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Production of chiral alcohols by enantioselective reduction with NADH-dependent phenylacetaldehyde reductase from *Corynebacterium* strain, ST-10

Nobuya Itoh [a,*], Naoyuki Mizuguchi [b], Mariko Mabuchi [a]

[a] *Biotechnology Research Center, Toyama Prefectural University, Kurokawa 5180, Kosugi, Toyama 939-0398, Japan*
[b] *Department of Applied Chemistry and Biotechnology, Faculty of Engineering, Fukui University, Bunkyo 3-9-1, Fukui 910-8507, Japan*

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Abstract

Phenylacetaldehyde reductase (PAR) (systematic name, 2-phenylethanol: NAD^+ oxidoreductase) isolated from styrene-assimilating *Corynebacterium* strain ST-10 was used to produce chiral alcohols. This enzyme with a broad substrate range reduced various prochiral 2-alkanones and aromatic ketones to yield optically active secondary alcohols with an enantiomeric purity of 87–100% enantiomeric excess (e.e.). The stereochemistry of PAR revealed that the pro-R hydrogen of NADH was transferred to carbonyl moiety of acetophenone derivatives or alkanones through its *re* face. The combination with a NADH-regenerating system using formate dehydrogenase and formate was able to practically produce optically pure alcohols.

Keywords: Chiral alcohol; (*S*)-1-Phenylethanol; (*S*)-2-Alkanol; Enzymatic reduction; Phenylacetaldehyde reductase (NADH); *Corynebacterium* sp.

1. Introduction

Enantioselective organic syntheses are in great demand in pharmaceuticals, agricultural chemicals and liquid crystals [1]. Routes to optically pure compounds include enantiomer separation from a racemic mixture, derivation of natural substances, and asymmetric synthesis. Chiral metal complexes have been successfully applied as catalysts for a number of enantioselective syntheses [2,3]; however, in many reactions difficulties remain in attaining sufficient optical purity and practical usage compared with enzymatic catalysis. The two biotransformation systems with baker's yeast [4] and lipase [5] have been widely used as a useful means in obtaining optically active compounds.

Chemical reductions of ketones using a BINAP-Ru(II) complex-chiral diamine–KOH system gave the (*R*)-/(*S*)-alcohols with an optical purity of 87–99% e.e. [3]. On the other hand, baker's yeast reductions of the 4-substituted acetophenones were carried out, and the corresponding (*S*)-alcohols with an enantiomeric purity of 82–96% e.e. were obtained [6]. Acetone powder of the fungus *Geotrichum candidum* cells gave (*S*)-alcohols from acetophenone

* Corresponding author. Tel.: +81-766-56-7500, ext. 560; Fax: +81-766-56-2498; E-mail: itoh@pu-toyama.ac.jp

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derivatives with an optical purity of 99% e.e. [7,8]. Akakabe et al. reported the production of the (*S*)-alcohols from 4-substituted acetophenones and heterocyclic aromatic ketones with more than 96% e.e. using immobilized carrot cells [9].

Direct usage of oxidoreductase for the preparation of chiral alcohols has been reported with alcohol dehydrogenases from yeast and horse liver [10]. However, they have some disadvantages, such as narrow substrate specificity, instability above 30°C, and sensitivity to organic solvents. Therefore, there have been many efforts undertaken to search for novel dehydrogenases and reductases suitable for the production of chiral alcohols from prochiral ketones. The known enzymes reducing various carbonyl compounds generally utilize NADPH as coenzyme. Aldehyde reductase (EC 1.1.1.2) from *Sporobolomyces salmonicolor* [11], aldo-keto reductase from *Saccharomyces cerevisiae* [12], and carbonyl reductase (EC 1.1.1.184) from *Candida macedoniensi* [13] belong to this type of enzyme. Recently, NADH-dependent carbonyl reductase and NAD^+-dependent alcohol dehydrogenase with a broad substrate range have been reported in *C. parapsilosis* [14] and *Pseudomonas* sp. [15], respectively. We have also found a novel NADH-dependent reductase, named phenylacetaldehyde reductase (PAR), from styrene-assimilating *Corynebacterium* strains [16,17]. This enzyme shows a broad substrate range and catalyzes the reduction of not only various arylketones but also 2-alkanones [17]. Its physical properties, substrate- and stereo-specificities are rather different from those reported in *C. parapsilosis* [14] and *Pseudomonas* sp. [15]. The substrate specificity of PAR is also different from general NAD^+-dependent alcohol dehydrogenase (EC 1.1.1.1) [18], $NAD^+/NADP^+$ dependent aryl–alcohol dehydrogenases (EC 1.1.1.90 and 91) [19,20] and $NADP^+$-dependent (*R*)-1-phenylethanol dehydrogenase from *Lactobacillus kefir* [21], because PAR never catalyzes the reduction of acetaldehyde, acetone, and benzaldehyde [17].

