# Asymmetric Electroreduction of Ketone and Aldehyde Derivatives to the Corresponding Alcohols Using Alcohol Dehydrogenase as an Electrocatalyst

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Asymmetric electroreduction of ketone and aldehyde derivatives was examined for two electrochemical reduction systems using alcohol dehydrogenase (ADH) as an electrocatalyst. The reaction system A is concerned with reduction of substrates catalyzed by ADH coupled with regeneration of cofactors by another enzyme with assistance of methyl viologen as an electron mediator, and the reaction system B is concerned with the use of ADH as the sole enzyme which catalyzes both reduction of substrates and regeneration of cofactors. In the latter case, a redox couple of phenethyl alcohol/acetophenone is used as an electron mediator to induce the reaction. The electrolysis using the system A allowed asymmetric reduction of acetophenone, propiophenone, phenoxy-2-propanone, pyruvic acid, and 2-phenylpropionaldehyde to the corresponding optically active alcohols with the enantiomer excesses (ee) close to 100% and the current efficiencies larger than 92%, and the turnover number of the cofactor higher than 50 was obtained for electrochemical reduction of phenoxy-2-propanone for 30 h. The reaction system B gave 100% ee for reduction of propiophenone, phenoxy-2-propanone, and pyruvic acid. However, the amount of products obtained was very small for reduction of benzoylformic acid, and a low enantiomer excess was obtained for reduction of phenylpropionaldehyde. Discussion is made focusing on what substrates are suitable for asymmetric reduction induced by the reaction system B.

## Introduction

Enzymes catalyze almost all *in-vivo* reactions with extremely high selectivity. Many kinds of enzymes are commercially available nowadays, and their utilization as catalysts for *in-vitro* reactions has attracted considerable attention in relation to practical organic syntheses.[1−9] In the field of electrochemistry, high selectivity that enzymes possess has been widely utilized in electroorganic syntheses[10−15] and amperometric biosensors.[16−25] The electrochemical techniques are also useful to investigate mechanisms and kinetics of the enzymatic reactions.[26−29] We have developed some kinds of electrochemical reaction systems using enzymes as electrocatalysts, such as reductive fixation of carbon dioxide into organic molecules such as α-oxoglutaric acid[30] and pyruvic acid[31] and electrochemical reduction of carbon dioxide to methanol.[32,33] Furthermore, attempts have been made to use alcohol dehydrogenase (ADH), which is known as a stable enzyme, as an electrocatalyst to induce electrochemical conversion of ketone and aldehyde derivatives to the corresponding alcohols.[34,35] Recently, synthesis of chiral compounds is gaining popularity, and electrochemi-

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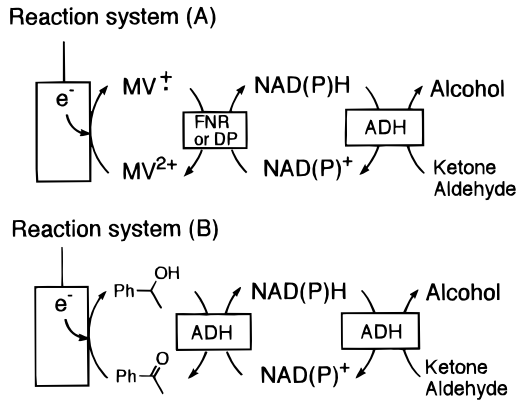


**Figure 1.** Two electrochemical reduction systems using alcohol dehydrogenase as an electrocatalyst.

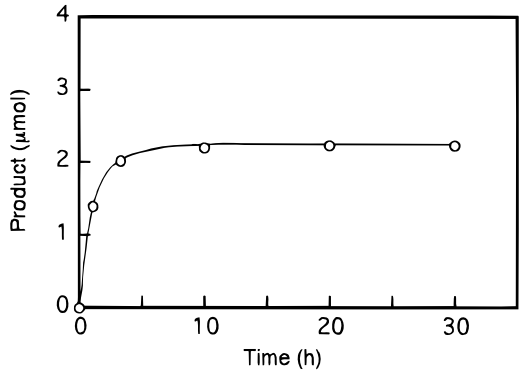


**Figure 2.** Time course of production of (*S*)-1-phenoxy-2-propanol obtained by adding 3 mmol $dm^{-3}$ NADPH to 2 mL of phosphate buffer (pH 7.1) containing 2.0 units of ADH (EC 1.1.1.2), 3.0 mmol $dm^{-3}$ phenoxy-2-propanone, and 3 vol % *tert*-butyl alcohol which served as a solubilizing agent for the substrate and product.

