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Resolution and Synthesis of Optically Active Alcohols with Immobilized Water-soluble Proteins from Green Pea, Soybean and Buckwheat as New Bio-catalysts

Hiroyuki NAGAOKA[1,3] and Hiroshi KAYAHARA[2]

[1]*The United Graduate School of Agricultural Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*
[2]*Department of Bioscience and Biotechnology, Shinshu University, 8304 Minamiminowa, Kamiina, Nagano Japan*
[3]*Sanyo Shokuhin Co., Ltd., 555-4 Asakura, Maebashi Gunma 371-0811, Japan*

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Kinetic resolution of racemic alcohols, (±)-1-(4-substituted phenyl)ethanol and (±)-1-(2-naphthyl)ethanol, was done with immobilized green pea, soybean, or buckwheat proteins. The resolution was done stereoselectively by oxidizing only one enantiomer of a racemic alcohol to leave an optically active alcohol with a high purity. In addition, each protein could be reused consecutively at least three times without any decrease of yield or optical purity.

Key words: immobilized green pea protein; immobilized soybean protein; immobilized buckwheat protein; (±)-1-(4-substituted phenyl)ethanol; (±)-1-(2-naphthyl)ethanol

Optically active alcohols are important as raw materials or intermediate raw materials of pharmaceuticals and agricultural chemicals as well as intermediates in the field of fine chemicals such as ferroelectric liquid crystals.

As to biological processes for producing optically active alcohol, the following bio-catalysts have been used microorganisms,[1] enzymes derived from microorganisms,[2] enzymes derived from animal tissues,[3] and cultured plant cells.[4]

However, a method for synthesis of optically active alcohol by using proteins from cereals and beans has not been reported as far as we know. Therefore, we have reported (*S*)-*5a* (>99%e.e., ~50%yield) was yielded by immobilized green pea (*Pisum sativum L.*) protein (IPP) from (±)-*5*, in which the (*R*)-*5* was selectively oxidized to *5b*. and we have suggested that pea protein powder may be very useful in stereoselectively catalytic reactions of the powder as a new biotransformation of chiral compounds.[5] To clarify whether such resolution activity is limited to the protein from green pea, we here tested the proteins from soybean (*Glycine max.*) and buckwheat (*Fagopyrum esculentum*). Consequently we report that immobilized protein powders from sources are usable as a new biocatalyst for optical resolution as well as IPP.

Materials and Methods

Extraction of water-soluble protein. Green pea, soybean, and buckwheat were crushed, and large pieces and the husks were removed. The component of green pea, soybean, and buckwheat water-soluble protein was dissolved in 9 times by weight distilled water by triturating the crush in water at ~40°C for ~45 min during with the pH was adjusted to 7.0 using aq. NaOH. When it is necessary to adjust the pH, this may be done using a food-grade acid such as H_2SO_4, HCl, or H_3PO_4 or a food-grade base such as NaOH. The precipitated food fiber was removed and the protein was precipitated at its isoelectric point by bringing the water-soluble protein portion to an acidic or basic condition (pH 4.5 for green pea and soybean, pH 9.5 for buckwheat). After redissolving the protein precipitate in distilled water at pH 7.0, spray-drying was done on the resulting green pea, soybean, or buckwheat water-soluble protein solution (sample concentration: 5.0%) to prepare powdered pea protein (PP), soybean protein (SP), or buckwheat protein (BP).