In this report we describe the application of PAR for the production of chiral alcohols from ketones including acetophenone derivatives and 2-alkanones with a high enantioselectivity. The kinetic property of the enzyme allowed it to be coupled with a coenzyme regeneration system such as formate/formate dehydrogenase for producing chiral alcohols with a high yield.

2. Experimental

2.1. Preparation of PAR

PAR was obtained from *Corynebacterium* sp. ST-10 cells grown on styrene, as previously reported [17]. Unless otherwise indicated, partially purified enzyme preparation produced by the sequential DEAE–Sepharose and Butyl–Toyopearl column chromatographies was used throughout the experiments [17].

2.2. Enzyme assay and kinetic measurements

PAR activity was assayed at 25°C by measuring the decrease in absorption at 340 nm of NADH as described in the previous paper [16]. One unit of enzyme was defined as the amount that converted 1 μmol NADH and phenylacetaldehyde in 1 min. Although PAR does not catalyze the oxidative reaction of 2-phenylethanol [17], it catalyzes the dehydrogenation of a few alcohols including 1-phenylethanol and 2-alkanol in the presence of NAD^+. Therefore, the equilibrium constant K_{eq} was determined for two substrate systems: acetophenone and (*S*)-1-phenylethanol, and heptanone and (*S*)-2-heptanol. The reaction mixture consisted of varying concentrations of (*S*)-1-phenylethanol and acetophenone or (*S*)-2-heptanol and heptanone, 1.0 mM NAD^+ and 0.05 units of PAR in 100 mM potassium phosphate buffer (pH 7.0) in a total volume of 1.5 ml. The reaction was monitored by a spectrophotometer on the basis of the increase in NADH at 340 nm at 25°C, and the concentration of each product in equilibrium condition was calculated from $\Delta A_{340\ nm}$ ($\varepsilon = 6220\ M^{-1}\ cm^{-1}$ of NADH).

2.3. Enzymatic reaction and reaction mixture analysis

The substrate specificity of PAR was spectrophotometrically measured by decreasing the absorption of NADH at 340 nm using the purified enzyme [17] and various ketones at 3 mM, and their relative activity was compared with acetophenone.

The reaction mixture for determining the products from ketones consisted of 2–6 μmol of each substrate, 10 μmol NADH, 0.1 mmol potassium phosphate buffer (pH 7.0), and 0.5 units of PAR solution in a total volume of 2 ml. The insoluble substrate had been previously suspended in the reaction mixture by ultrasonication (20 kHz, 100 W, for 2 min). The reaction proceeded for 2 h at 25°C, and the product was analyzed by gas chromatography (GC) using a Shimadzu GC-14A system equipped with a coiled column (3 mm × 2 m) packed with Thermon 1000 (5% on Chromosorb W) with an FID (flame ionization detector). GC was performed under the following conditions: column temperature 150°C, injection and detection temperatures 240°C, and a flow rate of 50 ml min^{-1} of N_2; the products and substrates showed the following retention times (min): phenylacetaldehyde, 2.2; 2-phenylethanol, 5.1; acetophenone, 2.3; 1-phenylethanol, 3.6; 2-chloroacetophenone (phenacyl chloride), 8.6; 2-chloro-1-phenylethanol, 13.0; 2′-chloroacetophenone, 4.1; 1-(2-chlorophenyl)ethanol, 8.9; 3′-chloroacetophenone, 4.0; 1-(3-chlorophenyl)ethanol, 10.1; 4′-chloroacetophenone, 4.2; 1-(4-chlorophenyl) ethanol, 9.9; 2′-bromoacetophenone, 7.0; 1-(2-bromophenyl)ethanol, 14.9; 3′-bromoacetophenone, 7.0; 1-(3-bromophenyl)ethanol, 17.3; 4′-bromoacetophenone, 6.9; 1-(4-bromophenyl) ethanol, 17.2; propiophenone, 2.9; 1-phenyl-1-propanol, 4.5; column temperature 130°C for benzylacetone, 6.7; 4-phenyl-2-butanol, 14.1, and column temperature 70°C, injection and detection temperatures 150°C for 2-heptanone, 1.9; 2-heptanol, 3.5; 2-octanone, 3.0; 2-octanol, 6.1.

