ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 15 (2001) 101–104

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Letter

Stereoselective reduction of keto esters with marine micro algae

Kohji Ishihara [a,*], Nobuyoshi Nakajima [b], Hitomi Yamaguchi [c], Hiroki Hamada [d], Yuu-shi Uchimura [e]

[a] *Department of Chemistry, Kyoto University of Education, Fushimi-ku, Kyoto 612-8522, Japan*
[b] *Department of Nutritional Science, Okayama Prefectural University, Soja, Okayama 719-1197, Japan*
[c] *Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan*
[d] *Department of Applied Science, Okayama University of Science, Ridai-cho, Okayama 700-0005, Japan*
[e] *Ehime Prefectural Fisheries Experimental Station, Uwazima, Ehime 798-0104, Japan*

Received 13 November 2000; received in revised form 28 November 2000; accepted 8 December 2000

Abstract

It was found that α- and β-keto esters were converted to the corresponding hydroxy esters by marine micro algae. Ethyl 2-methyl-3-oxobutanoate was reduced by *Nannochloropsis* sp. to the *anti*-hydroxy ester with excellent diastereo- (*syn*/*anti* = 1:99) and high enantioselectivity (*anti* >99%, *syn* 98%). The stereocontrolled reduction of ethyl 3-methyl-2-oxobutanoate was accomplished by *Nannochloropsis* sp. or *Chaetoceros gracilis* in the presence of L-lactic acid as an additive.

Keywords: Marine micro algae; Stereoselective reduction; Keto ester; Biocatalyst; Phytoplankton

The asymmetric reduction of carbonyl compounds by microorganisms is one of the methods widely used to obtain optically active alcohols [1–4]. This kind of biotransformation has been investigated with the specific use of bakers' yeast and fungi to effect stereoselective reduction of wide range of ketones such as α- and β-keto esters [3–9]. To date, several studies concerning the reduction of keto esters with other microorganisms (except yeast and fungi) have been reported [4,10,11]. However, little information is known about the reduction of carbonyl compounds using such microorganisms as biocatalysts. Until now, we reported that aerobic thermophilic bacteria such as *Bacillus stearothermophilus*, *Pseudonocardia thermophila*, and *Streptomyces thermocyaneoviolaceus* had high reducing abilities toward several keto esters and produced the corresponding chiral hydroxy esters [12–14]. Furthermore, we found that *Chlorella* strains, one of the micro green algae, catalyzed the stereoselective reduction of α- and β-keto esters [15]. In this paper, we would like to report that marine micro algae such as *Chaetoceros* and *Nannochloropsis* are available as a tool for stereoselective reduction in organic asymmetric synthesis (Fig. 1).

Ethyl pyruvate (**1a**) and ethyl 2-methyl-3-oxobutanoate (**3**) were purchased from Wako Pure Chemical Industries, Ltd., Japan. Ethyl 3-methyl-2-oxobutanoate (**1f**) was purchased from Aldrich, USA. Ethyl benzoylformate (**1g**) was obtained from Tokyo Kasei Kogyo, Japan. Ethyl 2-oxobutanoate (**1b**),

* Corresponding author. Tel: +81-75-644-8265; fax: +81-75-645-1734.
E-mail address: kishi@kyokyo-u.ac.jp (K. Ishihara).

1381-1177/01/$ – see front matter
PII: S1381-1177(00)00247-2

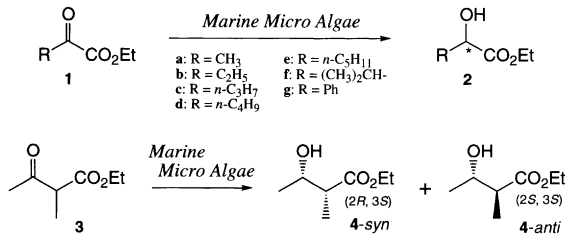

Fig. 1. Reduction of α- and β-keto esters by marine micro algae.

