buffer solution(pH 7.0) containing 1 mmol dm^{-3} either NADH or NADPH and one of the above mentioned substrates in 10 mmol dm^{-3} was prepared, and the reaction was initiated by addition of 100 units of the appropriate ADH. The chemical reaction was spectrophotometrically monitored at 360 nm where NAD^+ and $NADP^+$ show intense absorption but their reduced form, NADH and NADPH, do not. Figure 3 shows results obtained for formaldehyde. Apparently the oxidation of NADH to NAD^+ occurred with a rate faster than that of NADPH to $NADP^+$, suggesting that ADH(EC1.1.1.1) has a higher catalytic activity for the reduction of formaldehyde than ADH(EC1.1.1.2). To our knowledge, no paper has been published concerning the reduction of formaldehyde catalyzed by ADH(EC 1.1.1.1). Since this reaction occurs with second orders with respect to the substrate and the reducing agent, the amount of decrease in the concentration (C_d) of both substrate and reducing agent with the reaction time can be formulated by[10]

$$[1/(C_s^o - C_r^o)]\ln[(C_r^o/C_s^o)(C_s^o - C_d)/(C_r^o - C_d)] = kt, \qquad (1)$$

where C_s^o and C_r^o denote the initial concentration of the substrate (= 10 mmol dm^{-3}) and the reducing agent (= 1 mmol dm^{-3}), respectively, and k and t are the rate constant of the reaction and the reaction time, respectively. Application of the results shown in Fig. 1 to eq. (1) gave a good linear relation with the correlation coefficient more than 0.998 between the left hand side given in eq. (1) and the reaction time, from which the rate constants of the reactions were obtained. The rate constants of all combinations of the enzymes and the substrates obtained are given in Table 1. The ADH(EC 1.1.1.1) showed the highest activity for the reduction of acetaldehyde but no activity for acetone. The observed activities for the reduction of the three kinds of substrates are in good accordance with the tendency of alcohol oxidations catalyzed by this enzyme; the enzyme has a higher activity for oxidation of primary alcohols than that of secondary alcohols.[6)] In contrast, it was found that the activity of ADH (EC 1.1.1.2) was the highest for the reduction of acetone and it decreased in the order of acetaldehyde and formaldehyde.

Table 1. Rate constants of chemical reduction of formaldehyde, acetaldehyde, and acetone catalyzed by ADH

Enzyme	Rate constant (mol^{-1} dm^3 s^{-1})		
Substrate/	Formaldehyde	Acetaldehyde	Acetone
ADH(EC1.1.1.1)[a)]	1.73	54.6	0
ADH(EC1.1.1.2)[b)]	0.0470	1.61	2.32

a) NADH was used as a reducing agent.
b) NADPH was used as a reducing agent.

Based on the results shown above for chemical reduction reactions, electrochemical reduction of the substrates was attempted using the reaction scheme given in Fig. 2. All electrolysis experiments were carried out using a two compartment cell separated by a cation exchange membrane (Nafion 117, Aldrich). A glassy carbon plate having 1 cm^2 of exposed area and a Pt foil of 3 cm^2 were used as the test and the counter electrode, respectively. The electrolyte solution in the test electrode compartment contained methylviologen (MV^{2+}) as an electron relay, either ADH(EC 1.1.1.1) or ADH(EC 1.1.1.2), the corresponding cofactor (NAD^+ or $NADP^+$), diaphorase or FNR depending on the kind of cofactor used, and one of the substrates, and was magnetically stirred during the course of electrolysis. The amount of products was determined by gas chromatography. The current efficiency was evaluated based on the net electrolysis charges involved in the reduction of the substrate, which was obtained by subtracting the theoretical charges required for the reduction of all MV^{2+} in the electrolyte solution from the total electrolysis charges.[3-5)]