Immobilization of proteins. In the second step, the PP, SP, and BP were immobilized as follows: the protein powder (20 g) was dissolved in distilled water (200 ml), and 5% aq. Na arginate (250 ml) was poured into the solution with stirring. When the homogeneous solution was added dropwise to 0.6% aq. $CaCl_2$ (2000 ml), calcium arginate gel beads (ϕ =

To whom correspondence should be addressed. Hiroshi KAYAHARA, Tel: 81-265-77-1600; Fax: 81-265-77-1629; E-mail: kaya126@gipmc.shinshu-u.ac.jp

4–5 mm) involving the PP, SP, and BP were made. After the beads were kept in the 0.6% aq. $CaCl_2$ for 5 h more, the beads were separated from the solution and washed with distilled water.[5]

Resolution of alcohols. Then, the immobilized proteins (IPP, ISP, and IBP) were used for the resolution of substrate alcohols; (±)-*1-5*. That is, 199～218 mg of substrate (±)-*1-5* were added to distilled water (400 ml) containing IPP, ISP, and IBP at 33～37°C and the solution incubated 2–17 days at 35°C on a rotary shaker (55 rpm).

The substrate alcohol and the produced ketones were monitored with an interval of 12 hours using GC and the reaction was stopped after confirming the end of conversion. Each extract was analyzed by GC(FID) with a PEG-20M 25 m × 0.25 mm TC-5HT fused silica capillary column (GL Science, Tokyo, Japan) at 150°C～180°C (carrier gas, He 0.48 ml min^{-1}; split ratio, 1/55).

Finally, IPP, ISP, and IBP were removed from the reaction solution, and reactant and reaction product that were included in the reaction solution were extracted using diethyl ether. After the ether was washed with a sat. NaCl solution, dried over Na_2SO_4, and evaporated, the target optically active alcohol was isolated by silica gel chromatography (70–230 mesh, hexane:ethyl acetate = 9:1).

General procedure. The steric conformation of an isolated optically active alcohol was identified from a comparison between the values (+ or −) for the specific rotation ($[\alpha]_D^{20}$ in $CHCl_3$) obtained by referring to the literature.[4]

The IR and ^1H NMR spectra of *1a-5a* were identical with those of (±)-*1-5*.

The e.e. (= enantiomer excess) of *1a-5a* were calculated from chiral HPLC analyses [column, Chiralcel OB 4.6 × 250 mm; eluent, hexane:2-propanol (9:1); flow rate 0.5 cm^3 min^{-1} for (±)-*1,2,5*, 1.0 cm^3 min^{-1} for (±)-*3,4*; detection light 220 nm for (±)-*1,2*, 254 nm for (±)-*3,4,5*, reaction time (t_R) is shown Table 1].

Results

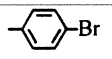
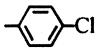
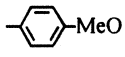
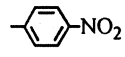
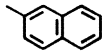
The production process for obtaining optically active alcohol of high optical purity by using the above-mentioned optical separation catalyst in this study includes a process comprising selectively oxidizing a substrate in the form of one enantiomer of a racemic to obtain a ketone, allowing the other enantiomer to remain unreacted, and separating the optically active alcohol.

Then, the immobilized green pea protein(IPP), soybean protein (ISP), and buckwheat protein (IBP) were used for the resolution of such racemic alcohols as in the form of 1-(4-bromophenyl)ethanol (*1*), 1-(4-chlorophenyl)ethanol (*2*), 1-(4-methoxyphenyl) ethanol (*3*), 1-(4-nitrophenyl)ethanol (*4*), and 1-(2-naphthyl)ethanol (*5*).

Process of producing optically active alcohol by selectively oxidizing one enantiomer of a racemic alcohol with green pea (Pisum sativum L.) protein

As shown here, the biochemical conversion reac-

Table 1. Biotransformation of Substrate *1-5* with Immobilized Green Pea Protein (IPP), Soybean Protein (ISP), Buckwheat Protein (IBP)