ethyl 2-oxopentanoate (**1c**), ethyl 2-oxohexanoate (**1d**), ethyl 2-oxoheptanoate (**1e**), and α-hydroxy esters (**2a–g**) were prepared according to the literature procedure [16]. *Chaetoceros gracilis* (EPFES-YU-1), *Chaetoceros* sp. (EPFES-YU-2), *Nannochloropsis* sp. (EPFES-YU-3), and *Pavlova lutheri* (EPFES-YU-4) were type cultures from Ehime Prefectural Fisheries Experimental Station, Uwazima, Ehime, Japan. The marine algae were photoautotrophically cultivated in a synthetic seawater (1 l) for 2 weeks at 20°C with constant aeration by air (2 l/min) in baffled 1 l flasks with illumination by white fluorescent light (1000 lx, one side). The synthetic seawater is comprised of 20.747 g NaCl, 0.8 μg $MnCl_2{\cdot}4H_2O$, 9.474 g $MgCl_2{\cdot}6H_2O$, 1.326 g $CaCl_2{\cdot}6H_2O$, 3.505 g Na_2SO_4, 597 mg KCl, 171 mg $NaHCO_3$, 85 mg KBr, 34 mg $Na_2B_4O_7{\cdot}10H_2O$, 12 mg $SrCl_2$, 3 mg NaF, 1 mg LiCl, 0.07 mg KI, 0.2 μg $CoCl_2{\cdot}6H_2O$, 8 μg $AlCl_3{\cdot}6H_2O$, 5 μg $FeCl_3{\cdot}6H_2O$, 0.2 μg $Na_2WO_4{\cdot}2H_2O$, 0.02 mg $(NH_4)_6Mo_7O_{24}$, and 1.07 ml of NM solution per 1 l of distilled water. The NM solution is one of the vitamin solutions: $NaNO_3$ (150 g), Na_2HO_4 (10 g), EDTA-2Na (0.9 g), Vitamin B_{12} (1.5 mg), thiamine·HCl (75 mg), biotin (1 mg), EDTA-Fe (2.5 g), and $H_2NC(CH_3OH)_3$ (5 g) were dissolved in 1 l of distilled water. *Chaetoceros* and *Pavlova* were grown in the synthetic seawater containing 0.0045% sodium silicate (Na_2SiO_3). The wet cells were collected by centrifugation at 8000 × *g* for 15 min (about 3 g of wet cells was obtained from 500 ml of the each cultures). The seawater-washed cells were resuspended in a large test tube (ϕ 30 mm × 200 mm) containing 20 ml of the seawater; then the substrate (0.15 mmol, 7.5 mM) was added and incubated at 20°C with aerobic shaking under illumination by white fluorescent light (1000 lx, one side). A portion of the reaction mixtures was filtered using an Extrelut® short column, extracted with diethyl ether, and then concentrated under reduced pressure. The conversion of products (**2a–g**, **4**) was determined using a GLC equipped with a capillary DB-WAX column (0.25 mm × 30 m, 110–150°C). The enantiomeric excesses (e.e.) of the products were measured using a GLC equipped with a chiral stationary phase capillary CP-Chirasil-DEX CB (0.25 mm × 25 m, 80–150°C) column (**2a–e**, **2g**, and **4**) and a Chiraldex G-TA (0.25 mm × 40 m, 90°C) column (**2f**). The absolute configuration of the hydroxy esters **2a–f**, **4**-*syn*, and **4**-*anti* were determined by comparing their retention time with those of authentic samples [16].

Four selected marine algae were tested for reducing abilities toward α- and β-keto esters. The results of the reduction of α-keto esters (**1a–g**) and a β-keto ester (**3**) are summarized in Tables 1 and 2, respectively.

It was found that seven α-keto esters were converted to the corresponding α-hydroxy esters by the four marine algae. All marine algae reduced ethyl benzoylformate (**1g**) to the corresponding alcohol (**2g**) with a high conversion ratio; however, the enantioselectivity of the product **2g** showed a low enantiomeric excess (e.e.). The reduction of ethyl 2-oxoheptanoate (**1e**) by *C. gracilis* gave the corresponding alcohol **2e** in high e.e. Ethyl 3-methyl-2-oxobutanoate (**1f**) was reduced by *Nannochloropsis* sp. to (*R*)-**2f** in high e.e. (98%) with high conversion ratio (99%, GC yield: 78%).