cal synthesis using enzymes as the electrocatalysts may provide one promising route to achieve this.[36] As an extension of our studies, asymmetric reduction of ketone and aldehyde derivatives has been investigated using ADH as the electrocatalyst.

Alcohol dehydrogenases which belong to EC 1.1.1.1 and EC 1.1.1.2 catalyze *in-vivo* oxidation of primary alcohols and secondary alcohols with assistance of nicotinamide adenine dinucleotide ($NAD^+$) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) as electron acceptors, respectively. Since those reactions catalyzed by ADH proceed under equilibrium conditions, it is expected that their reverse reactions also occur in the presence of a sufficient amount of the reduced forms of cofactors, *i.e.*, NADH and NADPH. This implies that the reduction reactions catalyzed by ADH can be utilized for organic syntheses by using NADH or NADPH, which will be denoted here as NAD(P)H, as a reducing agent. Since it is undesirable to consume the stoichiometric or a greater amount of expensive cofactors, several attempts have been made to regenerate NAD(P)H by chemical[6,37–40] and electrochemical[24,25,41–43] means. In our previous studies,[34,35] two kinds of electrochemical reaction systems, as schematically shown in Figure 1, were fabricated in order to induce the reduction reactions catalyzed by ADH with regeneration of NAD(P)H. In the reaction system A of Figure 1, NAD(P)H is regenerated from $NAD(P)^+$ using either ferredoxin−$NADP^+$ reductase (FNR) for NADPH or diaphorase (DP) for NADH. Electrochemical communication between the electrode and FNR (or DP) is achieved using methyl viologen ($MV^{2+}$) as an electron mediator.[42,43] The other method of the system B shown in Figure 1 uses ADH as the sole electrocatalyst which catalyzes both regeneration of NAD(P)H and reduction of substrates. As mentioned above, ADH originally catalyzes oxidation of alcohol which is accompanied by reduction of the cofactors. We found that phenethyl alcohol is oxidized by ADH accompanied by reduction of $NADP^+$ to NADPH and its oxidation product, acetophenone, is reduced electrochemically at a glassy carbon cathode. By combining this electrochemical regeneration reaction of NAD(P)H with the reduction of substrates catalyzed by ADH, the system B shown in Figure 1 is constructed.

The purpose of the present study is to examine asymmetric electroorganic syntheses using the reaction systems shown in Figure 1. As will be shown below, both systems were found to work well for inducing the asymmetric electroreduction of several ketone and aldehyde derivatives, reflecting the high enantioselectivity of the enzyme used.

## Results and Discussion

**Electrochemical Reduction of Ketone and Aldehyde Derivatives Using the Reaction System A.** Chemical reduction of phenoxy-2-propanone caused by NADPH as a reducing agent in the presence of ADH (EC 1.1.1.2) as a catalyst was investigated by determining the amount of (*S*)-1-phenoxy-2-propanol as a function of time after addition of NADPH to a phenyl-2-propanone solution. As shown in Figure 2, the product of *S*-configuration alone increased with the reaction time up to the initial 10 h where its amount was saturated, whereas (*R*)-1-phenyl-2-propanol was not obtained, giving the enantiomer excess of 100% for asymmetric reduction of phenoxy-2-propanone to (*S*)-1-phenoxy-2-propanol, which is one of the starting materials for synthesis of various optically active compounds.[44] Since the quantitative analysis adopted here allowed determination in a precision of 0.01 $\mu$mol or less of the experimental error, the enantiomer excess is said to have been obtained with the error smaller than 0.5%. If another 3.0 mmol $dm^{-3}$ substrate was added to the solution when the production of (*S*)-1-phenoxy-2-propanol came to a halt, the reduction product began to increase again. Therefore, the saturation of the production shown in Figure 2 seemed not due to the decrease in the enzyme activity but to the attainment of the reaction equilibrium which is given by