Substrate		Biocatalyst	Reaction Time (days)	Product			
Compd	Ar			Compd	%yield	%e.e.	$[\alpha]_D^{20}$
1		IPP	8	(*S*)-*1a*	57	88.2[a]	−35.70
		ISP	2	(*R*)-*1a*	54	82.2	+32.01
		IBS	11	(*R*)-*1a*	57	88.4	+36.11
2		IPP	8	(*S*)-*2a*	42	87.0[b]	−43.80
		ISP	3	(*R*)-*2a*	51	91.6	+45.54
		IBP	13	(*R*)-*2a*	58	96.3	+48.73
3		IPP	7	(*S*)-*3a*	48	95.4[c]	−55.44
		ISP	2	(*R*)-*3a*	50	99.2	+57.23
		IBP	6	(*R*)-*3a*	46	99.3	+57.16
4		IPP	4	(*R*)-*4a*	62	54.6[d]	+16.21
		ISP	5	(*S*)-*4a*	45	99.4	−28.27
		IBP	17	(*S*)-*4a*	25	99.7	−27.95
5		IPP	4	(*S*)-*5a*	50	99.8[e]	−51.81
		ISP	4	(*S*)-*5a*	49	99.2	−51.78
		IBP	4	(*S*)-*5a*	50	99.5	−51.90

[a] Determind by chiral HPLC t_R (*S*) 10.45, t_R (*R*) 11.03 (flow 0.5 cm^3 min^{1-}, detection 220 nm.)
[b] Determind by chiral HPLC t_R (*S*) 9.94, t_R (*R*) 10.36 (flow 0.5 cm^3 min^{-1}, detection 220 nm.)
[c] Determind by chiral HPLC t_R (*S*) 9.17, t_R (*R*) 10.78 (flow 1.0 cm^3 min^{-1}, detection 254 nm.)
[d] Determind by chiral HPLC t_R (*R*) 18.92, t_R (*S*) 19.56 (flow 1.0 cm^3 min^{-1}, detection 254 nm.)
[e] Determind by chiral HPLC t_R (*S*) 15.70, t_R (*R*) 17.05 (flow 0.5 cm^3 min^{-1}, detection 254 nm.)

tion of IPP for (±)-*1*, *2*, *3*, *4*, *5* (199–202 mg) required 8 for *1*, 8 for *2*, 7 for *3*, 4 for *4*, 4 for *5* (days) by going through bioconversion to 4-substituted acetophenones (*1b-4b*) and 2-acetonaphthone (*5b*) (86 mg for *1b*, 103 mg for *2b*, 101 mg for *3b*, 75 mg for *4b*, 99 mg for *5b*) accompanying sterically selective oxidation of (*R*)-*1*, (*R*)-*2*, (*R*)-*3*, (*S*)-*4*, (*R*)-*5* to obtain 114 mg of (*S*)-*1a*, 84 mg of (*S*)-*2a*, 96 mg of (*S*)-*3a*, 124 mg of (*R*)-*4a*, 100 mg of (*S*)-*5a* at % yield of 57%, 42%, 48%, 62%, 50%. The resulted yield and optical purity are shown in Table 1, 2.

Process of producing optically active alcohol by selectively oxidizing one enantiomer of a racemic alcohol with soybean (Glycine max.) protein

The first step of extraction of soybean water-soluble protein and the second step of immobilization were done by the same way as in green pea protein. In the third step, after washing soybean-calcium arginate gel beads with distilled water at 35°C, respective racemic alcohols, namely (±)-*1-5* were added followed by substrate conversion. The results are shown in Table 1, 2.

Process of producing optically active alcohol with buckwheat (Fagopyrum esculentum.) protein

Immobilized buckwheat protein (IBP) was also obtained by the same procedure as in the case of green pea protein and respective racemic alcohols, (±)-*1-5* was done. The results are shown in Table 1, 2.

Effectiveness of continuous recycling of green pea, soybean and buckwheat proteins

IPP, ISP, IBP could be reused consecutively at least three times without any decrease in yield and optical purity in the case of bioconversion of (±)-*5*, (±)-*3*, (±)-*5* respectively. The results obtained above are shown in Table 3.

Discussions

These results demonstrated that stereoselective oxidation of only (*R*)-*5* of a substrate (±)-*5* to *5b* left (*S*)-*5a* with an optical purity higher than 99%e.e. and either of ISP or IBP was effective for such resolution as well as the proteins from IPP (Scheme 1).