For determining the absolute configuration of alcohol produced from ketone, the product extracted with ethyl acetate from the reaction mixture was analyzed using GC equipped with a CP-cyclodextrin-β-236-N19 chiral column (0.25 mm × 25 m, 0.25-μm film, Chrompack, The Netherlands) and FID. Helium gas was used as a carrier at 3 kg cm^{-2}, split ratio 100, injection and detection temperatures were 250°C, and the column temperature was at 100°C for 1 min then raised up to 200°C at 10°C min^{-1} and maintained at 200°C for 3 min. The produced alcohol was compared with the authentic racemic and chiral ones. The enantiomeric purity (% e.e.) of the product was determined by GC analysis.

In order to determine the absolute configuration of the produced 2-alkanols, they were converted into benzoyl derivatives with benzoyl chloride and analyzed by HPLC with a chiral column. First, 2-alkanol obtained was dissolved in pyridine (about 1:20 by volume) and mixed with benzoyl chloride (1.2 times equimolar to 2-alkanol) and a small amount of *N,N′*-dimethylaminopyridine. After 3-h reaction at room temperature, the reaction mixture was poured into a 1 N HCl solution to eliminate excess pyridine. The product was then extracted in ethyl acetate, washed with saturated $NaHCO_3$ solution, dried with Na_2SO_4, and evaporated. The obtained benzoyl derivative was further purified by thin layer silica gel chromatography (TLC). Analytical HPLC proceeded by a Tosoh CCPE-II system equipped with a Chiralcel OB-H (4.6 × 250 mm, Daicel Chemical Industries, Japan). The mobile phase was a hexane/2-pronanol (49:1) and flowed at rate of 0.5 ml min^{-1}. The product was spectrophotometrically detected at 254 nm.

2.4. Stereochemistry of PAR

In order to determine the stereochemistry of PAR, deuterium (D)-containing NADH (NADD) at pro-R position in nicotinamide moiety was

enzymatically synthesized from NAD^+ and CH_3CD_2OH with yeast alcohol dehydrogenase. The reaction mixture contained 390 mmol CH_3CD_2OH, 460 μmol NAD^+ (320 mg), 200 units of yeast alcohol dehydrogenase and 50 mmol ammonium bicarbonate buffer (pH 9.0) in a total volume of 50 ml, and the reaction proceeded at 25°C for 48 h. After lyophilization of the reaction mixture, the yellowish powder obtained was applied to DEAE–Sepharose column (1.5×45 cm) previously equilibrated with 50 mM ammonium bicarbonate buffer (pH 8.0). The column was washed enough with the buffer and the NADD was eluted with a linear gradient of ammonium bicarbonate buffer from 50 to 300 mM (pH 8.0) and monitored at 340 nm. The fractions containing NADD were collected and lyophilized again. The white powder was applied to Sephadex G-25 column (1.5×45 cm) equilibrated with 20 mM ammonium bicarbonate buffer (pH 8.0), and NADD was eluted with the buffer. The product (120 mg) showed the single peak of pro-S hydrogen at 2.52 ppm in ^1H NMR (D_2O).

Acetophenone was reduced by the purified PAR using this cofactor as follows; the reaction mixture consisted of 75 μmol acetophenone, 105 μmol NADD, 7 units of PAR and 1.5 mmol potassium phosphate buffer (pH 7.0) in a total volume of 15 ml. The reaction proceeded at 25°C for 48 h. The produced 1-phenylethanol was purified by extraction with ethyl acetate from the mixture and silica gel chromatography according to the methods described in Section 2.5, and analyzed by ^1H NMR ($CDCl_3$) and GC-mass spectrometry (MS) (QP-5000 GC-MS, Shimadzu, Japan).