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$$C_6H_5OCH_2C(O)CH_3 + NHDPH + H^+ \underset{K_{eq}}{\overset{(ADH)}{\rightleftharpoons}} C_6H_5OCH_2CH(OH)CH_3 + NADP^+$$

$$K_{eq} = \frac{[\text{product}][NADP^+]}{[\text{substrate}][NADPH][H^+]} \quad (1)$$

where $K_{eq}$ is the equilibrium constant. The concentration of phenoxy-2-propanone, (*S*)-phenoxy-2-propanol, NADPH, and $NADP^+$ obtained under equilibrium of the reaction, which was attained at a reaction time of 30 h, allowed the determination of $K_{eq}$ of $3.98 \times 10^6$ mol$^{-1}$ dm$^3$ at pH 7.1, from which it is known that if 90% conversion of the reaction substrate to the product is required, then NADPH is used as the reducing agent in an amount of about 25 times that of the reaction substrate.

The conversion of phenoxy-2-propanone to 1-phenoxy-2-propanol can also be made by direct electrolysis.[45] When 10 mL of phosphate buffer (pH 7.1) containing 3 mmol dm$^{-3}$ phenoxy-2-propanone and 3 vol % *tert*-butyl alcohol was used as an electrolyte solution, racemic 1-phenoxy-2-propanol was produced by electrolysis using a glassy carbon electrode polarized at −1.0 V vs Ag/AgCl. The amount of the product and its current efficiency obtained by the electrolysis for 30 h were 1.8 $\mu$mol and 96%, respectively, indicating that almost no side reactions took place under the experimental conditions used. It was confirmed that no reduction of this substrate took place if the applied potential was shifted to −0.9 V vs Ag/AgCl. In contrast, the electrochemical reduction of phenoxy-2-propanone using the reaction system A shown in Figure 1 gave different results as shown in Figure 3. The applied potential chosen was −0.65 V vs Ag/AgCl which was negative enough to reduce $MV^{2+}$ but too positive to induce the direct reduction of the substrate as mentioned above. As given in Figure 3, (*S*)-1-phenoxy-2-propanol was selectively produced with 100% ee, and the current efficiency obtained at 30 h was also 100%, indicating that the reaction selectivity of ADH was completely preserved in the electrolysis system. It is noteworthy that a conversion yield greater than 50% was obtained by electrolysis for 40 h, although the amount of $NADP^+$ used was one-thirtieth of that of the reaction substrate. The electrolysis of phenoxy-2-propanone was made under the same electrolysis conditions except for changing the concentration of $NADP^+$ from 0.1 to 0.05 and 0.02 mmol dm$^{-3}$. The production rate of (*S*)-1-phenoxy-2-propanol was not varied by changing the concentration of $NADP^+$ from 0.1 to 0.05 mmol dm$^{-3}$, but a little decrease in the reaction rate was observed when the $NADP^+$ concentration was reduced to 0.02 mmol dm$^{-3}$, suggesting that the rate-determining step of the reaction was changed from the reduction of the reaction substrate by ADH to the generation of NADPH by FNR when the concentration of $NADP^+$ dropped from 0.05 to 0.02 mmol dm$^{-3}$. Figure 4 shows the comparison of the turnover numbers of $NADP^+$ and the enantiomer excesses obtained by the electrolysis for 30 h. Since decrease in the reaction rate was small even if the concentration of $NADP^+$ was reduced to 0.02 mol dm$^{-3}$, the turnover number of $NADP^+$ increased to as high as 50 in that concentration without any appreciable change in the enantiomer excess.

