Bioscience, Biotechnology, and Biochemistry

ISSN: 0916-8451 (Print) 1347-6947 (Online) Journal homepage: http://www.tandfonline.com/loi/tbbb20

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To cite this article: Hiroyuki NAGAOKA, Hiroshi KAYAHARA & Yasushi WAKABAYASHI (2001) Resolution and Synthesis of Optically Active Alcohols with Immobilized Ovalbumin and Pea Protein as New Bio-catalysts, Bioscience, Biotechnology, and Biochemistry, 65:3, 634-637, DOI: 10.1271/bbb.65.634

To link to this article: http://dx.doi.org/10.1271/bbb.65.634

Published online: 22 May 2014.

Biosci. Biotechnol. Biochem., **65** (3), 634–637, 2001

Resolution and Synthesis of Optically Active Alcohols with Immobilized Ovalbumin and Pea Protein as New Bio-catalysts

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Received October 11, 2000; Accepted November 28, 2000

It was found that ovalbumin stereoselectively oxidized one of the enantiomers of p-substituted racemic alcohols, thereby providing optically active alcohols with high optical purities. It was found out that, when used appropriately in combination with immobilized pea protein, immobilized ovalbumin made it possible to resolve and synthesize racemic 1-(2-naphthyl)ethanol, 1-phenylethanol, and 1-phenyl-1-propanol. Immobilized ovalbumin could be continuously recycled at least three times without lowering the yield and purity of the products. These results suggested that cereals, beans, and ovalbumin might have additional fourth function among conventional foods. Namely, there might contain nutritional, sensory, biologically regulatory and bio-catalytic functions in conventional foods.

Key words: immobilized ovalbmin; 1-(4-substituted-phenyl) ethanol; 1-(2-naphthyl)ethanol; 1-phenylethanol; 1-phenyl-1-propanol

In recent years, optically active alcohols have become progressively more important as intermediates in synthesizing drugs, pesticides, perfumes, liquid crystal materials, and also in the field of fine chemistry.

The methods for synthesizing these optically active alcohols have been reported in such ways as to use microbial cells,[1)] microbial cells with overexpressed enzymes,[2)] enzyme originating in animal tissues such as swine pancreatic lipase,[3)] and cultured plant cells.[4)] Under these circumstances, we have recently reported a novel synthesis method which comprises preparing a powdery crude protein fraction from cereal and bean tissues, treating substrate racemic alcohols with these fractions, and thus stereoselectively oxidizing one of enantiomers to thereby resolve optically active alcohols with high optical purity.[5)] In those reports, we pointed out that immobilized cereal and bean proteins can be continuously recycled at least three times, and that enantiomers can be resolved by using proper cereal and bean varieties. Moreover, we suggested that cereal and bean powders obtaind by two steps including isoelectric precipitation and spray-drying are usable as asymmetric catalysts.

In this study, we have paid attention to an ovalbumin powder prepared from chicken eggs by two steps including ammonium sulfate treatment and spray-drying, and attempted to use it effectively as a new asymmetric catalyst.

Recently, ovalbumin and cereal and bean protein fractions have come to be considered as allergens. Therefore, it is seemingly highly interesting to point out that these substances are effectively usable not only as foods but also as biological catalysts in the field of synthetic organic chemistry.

Materials and Methods

Extraction of ovalbumin and pea protein. Albumin was isolated roughly from chicken egg. After addition of distilled water to the albumin, ammonium sulfate was added and the mixture was centrifuged (6000 rpm, 30 min). Thus ovalbumin was obtained as the precipitate. Distilled water was added to the precipitate fraction to give an aqueous ovalbumin solution (0.1 to 5%) which was then spray-dried, thereby giving powdered ovalbumin (OA).

On the other hand, green peas were crushed, and the large pieces and the husks were removed. Water-soluble protein was dissolved in distilled water at pH 7.0. The precipitated food fiber was removed and the protein was precipitated at its isoelectric point by bringing the water-soluble protein to pH 4.5. After redissolving the protein precipitate in distilled water at pH 7.0, spray-dring was done on the resulting green pea water-soluble protein solution to prepare powdered pea protein (PP).[5)]

Immobilization of pea protein and ovalbumin. The OA and PP were immobilized separately as follows:

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OA (20 g) or PP (20 g) was dissolved in distilled water (200 ml), and 5% aq. Na alginate (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 = 4–5 mm) including the OA or PP were yielded. After the beads were kept in the 0.6% aq. $CaCl_2$ for more 5 hours, the beads were separated from the solution and then washed with distilled water.

Resolution of alcohols. The immobilized OA and PP were used for the resolution of substrate alcohols; IOA for (±)-**1**–**7** and IPP for (±)-**5**–**7**. That is, 201 ~ 213 mg of substrate (±)-**1**–**7** was added to distilled water (400 ml) containing IOA or IPP at 33 ~ 37°C and for each, the reaction solution was incubated 24–168 hours at 35°C on a rotary shaker (55 rpm).