2.5. PAR-catalyzed reduction coupling to NADH regeneration system with formate dehydrogenase

For determining the enantiomeric purity of some products and assessing the coupling system of NADH regeneration, we constructed the PAR and formate dehydrogenase (FDH) system (Fig. 1). The reaction mixture consisted of 5 mmol potassium phosphate buffer (pH 6.5), 0.2–0.5 mmol of each substrate, 10 mmol formate, 25 μmol NADH, 2.5 units of PAR and 2.5 units FDH in a total volume of 50 ml. The reaction proceeded for between 7 and 75 h at 25°C. Conversion of the substrate was monitored by TLC or GC. The conversion ratios were determined on the basis of the peak areas of ketone substrates and alcohol products on the GC. After the reaction, the product was extracted twice with ethyl acetate from the reaction mixture. The combined extracts were dried with Na_2SO_4 and then evaporated. The product was subjected to silica gel (Wako gel C-400) chromatography and was eluted with *n*-hexane/ethyl acestate (3:2). The purified product was obtained by the evaporation of fractions containing the product. The optical rotation of the product was measured with a JASCO DIP-370 polarimeter using a 1-ml cell at 589 nm of Na–D line using chloroform or ethanol as a solvent.

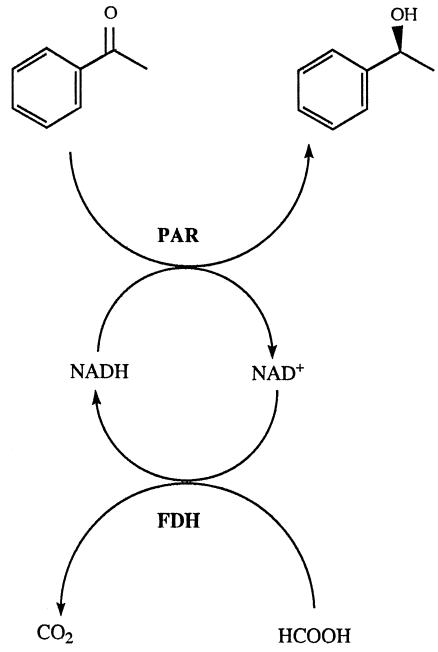


Fig. 1. acetophenone reduction by PAR with an NADH-regenerating system with formate dehydrogenase.

2.6. Chemicals

Acetophenone, 2-chloroacetophenone (phenacyl chloride), 2-bromoacetophenone, 2′-chloroacetophenone, 3′-chloroacetophenone, 4′-chloroacetophenone, 3′-bromoacetophenone, 4′-bromoacetophenone, propiophenone, 2-heptanone, 2-octanone, (*R,S*)-1-phenylethanol, (*R,S*)-2-chloro-1-phenylethanol, (*R,S*)-1-(2-chlorophenyl)ethanol, (*R,S*)-1-(3-chlorophenyl)ethanol, (*R,S*)-1-(4-chlorophenyl)ethanol, (*R,S*)-1-phenyl-1-propanol, (*R,S*)-2-octanol and (*S*)-1-phenylethanol were purchased from Tokyo Kasei, Tokyo, Japan. 2′-Bromoacetophenone, (*R*)- and (*S*)-2-chloro-1-phenylethanols, (*S*)-1-phenyl-1-ethanol, (*R,S*)-1-(4-bromophenyl)ethanol, (*S*)-1-(2-bromophenyl)ethanol, (*R*)-2-heptanol and (*R*)-2-octanol were obtained from Aldrich Chemical, USA, and (*R,S*)-2-heptanol, (*S*)-2-heptanol, (*R,S*)-2-octanol and (*S*)-2-octanol from Wako Pure Chemicals, Osaka, Japan. Formate dehydrogenase from *Xylaria digitata* (*C. boidinii*) and alcohol dehydrogenase from yeast were supplied from Boehringer Mannheim Japan, Other reagents used were all of analytical grade.