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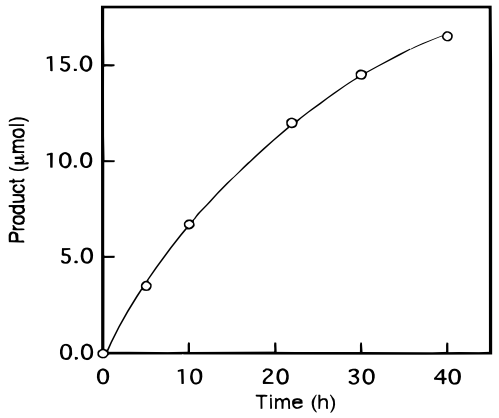



**Figure 3.** Production of (*S*)-1-phenoxy-2-propanol by electrochemical reduction at −0.65 V vs Ag/AgCl of 10 mL of phosphate buffer (pH 7.1) containing 3.0 mmol dm$^{-3}$ phenoxy-2-propanone, 10 units of ADH (EC 1.1.1.2), 0.5 unit of FNR, 0.1 mmol dm$^{-3}$ $NADP^+$, 0.1 mmol dm$^{-3}$ $MV^{2+}$, and 3.0 vol % *tert*-butyl alcohol.

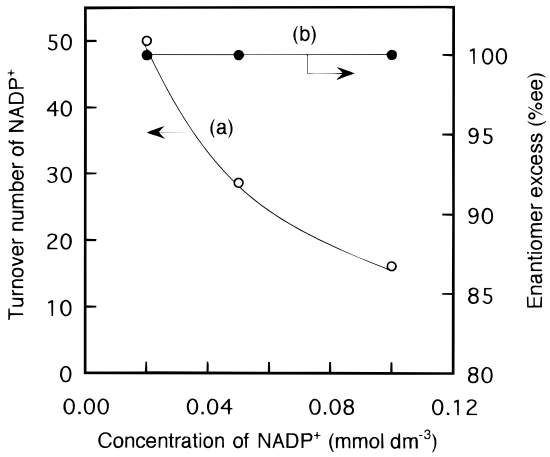
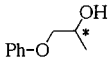
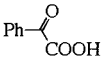
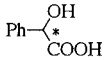
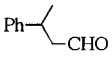
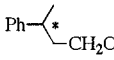



**Figure 4.** Plots of (a) turnover number of $NADP^+$ and (b) enantiomer excess obtained by the electrochemical reduction of phenoxy-2-propanone to (*S*)-1-phenoxy-2-propanol for 30 h using the reaction system A shown in Figure 1 as a function of concentration of $NADP^+$ in the electrolyte solution. The electrolysis conditions were the same as those given in the figure caption of Figure 3 except for the concentration of $NADP^+$.

The electrochemical reduction of several ketone and aldehyde derivatives was attempted using the reaction system A shown in Figure 1, and the results obtained by the electrolysis for 30 h are summarized in Table 1. This table also includes results obtained by the electrolysis system B, which will be explained in the next section. The expected products were obtained with the current efficiencies ranging from 89% to 100%, suggesting that the reaction selectivity of ADH was maintained for all cases. Attempts were made to determine byproducts by HPLC analyses when the current efficiencies were smaller than 100%, but no definite product was obtained. The enantiomer excess greater than 98% was obtained for the products from acetophenone, propiophenone, phenoxy-2-propanone, pyruvic acid, and 2-phenylpropionaldehyde, whereas the reduction of benzoylformic acid and 3-phenylbutyl aldehyde gave racemic products.

The stereochemical rule of the reactions catalyzed by ADH was investigated in detail using ADH (EC 1.1.1.1) from Bakers yeast and equine liver,[46] and the proposed rule can be schematically shown in Figure 5 where $S_S$

**Table 1. Electroreduction of Ketone and Aldehyde Derivatives with Use of the Electrochemical Reaction Systems Shown in Figure 1[a]**

| Substrate | Product | ADH[b] enzyme No. | Reaction system (A)[c] | | | Reaction system (B)[d] | | |
|---|---|---|---|---|---|---|---|---|
| | | | Configuration and enantiomer excess (%ee) | Amount of product (μmol) | Current efficiency (%) | Configuration and enantiomer excess (%ee) | Amount of product (μmol) | Current efficiency (%) |
|  |  | 1.1.1.2 | *R* / 98 | 18.2 | 92 | | | |
|  |  | 1.1.1.2 | *R* / 100 | 12.5 | 97 | *R* / 100 | 3.4 | 91 |
|  |  | 1.1.1.2 | *S* / 100 | 14.5 | 100 | *S* / 100 | 2.1 | 83 |
|  |  | 1.1.1.2 | *R* / 100 | 8.3 | 89 | *R* / 100 | 3.6 | 86 |
|  |  | 1.1.1.2 | *RS* / ~ 0 | 5.5 | 93 | *RS* / ~ 0 | > 0.3 | 76 |
|  |  | 1.1.1.1 | *S* / 98 | 9.6 | 96 | *RS* / ~ 0 | 4.7 | 90 |
|  |  | 1.1.1.1 | *RS* / ~ 0 | 3.2 | 100 | | | |