Figure 4 shows the time course of methanol production from formaldehyde obtained by the electrolysis at −0.7 V *vs.* SCE in the presence of ADH (EC 1.1.1.1), NAD^+, and MV^{2+}. The production of methanol occurred from the beginning of the electrolysis and its amount increased linearly with the electrolysis time for the initial 6 h, beyond which the production rate decreased with a decrease in the concentration of formaldehyde. As shown in this figure, the electrolysis for 16 h resulted in the production of methanol of 50 μmol which gives the production yield of 100%. The current efficiency obtained in this case was as high as 98%. The experiments have been carried out for the three kinds of substrates with the use of the two kinds of enzymes. The results are summarized in Table 2. Except for the reduction of acetone using ADH (EC 1.1.1.1) where no product was obtained, other electrolyses gave a linear increase in the respective product with the electrolysis time for initial few hours,

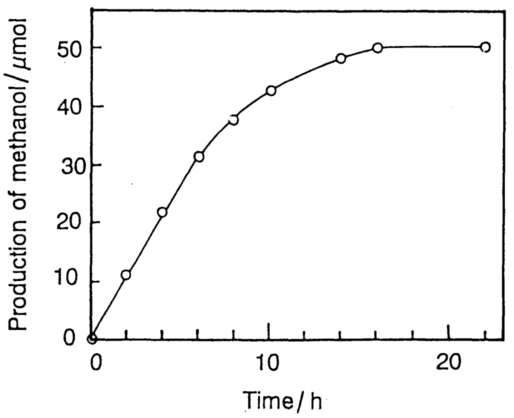

Fig. 4. Time course of methanol production by the electrolysis at -0.7 V *vs.* SCE of 5 cm^3 of 0.5 mol dm^{-3} phosphate buffer solution containing 10 mmol dm^{-3} formaldehyde, 1 mmol dm^{-3} MV^{2+}, 1 mmol dm^{-3} NAD^+, 500 units of diaphorase and 100 units of ADH(EC 1.1.1.1).

allowing the determination of their production rates. The current efficiency obtained for each electrolysis is also given in this table. In the cases where the reduction product was obtained, the current efficiency more than 98% was obtained in the all cases regardless of the differences in the production rates, indicating that the high reaction selectivity of the enzymes was kept even under the electrolysis conditions. The current efficiencies obtained were reproducible within experimental errors of ±2%. Besides the reduction of formaldehyde using ADH(EC 1.1.1.1), the reduction of formaldehyde, acetaldehyde and acetone using ADH(EC1.1.1.2) also gave the production yield of 100% by the electrolyses for 30, 6, and 14 h, respectively, with high reproducibility. In the case of the reduction of acetaldehyde using ADH(EC1.1.1.1), the amount of ethanol as a product increased linearly for 70 h, giving the production yield of 76%. These results suggest that the enzymes used were stable under electrolysis conditions for at least few days. By comparing the production rates shown in Table 2 with the rate constants of the chemical reactions shown in Table 1 for the reduction of formaldehyde and acetone, it was found that good correlations are seen between the two, suggesting that the reduction of the substrates at ADH determines the reaction rate in these electrolyses and the generation of NADH or NADPH at diaphorase or FNR does not. However, disagreement is seen for the reduction behaviors of acetaldehyde; ADH(EC 1.1.1.1) that gave the highest rate of the chemical reduction (see Table 1) gave a very low reduction rate in the electrolysis, while ADH(EC 1.1.1.2) that gave the rate of chemical reduction lower than that obtained for the chemical reduction of acetone showed the highest electrochemical reduction. These results suggest that the presence of MV^{2+} and/or its reduced species influenced the reduction of acetaldehyde at both kinds of ADHs, and it is under investigation now.

Table 2. Electrochemical reduction rate (ν) and current efficiency (η) obtained for formaldehyde, acetaldehyde, and acetone with the use of ADH as electrocatalysts[a)]

Enzyme number	ν (μmol h^{-1}) and η (%)					
Substrate/	Formaldehyde		Acetaldehyde		Acetone	
	ν	η	ν	η	ν	η
EC1.1.1.1[b)]	3.9	98	0.6	98	0	-
EC1.1.1.2[c)]	1.7	99	15.2	100	4.3	99

a) Electrolysis was carried out at -0.7 V *vs.* SCE.
b) NAD^+ and FNR were dissolved in the electrolyte solution.
c) $NADP^+$ and diaphorase were dissolved in the electrolyte solution.

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