3. Results and discussion

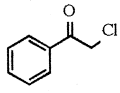
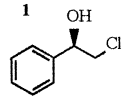
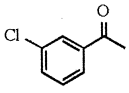
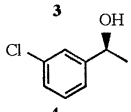
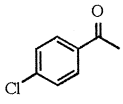
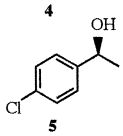
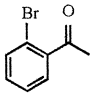
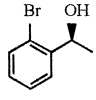
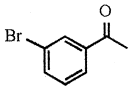
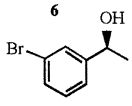
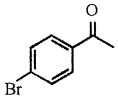
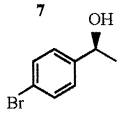
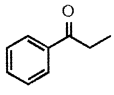
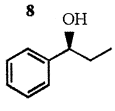
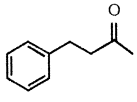
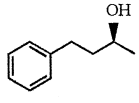
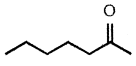
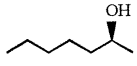
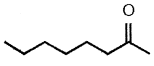
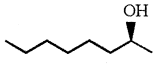
3.1. Substrate specificity and kinetic properties of PAR

As previously described, PAR is a novel NADH-dependent reductase with a broad substrate range [17]. The enzyme showed high activity toward medium-chain normal aldehydes and 2-alkanones between C_5 and C_{10}, and various arylaldehydes and acetophenone derivatives, although it did not act on short-chain normal alkylaldehydes and 2-alkanones, and benzaldehyde. For arylketones, acetophenone was reduced to (*S*)-1-phenylethanol with a relative activity 35% of that for phenylacetaldehyde, and 3′- and 4′-halogenated acetophenones were also converted into the corresponding alcohols. Therefore, PAR was thought to be a suitable biocatalyst for producing the chiral alcohols. The stereospecificities of several acetophenone derivatives and medium-chain 2-alkanones catalyzed by PAR are summarized in Table 1. PAR produced (*S*)-form alcohols from acetophenone derivatives, (*R*)-halohydrin from 2-chloroacetophenone (phenacyl chloride), and (*S*)-alkanols from 2-alkanones with an enantiomeric purity more than 96% e.e., except for 2′-bromoacetophenone (87% e.e.).

PAR in *Corynebacterium* strains has a physiological role to reduce phenylacetaldehyde to 2-phenylethanol in styrene or styrene oxide metabolism [16,17]. Under practical reaction conditions, however, PAR does not catalyze the reverse reaction: dehydrogenation of 2-phenylethanol to phenylacetaldehyde in the presence of NAD^+ [17]. Therefore, we checked whether or not PAR did catalyze the reverse reaction for other substrates. Table 2 shows the relative activity for the oxidative reaction of PAR with NAD^+ for some alcohols, when that for 1-phenylethanol is 100%. (*S*)-1-phenylethanol was a good substrate of PAR. (*R*)-1-phenylethanol, however, did not serve as a substrate. This coincided with the stereospecificity of PAR's reductive reaction producing (*S*)-form 1-phenylethanol derivatives (Table 1). In contrast to 1-phenylethanol, PAR acted on (*R*)-form alkanols with a lower activity than (*S*)-form alkanols. The equilibrium of the PAR-catalyzed alkanol oxidation was shifted in the reverse reaction as described below. In addition to the shifted equilibrium, difference in the k_{cat}/K_m of PAR toward (*S*)- and (*R*)-form alkanols probably reflects the fact that PAR produced only (*S*)-form alkanol from alkanone. In comparison with 2-alcohols, only slight or no activities were observed for 1-alkyl- and 1-arylalcohols, in spite of the high activity of PAR toward the corresponding aldehyde in the reductive reactions [17].