[a] The electrolysis time was 30 h. [b] ADH (EC 1.1.1.2) was obtained from *T. brockii*, and ADH (EC 1.1.1.1) was from equine liver. [c] The electrolyte solution used was 10 mL of phosphate buffer (pH 7.1) containing 3.0 mmol $dm^{-3}$ reaction substrate, 10 units of ADH, 0.1 mmol $dm^{-3}$ $NAD(P)^+$, 0.1 mmol $dm^{-3}$ $MV^{2+}$, 3.0 vol % *tert*-butyl alcohol and 0.5 unit of FNR or 10 units of DP. In the case of the electrolysis using ADH (EC 1.1.1.2), $NADP^+$ and FNR were dissolved in the electrolyte solution, whereas $NAD^+$ and DP were used for the electrolysis using ADH (EC 1.1.1.1). Applied potential chosen was −0.65 V vs Ag/AgCl. [d] The electrolyte solution used was 10 mL of phosphate buffer (pH 7.1) containing 3.0 mmol $dm^{-3}$ reaction substrate, 0.1 mmol $dm^{-3}$ acetophenone, 20 units of ADH, 0.1 mmol $dm^{-3}$ $NAD(P)^+$, and 3 vol % *tert*-butyl alcohol. Applied potential chosen was −0.85 V vs Ag/AgCl.

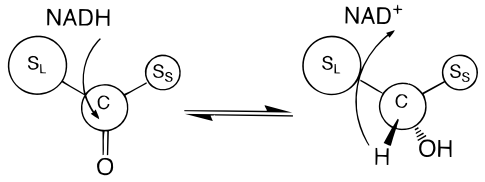



**Figure 5.** Schematic illustration of the stereoselective rule proposed for oxidation and reduction reactions catalyzed by ADH (EC 1.1.1.1) obtained from Bakers yeast and equine liver.[46]

and $S_L$ denote substituents of a carbonyl compound whose sterical size is $S_L > S_S$. If $S_S$, oxygen, and $S_L$ of the carbonyl compound are depicted clockwise around the carbon of the carbonyl group as shown in Figure 5, hydride transfer from NADH to the carbonyl group and its reverse reaction take place on the front side of the molecule. Let $S_L$ and $S_S$ be a phenyl group and a short alkyl group, respectively. If this ketone is reduced according to the rule shown in Figure 5, the resulting alcohol must have *S*-configuration. In contrast, if ADH obtained from *Thermoanaerobium brockii* was used, the reduction of acetophenone, propiophenone, and pyruvic acid yielded the corresponding alcohols of *R*-configuration as given in Table 1, suggesting that the reaction catalyzed by this enzyme obeys the reverse of the stereochemical rule observed for other kinds of ADH enzymes. The similar results were obtained by other investigators for chemical reduction of 2-pentanone using ADH obtained from *T. brockii* and NADPH as a reducing agent,[47] where (*R*)-2-pentanol was obtained as a main product. When phenoxy-2-propanone is reduced to (*S*)-1-phenoxy-2-propanol, ADH must recognize the phenoxy group as $S_L$ to catalyze its enantioselective reduction, but the Cahn−Ingold−Prelog priority sequence of phenoxy group > OH > $CH_3$ favors the *S*-configuration of the *R*/*S* convention of the resulting product. As in the case of the electrochemical reduction of benzoylformic acid, the chemical reduction of benzoylformic acid using ADH and NADPH gave racemic mandelic acid, suggesting that it is difficult for ADH (EC 1.1.1.2) from *T. brockii* to recognize the difference in the sterical sizes between phenyl and carboxylic groups.