In addition, the resolution for other racemic substrate; (±)-*1*, (±)-*2*, (±)-*3*, (±)-*4* was attempt and the optical active alcohols were obtained from these substrates as well as (*S*)-*5a* as shown in Table 1. These results suggested that specific use for each enantiomer of the racemic alcohols; (±)-*1*, (±)-*2*, (±)-*3* and (±)-*4* become possible through a selective use of IPP, ISP, IBP (Scheme 1).

Table 2. Biochemical Conversion of IPP, ISP, IBP for Substrate *1-5* by Going through Bioconversion to *1b-5b* Accompanying Sterically Selective Oxidation of Only One Isomer of One to Obtain Optically Active Alcohols

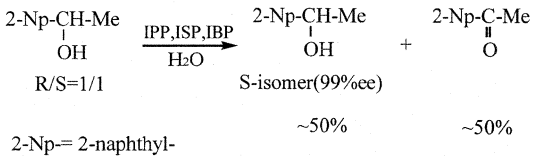
Substrate		Biocatalyst	Reaction Time (days)	Oxidized isomer	Product (*1b-5b*)		
Compd	mg				Compd	mg	%yield
1	200	IPP	8	(*R*)-*1*	*1b*	86	43
	204	ISP	2	(*S*)-*1*	*1b*	91	45
	200	IBP	11	(*S*)-*1*	*1b*	82	41
2	200	IPP	8	(*R*)-*2*	*2b*	103	52
	202	ISP	3	(*S*)-*2*	*2b*	98	49
	207	IBP	13	(*S*)-*2*	*2b*	82	40
3	200	IPP	7	(*R*)-*3*	*3b*	101	51
	201	ISP	2	(*S*)-*3*	*3b*	99	49
	207	IBP	6	(*S*)-*3*	*3b*	106	51
4	200	IPP	4	(*S*)-*4*	*4b*	75	38
	218	ISP	5	(*R*)-*4*	*4b*	103	47
	204	IBP	17	(*R*)-*4*	*4b*	121	59
5	199	IPP	4	(*R*)-*5*	*5b*	99	50
	203	ISP	4	(*R*)-*5*	*5b*	102	51
	212	IBP	4	(*R*)-*5*	*5b*	99	47

Table 3. Chiral Resolution of (±)-*5*, (±)-*3*, (±)-*5* Using Immobilized IPP, ISP, IBP and Consecutive Reuse (2nd, 3rd) of One

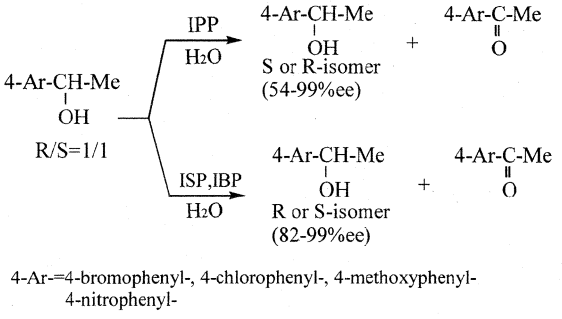
Catalysts	Reused Times	Time/h[a]	Config.	CY/%[b]	OP/%e.e.[c]
IPP	1st/2nd/3rd	96/48/36	*S*/*S*/*S*	50/50/50	>99/>99/>99
ISP	1st/2nd/3rd	48/24/24	*R*/*R*/*R*	50/50/50	>99/>99/>99
IBP	1st/2nd/3rd	96/96/96	*S*/*S*/*S*	50/50/50	>99/>99/>99

[a] Reaction time.
[b] Chemical yield.
[c] Optical purity measured by HPLC.

Example 1 (substrate; (±)-5)



Example 2 (substrate; (±)-1-4)



Scheme 1. A Possible Pathway of the Biotransformation of (±)-*1-5* with Immobilyzed Pea Protein (IPP), Soybean Protein (ISP), Buckwheat Protein (IBP).