The equilibrium constant, defined as the following equation, $K_{eq} = [\text{Alcohol}][NAD^+]/[\text{Ketone}][\text{NADH}][H^+]$, is important to evaluate the

Table 1
Enantioselective reduction of aromatic ketones and 2-alkanones by PAR

substrate	relative activity (%)	product	enantiomeric excess (%)	reaction time (hr)	conversion yield (%)	chemical yield (%)
	100	 **1**	96	19	100	86
	23	 **2**	99	N.T.[a]	N.T.	N.T.
	49	 **3**	100	75	56[b]	29
	1925	 **4**	100	7	100	52
	970	 **5**	100	16	100	91
	14	 **6**	100	N.T.	N.T.	N.T.
	2320	 **7**	87	9.5	100	55
	1090	 **8**	100	18	100	65
	6	 **9**	100	N.T.	N.T.	N.T.
	83	 **10**	100	N.T.	N.T.	N.T.
	2170	 **11**	100	9	100	65
	1490	 **12**	100	9	100	74

[a] Not tested.
[b] Not optimized. Higher yield can be obtained with more enzyme.

Table 2
Oxidative reaction of PAR for some alcohols

Substrate	Relative activity (%)
(*S*)-(−)-1-Phenylethanol	100
(*R*)-(+)-1-Phenylethanol	0
2-Phenylethanol	0
Cinnamyl alcohol	20
(*S*)-(−)-1-Phenyl-1-propanol	5
Cyclohexanol	0
1-Heptanol	6
(*S*)-(+)-2-Heptanol	436
(*R*)-(−)-2-Heptanol	187
(*R,S*)-2-Heptanol	410
3-Heptanol	18
4-Heptanol	0
1-Octanol	0
(*S*)-(+)-2-Octanol	454
(*R*)-(−)-2-Octanol	81
(*R,S*)-2-Octanol	422
5-Nonanol	0

yield of the product in the PAR reaction. The K_{eq} values were determined for two alcohol and ketone systems: (*S*)-1-phenylethanol and acetophenone, and (*S*)-2-heptanol and 2-heptanone. The concentrations of the reaction components are shown in Table 3. The average K_{eq} values from three separate experiments were 5.7×10^7 M^{-1} for the (*S*)-1-phenylethanol and acetophenone system and 39×10^7 M^{-1} for the (*S*)-2-heptanol and 2-heptanone system. The results showed that the PAR is favorable to reducing ketones under regular reaction conditions in both cases. For example, 98.8% yield of 1-phenylethanol (2.964 mM) should be obtained at equilibrium conditions, if the initial reaction mixture contained 3 mM acetophenone and 5 mM NADH at pH 6.0.

3.2. Stereochemistry of PAR

As shown in Table 1, PAR converted acetophenone derivatives and 2-alkanones into (*S*)-(−)-1-phenylethanol derivatives and (*S*)-(+)-2-alkanols, respectively, with a high optical purity of more than 87% e.e. The results indicated that the hydride anion from NADH is transferred to the *re* face of the carbonyl, as illustrated in Fig. 2. This suggests that the stereochemistry of PAR follows Prelog's rule [22], the same as yeast and horse liver alcohol dehydrogenases [15].

On the other hand, the stereochemistry with respect to NADH was determined by the PAR-catalyzing transfer of deutride (D) or hydride from the D-labeled NADH at pro-R position (NADD), which was obtained by the reaction of yeast dehydrogenase (YADH) and CH_3CD_2OH as shown in Fig. 2. ^1H NMR analysis of the 1-phenylethanol produced by PAR with the NADD gave the disappearance of C-1 proton peak at 4.8 ppm. The GC-MS also showed the presence of M^+ ion of C_8ODH_9 (Mr:123) and other fragment ion peaks (108, 80, 51 and 43). These results indicated that the C-1 hydrogen of 1-phenylethanol was replaced by deuterium through PAR reduction. Therefore, PAR transfers the pro-R hydrogen of NADH to the *re* face of the substrate. Thus, the overall stereo-

Table 3
Equilibrium constants of PAR reactions in two substrate systems: (a) acetophenone and (*S*)-1-phenylethanol, (b) heptanone and (*S*)-2-heptanol

Concentration (mM)			ΔA340	$[NADH]_{eq}$ (μM)	K_{eq} (M^{-1})
(a) (*S*)-1-phenyl-ethanol	acetophenone	NAD^+			
0.3	0.1	1	0.748	120	6.00×10^7
0.3	0.3	1	0.568	91	5.34×10^7
0.6	0.3	1	0.914	147	5.95×10^7
(b) (*S*)-1-heptanol	heptanone	NAD^+			
0.3	0.05	1	0.342	56	40.1×10^7
0.3	0.1	1	0.285	45	36.3×10^7
0.6	0.1	1	0.444	71	40.5×10^7