Both 2-phenylpropionaldehyde and its reduction product possess the chiral center. The electrolysis experiment of racemic 2-phenylpropionaldehyde in the presence of ADH (EC 1.1.1.1) obtained from equine liver, DP, $NAD^+$, and $MV^{2+}$ gave selective production of (*S*)-2-phenyl-1-propanol. As well known, 2-phenylpropionaldehyde exhibits structure changes in aqueous solution due to keto−enol tautomerism given by


If the enzyme recognizes selectively the C=C in the enol form instead of C=O of the formyl group as double bonds to be hydrated and the phenyl group and methyl group as $S_L$ and $S_S$, respectively, then the hydride transfer with the rule shown in Figure 5 gives the product of *S*-configuration, in good accordance with the electrolysis result given in Table 1. Therefore, it can be understood why no asymmetric reaction took place for the reduction of 3-phenylbutyl aldehyde which does not exhibit the keto−enol tautomerism.

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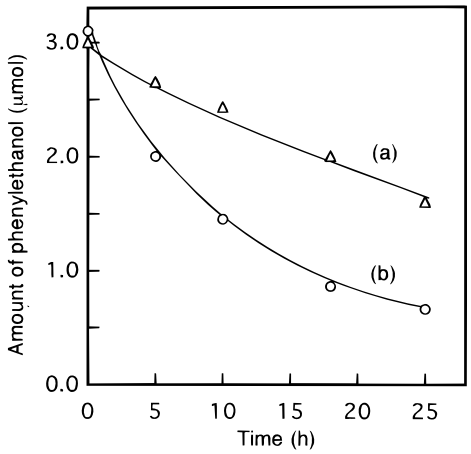


**Figure 6.** Time course of the decrease in the amount of phenylethanol of (a) *S*-configuration and (b) *R*-configuration caused by adding 3 mmol $dm^{-3}$ $NADP^+$ to 2 mL of phosphate buffer (pH 7.1) containing 2 units of ADH (EC 1.1.1.2), 3 mmol $dm^{-3}$ racemic phenethyl alcohol, and 3 vol % *tert*-butyl alcohol.

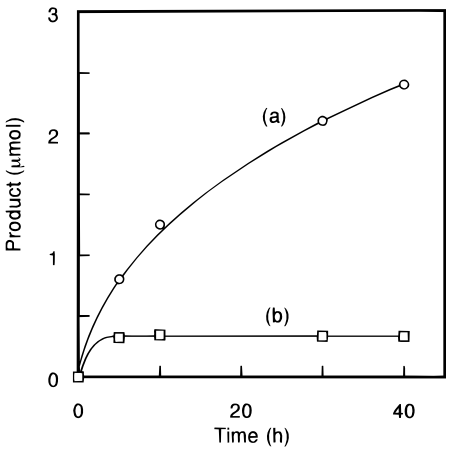


**Figure 7.** Production of (a) (*S*)-1-phenoxy-2-propanol and (b) (*S*)-phenethyl alcohol by electrolysis at −0.85 V vs Ag/AgCl of 10 mL of phosphate buffer (pH 7.1) containing 3 mmol $dm^{-3}$ phenoxy-2-propanone, 0.1 mmol $dm^{-3}$ acetophenone, 20 units of ADH (EC 1.1.1.2), 0.1 mmol $dm^{-3}$ $NADP^+$, and 3 vol % *tert*-butyl alcohol.