In respect of consecutive reuse of the ISP, IBP, it was demonstrated that those are usable at least three times with no reduction in yield and optical purity (Table 3).

We reported here, a new method to produce an optically active alcohol which specifically oxidizes only one enantiomer of a racemic alcohol with new biological catalysts; water-soluble proteins from cereals and beans.

References

1) a) Nakamura, K. and Matsuda, T. J., Asymmetric reduction of ketones by the acetone powder of *Geotrichum candidum*. *Org. Chem.*, **63**, 8957–8964 (1998).
b) Puntambekar, H. M. and Naik, D. G., Geotrichum candidum assisted synthesis of sitophilate, male aggregation pheromone of granary weevil. *Synth. Commun.*, **28**, 2399–2406 (1998).
c) Salvi, N. A., Udupa, S. R., and Banerji, A., Chiral synthesis of α-phenylpyridylmethanols with *Rhizopus arrhizus*. *Biotechnol. Lett.*, **20**, 201–203 (1998).

2) a) Kataoka, M., Yamamoto, K., Kawabata, H., Wada, M., and Shimizu, S., Stereoselective reduction of ethyl 4-chloro-3-oxobutanoate by *Escherichia coli* transformant cells coexpressing the aldehyde reductase and glucose dehydrogenase genes. *Appl. Microbiol. Biotechno.*, **51**, 486–490 (1999).
b) Kataoka, M., Rohani, L. P. S., Yamamoto, K., Wada, M., Kawabata, H., Shimizu, S., Kita, K., and Yanase, H., Enzymatic production of ethyl (*R*)-4-chloro-3-hydroxybutanoate: asymmetric reduction of ethyl 4-chloro-3-oxobutanoate by an *Escherichia coli* transformant expressing the aldehyde reductase gene from yeast. *Appl. Microbiol. Biotechnol.*, **48**, 699–703 (1997).

3) a) Matumoto, K., Kitajima, H., and Nakata, T., Enantioselectivity-promoting factor in enzyme-mediated asymmetric hydrolysis of enol esters. *J. Mol. Catal.*, **1**, 17–21 (1995).
b) Ozasa, N., Tokioka, K., and Yamamoto, Y., Asymmetric trans-esterification of meso-2,5-dibromoadipate and synthesis of optically active. *Biosci. Biotechnol. Biochem.*, **59**, 1905–1906 (1995).
c) Naemura, K., Fukuda, R., Takahasi, N., Konishi, I. M., Hirose, Y., and Tobe, Y., Enzyme-catalyzed asymmetric acylation and hydrolysis of *cis*-2,5-disubstituted tetrahydrofuran derivatives: contribution to development of models for reactions catalyzed by porcine liver esterase and porcine pancreatic lipase. *Tetrahedron. Asym.*, **4**, 911–918 (1993).

4) a) Naoshima, Y. and Akakabe, Y., Biotransformation of aromatic ketones with cell cultures of carrot, tobacco, and gardenia. *Phytochemistry*, **30**, 3595–3597 (1991).
b) Akakabe, Y., Takahashi, M., Kamezawa, M., Kikuchi, K., Tachibana, H., Ohtani, T. and Naoshima, Y., Biocatalytic preparation of chiral alcohols by enantioselective reduction with immobilized cells of carrot. *J. Chem. Soc. Perkin Trans 1*, 1295–1995 (1995).
c) Takemoto, M., Moriyasu, Y. and Achiwa, K., Synthesis of optically active α-phenylpyridylmethanols with cell cultures of *Nicotiana tabacum*. *Chem. Pharm Bull.*, **43**, 1458–1461 (1995).

5) Nagaoka, H. and Kayahara, H., Resolution and synthesis of (±)-1-(2-naphthyl)ethanol with immobilized pea protein: as a new biocatalyst. *Biosci. Biotechnol. Biochem.*, **63**, 1991–1992 (1999).