The substrate alcohols and the produced ketones were monitored with an interval of 4 hours using GC and the reaction was stopped after confirming the end of conversion. Each extract was analyzed by GC with a PEG-20M 25 m × 0.25 mm TC-5HT fused silica capillary column at 150°C–180°C.

Finally, the IOA or IPP was removed from the reaction solution, and the reactant and reaction product 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.[5]

General detection procedure of optical purity. The stereochemistry 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.[4,5]

The IR and 1H NMR spectra of *1a–7a* were identical with those of (±)-*1–7*.

The e.e. (= enantiomeric excess) of *1a–7a* 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*, *6*, *7*, 1.0 $cm^3\ min^{-1}$ for (±)-*3*, *4*; detection light 220 nm for (±)-*1*, *2*, 254 nm for (±)-*3*, *4*, *5*, *6*, *7*, retention time (t_R) is shown in Table 1].

Results

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

Then, the immobilized ovalbumin (IOA) was used for the resolution of racemic alcohols 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**), 1-phenylethanol (**6**), 1-phenyl-1-propanol (**7**). And immobilized pea protein (IPP) was used in the form of (±)-**5**, **6**, **7** (already reported for (±)-**1**–**4**[5]).

Process of producing optically active alcohol by selectively oxidizing one enantiomer of a racemic alcohol with ovalbumin

As shown here, the biochemical conversion reaction of IOA for (±)-**1**, **2**, **3**, **4**, **5**, **6** and **7** required 24 for **1**, 24 for **2**, 24 for **3**, 24 for **4**, 24 for **5**, 48 for **6**, 144 for **7** (hours) by going through bioconversion to **1b**, **2b**, **3b**, **4b**, **5b**, **6b**, **7b** accompanying stereoselective oxidation of (S)-**1**, (S)-**2**, (S)-**3**, (R)-**4**, (S)-**5**, (S)-**6**, (S)-**7** to obtain (R)-**1a**, (R)-**2a**, (R)-**3a**, (S)-**4a**, (R)-**5a**, (R)-**6a**, (R)-**7a** at %yield of 27%, 26%, 26%, 25%, 24%, 41%, 45%. The resulted yield and optical purity are shown in Tables 1, 2.

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 reaction of IOA for (±)-**5**, **6** and **7** required 96 hours for **5**, 120 for **6**, and 168 for **7** by going through bioconversion to 2-acetonaphthone (**5b**) and 1-phenylalkylketone (**6b**–**7b**) accompanying sterically selective oxidation of (S)-**5**, (S)-**6**, and (S)-**7** to obtain (R)-**5a**, (R)-**6a**, and (R)-**7a** at %yields of 50%, 49%, and 49%. The resulted yields and optical purity are shown in Tables 1, 2.

Effectiveness of continuous recycling of ovalbumin

IOA could be reused consecutively at least three times without any decrease in yield and optical purity in the case of bioconversion of (±)-**1**–**3** and (±)-**5**, respectively. The results obtained above are shown in Table 3.

Discussion

It is clarified that immobilized ovalbumin asymmetrically oxidizes selectively one of the enantiomers of p-substituted racemic alcohols (**1**–**4**) to thereby provides optically active alcohols having high optical purity and that the reaction time of IOA is faster than one of IPP for the substrate (±)-**1**–**4** (Tables 1 and 2).

We further found out that immobilized ovalbumin can be continuously recycled at least three times without lowering the yield or optical purity and that the reaction time of IOA is shorted by the connected reuse (Table 3).

In addition, it is found out that the specific use for each enantiomer of the racemic alcohols; (±)-**5**, (±)-**6** and (±)-**7** become possible through a selective use of IOA and IPP (Scheme 1).

Table 1. Biotransformation of Substrate 1-7 with Immobilized Ovalbumin (IOA) and Pea Protein (IPP)

Substrate			Biocatalyst	Reaction time (hours)	Product			
Compd	Ar-	alkyl-			Compd	%Yield	%e.e.	$[\alpha]_D^{20}$
1		Me	IOA	24	(R)-1a	27	86.6[a]	+34.55
2		Me	IOA	24	(R)-2a	26	96.4[b]	+48.70
3		Me	IOA	24	(R)-3a	26	99.8[c]	+57.33
4		Me	IOA	24	(S)-4a	25	79.6[d]	−20.81
5		Me	IOA IPP	24 96	(R)-5a (S)-5a	24 50	85.8[e] 99.8	+45.95 −51.81
6		Me	IOA IPP	48 120	(R)-6a (S)-6a	41 49	93.2[f] 94.2	+55.06 −55.75
7		Et	IOA IPP	144 168	(R)-7a (S)-7a	45 49	90.2[g] 93.8	+45.11 −46.98

[a] Measured by chiral HPLC t_R(S) 10.45, t_R(R) 11.03 (flow 0.5 cm^3 min^{-1}, detection 220 nm).
[b] Measured by chiral HPLC t_R(S) 9.94, t_R(R) 10.36 (flow 0.5 cm^3 min^{-1}, detection 220 nm).
[c] Measured by chiral HPLC t_R(S) 9.17, t_R(R) 10.78 (flow 1.0 cm^3 min^{-1}, detection 254 nm).
[d] Measured by chiral HPLC t_R(R) 18.92, t_R(S) 19.56 (flow 1.0 cm^3 min^{-1}, detection 254 nm).
[e] Measured by chiral HPLC t_R(S) 15.70, t_R(R) 17.05 (flow 0.5 cm^3 min^{-1}, detection 254 nm).
[f] Measured by chiral HPLC t_R(S) 11.96, t_R(R) 13.13 (flow 0.5 cm^3 min^{-1}, detection 254 nm).
[g] Measured by chiral HPLC t_R(S) 8.19, t_R(R) 8.91, (flow 0.5 cm^3 min^{-1}, detection 254 nm).