electrochemical reduction of 10 mL of phosphate buffer (pH 7.1) containing 1 mmol $dm^{-3}$ acetophenone using the glassy carbon electrode polarized at −0.85 V vs Ag/AgCl produced racemic phenethyl alcohol whose amount increased linearly with increase in the electrolysis time. The amount of phenethyl alcohol and its current efficiency obtained by the electrolysis for 25 h were 2.4 μmol and 99%, respectively. In order to induce the electrochemical reaction system B shown in Figure 1, reoxidation of the produced phenethyl alcohol at ADH is essential for regeneration of NAD(P)H. Thus the oxidation of phenethyl alcohol catalyzed by ADH (EC 1.1.1.2) was examined by the chemical reaction using $NADP^+$ as an oxidizing agent. As recognized from the time course of the amount of phenethyl alcohol of *R*- and *S*-configurations shown in Figure 6, both (*R*)- and (*S*)-phenethyl alcohol were consumed although the reaction rate of the former was larger than that of the latter. This result was a marked contrast to the result obtained by the electrochemical reduction of acetophenone using the reaction system A where the enantiomer excess of 92% was obtained for the production of (*R*)-phenethyl alcohol as given in Table 1, suggesting that the stereoselectivity of ADH (EC 1.1.1.2) for the oxidation of phenethyl alcohol to acetophenone is much lower than that for its reverse reaction. This property of the enzyme seems favorable for efficient utilization of phenethyl alcohol as the electron mediator in the reaction system B. The amount of NADPH produced at the reaction time of 25 h was 3.49 μmol, which was in good accordance with the total amount of (*R*)- and (*S*)-phenethyl alcohol consumed by the reaction (3.50 μmol).

The time course of electrochemical reduction of phenoxy-2-propanone is shown in Figure 7, which was obtained by electrolysis at −0.85 V vs Ag/AgCl of 10 mL of a phenoxy-2-propanone solution. The potential chosen for electrolysis was negative enough to induce direct reduction of acetophenone but too positive to induce the direct reduction of phenoxy-2-propanone as mentioned above, allowing selective reduction of acetophenone at the cathode. The enantiomer excess of 100% was also obtained for the production of (*S*)-1-phenoxy-2-propanol, indicating that the enantioselectivity of the enzyme can also be utilized by the reaction system B. The finding that *R*-configuration was not produced and only phenethyl alcohol of *S*-configuration accumulated may result from the difference in the rates of consumption of these substances in the regeneration of NADPH catalyzed by ADH as shown in Figure 6. Furthermore, judging from the results shown in Figure 7, where the amount of (*S*)-phenethyl alcohol was not changed at all during the course of the electrolysis, both (*R*)- and (*S*)-phenethyl alcohol must have worked as the electron mediators. If the same electrolysis was made in the absence of acetophenone or $NADP^+$, no production of (*S*)-1-phenoxy-2-propanol was obtained, indicating validity of the reaction scheme shown by the reaction system B in Figure 1.

Attempts were made to reduce electrochemically the substrates given in Table 1 using the reaction system B except for acetophenone and 3-phenylbutyl aldehyde, and the results obtained by the electrolysis for 30 h are also given in Table 1. In addition to the electrochemical reduction of phenoxy-2-propanone, the enantiomer excess of 100% with the current efficiencies higher than 83% was obtained for the reduction of propiophenone and pyruvic acid. However, the amounts of these products were smaller than those obtained using the reaction system A, suggesting that the electrochemical reduction of acetophenone at the cathode or electron exchanges between phenethyl alcohol and ADH was the rate-determining step for the reaction system B. The electrochemical reduction of benzoylformic acid gave an extremely low amount of product, whereas racemic phenethyl alcohol of 0.98 μmol was obtained, which amounted to 98% conversion of acetophenone in the electrolyte solution. The chemical oxidation of racemic mandelic acid was made under the same condition as that given for phenethyl alcohol in the figure caption of Figure 6. The reaction rate observed was much higher than that obtained for the oxidation of phenethyl alcohol, and all of both (*R*)- and (*S*)-mandelic acid were completely consumed for 3 h. Therefore, in the case of the electrochemical reduction of benzoylformic acid to mandelic acid using the reaction system B, the reoxidation of the product by ADH took place in preference to that of phenethyl alcohol, resulting in retardation of the desired reaction. Quite different reduction behaviors were observed for the reduction of 2-phenylpropionaldehyde,

where racemic product was obtained, the results being in marked contrast to that obtained using the reaction system A. It was revealed by the electrolysis of 2-propionaldehyde in the absence of ADH and acetophenone that this substrate was directly reduced to racemic 2-phenyl-1-propanol at the cathode polarized at −0.85 V vs Ag/AgCl, and the reaction rate was a little higher than that of the electrochemical reduction of acetophenone. It is then suggested that direct reduction of 2-propionaldehyde to racemic 2-phenyl-1-propanol occurs more predominantly than that of acetophenone to phenethyl alcohol, and because of this reason, it was not easy to recognize the occurrence of the asymmetric reduction catalyzed by ADH.