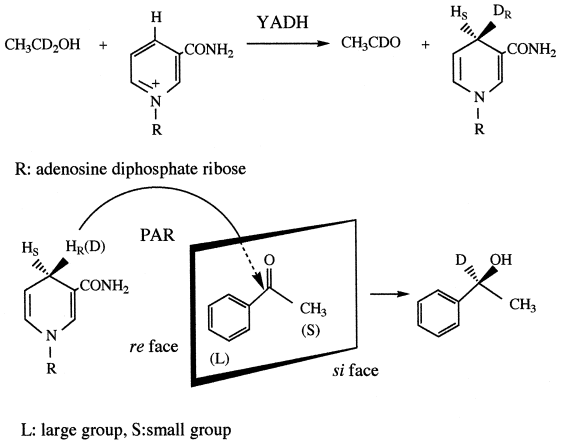


Fig. 2. Stereochemistry of PAR reaction.

chemistry of PAR is similar to that of yeast alcohol dehydrogenase and horse liver alcohol dehydrogenase [15].

3.3. Production of chiral alcohols by PAR coupling with formate dehydrogenase NADH-regeneration system

As exemplified in Table 1, the enantioselective reduction of the aromatic ketones and 2-alkanones by PAR coupled with formate dehydrogenase gave the corresponding chiral alcohols **1**–**12** with an enantiomeric purity of 87–100% e.e. Fig. 3 shows the time course of the biotransformation of acetophenone, indicating that (*S*)-1-phenylethanol **1** was yielded at almost 100% under the conditions established. Conversion yields of other acetophenone derivatives and two 2-alkanones tested were almost 100%, except for 2′-chloroacetophenone; the chemical yields of isolated alcohols ranged between 29 and 91%, although the reaction time varied between 7 and 75 h depending on each substrate. After the reaction, the product was isolated, purified by silica gel chromatography and analyzed. The analytical data for each product were as follows.

(*S*)-1-phenylethanol (**1**): 86% yield (52 mg) from acetophenone (60 mg); $[\alpha]_D^{20}$ −36.5 (c = 2, $CHCl_3$); 96% e.e. as determined by GC on a CP-cyclodextrin chiral column; retention times were 3.3 min for (−) (*S*) and 3.2 min for (+) (*R*).

(*S*)-1-(2-chlorophenyl)ethanol (**3**): 29% yield (22.5 mg) from 2′-chloroacetophenone (77.3

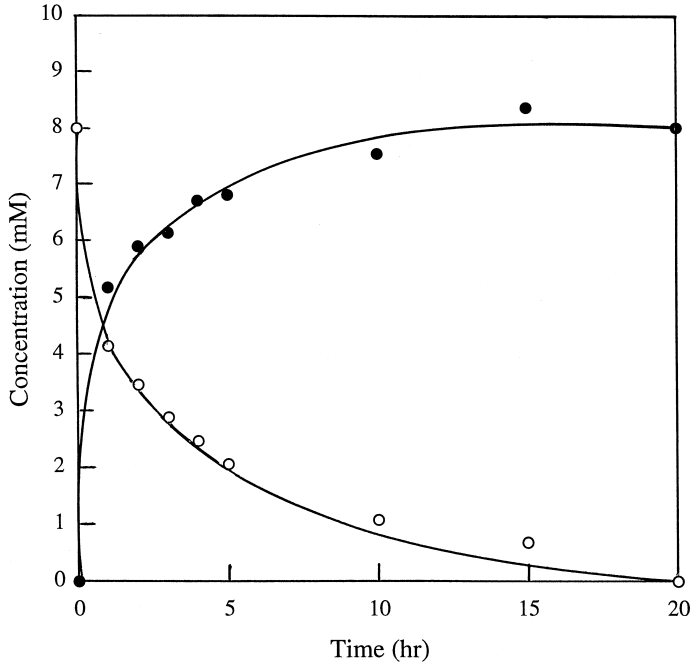


Fig. 3. Course of the conversion of acetophenone (○) to 1-phenylethanol (●) by PAR with an NADH-regenerating system.

mg); $[\alpha]_D^{20}$ −51.6 ($c = 1$, $CHCl_3$); 100% e.e. as determined by GC on a CP-cyclodextrin chiral column; retention times were 8.03 min for (−) (*S*) and 8.13 min for (+) (*R*).