Table 2. Biochemical Conversion of IOA and IPP for Substrate 1-7 by Going Through Bioconversion to 1b-7b Accompanying Sterically Selective Oxidation of Only One Isomer of One to Obtain Optically Active Alcohols

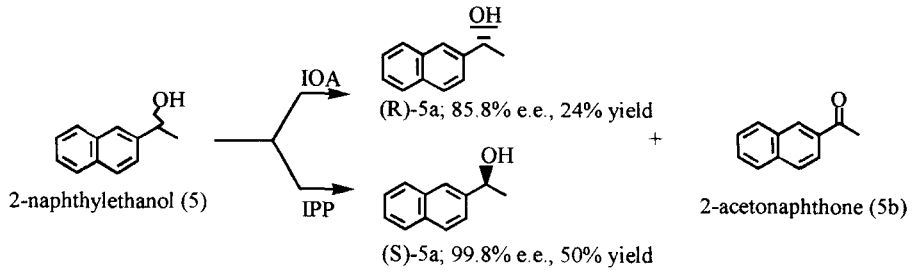
Substrate		Biocatalyst	Reaction time (hours)	Rreferred substrate	Product (1b-7b)		
Compd	mg				Compd	mg	%Yield
1	209	IOA	24	(S)-1a	1b	148.4	71
2	210	IOA	24	(S)-2a	2b	153.3	73
3	203	IOA	24	(S)-3a	3b	148.2	73
4	204	IOA	24	(R)-4a	4b	146.9	72
5	207	IOA	24	(S)-5a	5b	155.3	75
	201	IPP	96	(R)-5a	5b	98.5	49
6	210	IOA	48	(S)-6a	6b	119.7	57
	209	IPP	120	(R)-6a	6b	104.5	50
7	213	IOA	144	(S)-7a	7b	112.9	53
	210	IPP	168	(R)-7a	7b	105.0	50

Table 3. Biotransformation of Substrate 1-3 and 5 with Immobilized Ovalbumin (IOA)

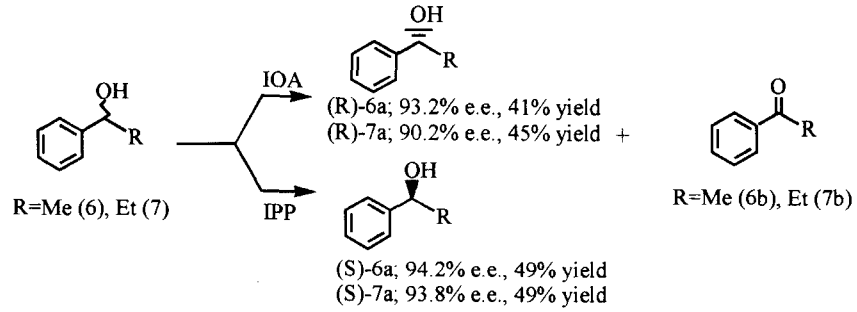
Substrate		Biocatalyst	Reused times	Time/h[a]	Product			
Compd	Ar-				Compd	CY/%[b]	OP/% e.e.[c]	$[\alpha]_D^{20}$
1		IOA	1st	24	(R)-1a	27	86.6	+34.55
			2nd	20	(R)-1a	27	91.2	+39.17
			3rd	16	(R)-1a	27	86.8	+34.81
2		IOA	1st	24	(R)-2a	26	96.4	+48.70
			2nd	20	(R)-2a	26	95.8	+48.10
			3rd	16	(R)-2a	26	96.2	+48.54
3		IOA	1st	24	(R)-3a	26	99.8	+57.33
			2nd	20	(R)-3a	27	99.6	+57.21
			3rd	16	(R)-3a	27	99.8	+57.40
5		IOA	1st	24	(R)-5a	24	85.8	+45.95
			2nd	12	(R)-5a	24	88.2	+47.55
			3rd	10	(R)-5a	24	88.6	+48.21

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

Example 1 (substrate 5)



Example 2 (substrate 6-7)



Scheme 1. A Possible Pathway of the Biotransformation of (±)-**5**-**7** with Immobilyzed Ovalbumin (IOA) and Pea Protein (IPP).

These results suggest that there should exist a fourth function, namely a bio-catalytic function in conventional foods. Furthermore, it was found out that allergens such as ovalbumin, cereal, and bean proteins are effectively usable as biological catalysts in the field of synthetic organic chemistry.

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Bioscience, Biotechnology, and Biochemistry 2001.65:634-637.