## Conclusion

We have demonstrated that the electrolysis systems A and B shown in Figure 1, which were fabricated using ADH as an electrocatalyst, are useful for inducing asymmetric electroreduction of ketone and aldehyde derivatives. A novel finding shown in this paper is that so long as an appropriate reaction substrate is chosen, optically active products with high enantiomer excess can be obtained by the reaction system B in which ADH is utilized as the sole electrocatalyst for regenerating NAD(P)H and reducing the substrate. Since the reaction system B uses only one kind of enzyme, it seems to be more suitable than the reaction system A in practical application. However, there are still some problems to be solved. Slow rate of reaction is the most serious. As already mentioned, the reaction rate achieved in the system B was much lower than that in the system A if the comparisons are made for the same reaction, and even if the reaction system A was used, the obtained reaction rates were unfortunately insufficient from the viewpoint of practical application. Since it was shown in our previous study[34] that the reduction of the substrates catalyzed by ADH is the rate-determining step in the reaction system A, the increase in the amount of ADH would be the most effective way to improve the reaction rate in this system. If this were economically inconvenient, another enzyme having higher catalytic activities for the desired reaction would need to be chosen. In the case of using the reaction system B, the rate of electron exchanges between the electron mediator and the electrode or ADH seems to determine the reaction rate. Then, the apparent reaction rate could be enhanced by increasing the amount of the electron mediator.

## Experimental Section

**Materials.** ADH (EC 1.1.1.2) from *T. brockii* (Sigma), ADH (EC 1.1.1.1) from equine liver (Sigma), FNR from spinach leaves (Sigma), DP from pig heart (Boehringer Mannheim), and cofactors of $NAD(P)^+$ and NAD(P)H (Oriental yeast) were used as received. Other chemicals used were of reagent grade from Wako Pure Chemicals and Tokyo Kasei Organic Chemicals. Water was purified by twice distillations of deionized water.

**General.** The amount of products and their enantiomer excesses obtained by the electrolyses were determined by using a JASCO high-performance liquid chromatography system composed of a PU-980 pump and an MD-910 multichannel detector. A Chiralcel OD-R column (Dicel Chemical) was used for determination of phenethyl alcohol, and a Sumichiral OA-5000 column (Sumika Chemical) was used for mandelic acid and lactic acid. Configuration of products was determined on the basis of chromatograms obtained by authentic samples. Determination of other products was made using a Chiralcel OB column (Dicel Chemical). The eluent used was a mixed solvent of $H_2O$ and acetonitrile (70/30) for the Chiralcel OD-R and OB columns and of $H_2O$ and 2-propanol (95/5) containing 2 mmol $dm^{-3}$ $CuSO_4$ for the Sumichiral OA-5000 column.

**Electrolysis.** The electrode used was a glassy carbon plate (Tokai Carbon) having an exposed area of 2.0 $cm^2$. Its surface was successively polished with emery papers (#1000 and #2000) and alumina particles (1.0 and 0.3 $\mu$m) to obtain the mirror-finished surface. The resulting electrode was subjected to ultrasonication in distilled water to get rid of adsorbed alumina particles. A platinum foil having 5 $cm^2$ area and an Ag/AgCl in saturated KCl solution served as a counter electrode and a reference electrode, respectively. The electrolyte solution used for the electrolysis experiments was phosphate buffer, pH 7.1, which was prepared by mixing 20 mmol $dm^{-3}$ $KH_2PO_4$ and 20 mmol $dm^{-3}$ $H_2PO_4$. An H-type two-compartment cell divided by a cation exchange membrane (Nafion 117, Aldrich) was used as an electrolytic cell. The electrolyte solution containing the enzymes and desired chemicals was put into the test electrode compartment, whereas the counter electrode compartment was filled with the phosphate buffer solution alone. After both catholyte and anolyte were bubbled with $N_2$ for at least 1 h, the electrolysis was initiated using a Hokuto Denko HA-301 potentiostat. The charge consumed in the electrolysis was measured with a Hokuto Denko HF-202D coulometer.

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