(*S*)-1-(3-chlorophenyl)ethanol (**4**): 52% yield (24.1 mg) from 3′-chloroacetophenone (46.3 mg); $[\alpha]_D^{20}$ −38.6 ($c = 1$, $CHCl_3$); 100% e.e. as determined by GC on a CP-cyclodextrin chiral column; retention times were 7.8 min for (−) (*S*) and 7.7 min for (+) (*R*).

(*S*)-1-(4-chlorophenyl)ethanol (**5**): 91% yield (42 mg) from 4′-chloroacetophenone (46.3 mg); $[\alpha]_D^{20}$ −19.3 ($c = 2$, $CHCl_3$); 100% e.e. as determined by GC on a CP-cyclodextrin chiral column; retention times were 7.9 min for (−) (*S*) and 7.7 min for (+) (*R*).

(*S*)-1-(3-bromophenyl)ethanol (**7**): 55% yield (54.6 mg) from 3′-bromoacetophenone (99.5 mg); $[\alpha]_D^{20}$ −23.6 ($c = 2$, $CHCl_3$); 87% e.e. as determined by GC on a CP-cyclodextrin chiral column; retention times were 6.1 min for (−) (*S*) and 6.0 min for (+) (*R*).

(*S*)-1-(4-bromophenyl)ethanol (**8**): 65% yield (64.4 mg) from 4′-bromoacetophenone (99.5 mg); $[\alpha]_D^{20}$ −14.9 ($c = 2$, $CHCl_3$); 100% e.e. as determined by GC on a CP-cyclodextrin chiral column; retention times were 6.1 min for (−) (*S*) and 6.0 min for (+) (*R*).

(*S*)-2-heptanol (**11**): 65% yield (38 mg) from 2-heptanone (58 mg); $[\alpha]_D^{20}$ +2.3 ($c = 2$, CH_3CH_2OH); 100% e.e. as a benzoyl derivative determined by HPLC on a Chiral OB-H column; hexane/2-propanol (49:1); 0.5 ml min^{-1}; retention times were 7.6 min for (−) (*R*) and 8.4 min for (+) (*S*).

(*S*)-2-octanol (**12**): 74% yield (46.7 mg) from 2-octanone (65.1 mg); $[\alpha]_D^{20}$ +2.5 ($c = 2$, CH_3CH_2OH); 100% e.e. as a benzoyl derivative determined by HPLC on a Chiral OB-H column; hexane/2-propanol (49:1); 0.5 ml min^{-1}; retention times were 7.4 min for (−) (*R*) and 8.1 min (+) (*S*).

Other products described in Table 1 were only examined for their e.e. values by GC, because the conversion rates were too low to isolate them.

(*R*)-2-chloro-1-phenylethanol (**2**): 100% e.e. as determined by GC on a CP-cyclodextrin chiral column; retention times were 8.13 min for (−) (*R*) and 8.02 min for (+) (*S*).

(*S*)-1-(2-bromophenyl)ethanol (**6**): 100% e.e. as determined by GC on a CP-cyclodextrin chiral column; retention times were 8.34 min for (−) (*S*) and 7.98 min for (+) (*R*).

(*S*)-1-phenyl-1-propanol (**9**): 100% e.e. as determined by GC on a CP-cyclodextrin chiral column; retention times were 6.15 min for (−) (*S*) and 6.08 min for (+) (*R*).

(*S*)-4-phenyl-2-butanol (**10**): 100% e.e. as determined by GC on a CP-cyclodextrin chiral column; retention times were 18.0 min for (−) (*S*) and 18.5 min for (+) (*R*).

The results showed that the enzymatic reduction of acetophenone derivatives and 2-alkanones by PAR with simultaneous NADH regeneration using formate dehydrogenase were practical routes to produce chiral alcohols.

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