The reduction of ethyl-2-methyl-3-oxobutanoate (**3**) by the micro algae gave the corresponding *anti*-hydroxy ester (*anti*-**4**) in low conversion ratios (25–68%). In particular, the substrate was reduced by *Nannochloropsis* sp. to the *anti*-hydroxy ester with excellent diastereo- (*syn*/*anti* = 1:99) and high enantioselectivity (>99%), when compared with the selectivity of **4** reduced by *Saccharomyces cerevisiae* [17], *Chlorella* [15], and *Glycine max* [18], which produced *syn*-hydroxy ester predominantly.

Furthermore, effects of additives on the conversion ratio and the stereochemistry of the product (**2f**) were investigated as shown in Table 3.

The reduction of **1f** by *C. gracilis* in the presence of glucose gave the corresponding α-hydroxy ester **2f** in a high conversion ratio (>99%); however, the enantioselectivity of the product was low (51%, *R*). In the algal reduction, the addition of DL-lactatic acid decreased the conversion ratio, while the enantioselectivity of the product increased. The enantioselectivity

Table 1
The reduction of α-keto esters by marine micro algae[a]

Products	*Chaetoceros gracilis*			*Chaetoceros* sp.			*Nannochloropsis* sp.			*Pavlova lutheri*		
	Conversion (%)[b]	e.e. (%)[c]	*R*/*S*[c]	Conversion (%)[b]	e.e. (%)[c]	*R*/*S*[c]	Conversion (%)[b]	e.e. (%)[c]	*R*/*S*[c]	Conversion (%)[b]	e.e. (%)[c]	*R*/*S*[c]
2a	99	50	*S*	99	9	*R*	99	16	*S*	48	48	*S*
2b	78	10	*S*	44	8	*S*	99	3	*R*	36	13	*S*
2c	64	15	*S*	51	27	*S*	99	52	*S*	28	25	*R*
2d	82	80	*S*	72	82	*S*	70	25	*S*	59	7	*S*
2e	42	89	*S*	40	56	*S*	71	3	*R*	18	8	*R*
2f	66	18	*S*	66	8	*S*	99	98	*R*	53	50	*S*
2g	99	23	*S*	99	17	*R*	99	21	*R*	99	4	*R*

[a] The synthetic seawater (20 ml) and α-keto ester (**1a–g**) (7.5 mM) were added to the wet cells (0.3 g) and the reaction mixtures were incubated at 20°C for 24 h under light (1000 lx).
[b] The conversion was measured by GLC with a capillary column DB-WAX (0.25 mm × 30 m).
[c] The e.e. (%) and configuration were determined by GLC with an optically active capillary column CP-Chirasil-DEX CB (0.25 mm × 25 m).

Table 2
The reduction of β-keto esters by marine micro algae[a]

Marine algae	Conversion (%)[b]	*syn*/*anti*[b]	e.e. (%)[c]	
			syn-(2*R*, 3*S*)	*anti*-(2*S*, 3*S*)
Chaetoceros gracilis	25	30/70	98	>99
Chaetoceros sp.	41	18/82	97	>99
Nannochloropsis sp.	68	1/99	98	>99
Pavlova lutheri	40	21/79	96	>99

[a] Synthetic seawater (20 ml) and β-keto ester (**3**) (7.5 mM) were added to the wet cells (0.3 g) and the reaction mixtures were incubated at 20°C for 48 h under light (1000 lx).
[b] The conversion and *syn*/*anti* ratio were measured by GLC with a capillary column.
[c] The e.e. and configuration were determined by GLC with an optically active capillary column.

Table 3
Effects of additives on the reduction of **1f**[a]

Additives	*Chaetoceros gracilis*		*Nannochloropsis* sp.	
	Conversion (%)[b]	e.e. (%)[c]	Conversion (%)[b]	e.e. (%)[c]
No additive	66	18 (*S*)	99	98 (*R*)
Glucose	>99	51 (*R*)	84	10 (*R*)
DL-Lactic acid	46	99 (*R*)	64	39 (*R*)
Lithium D-lactate	0	–	0	–
L-Lactic acid	89	99 (*R*)	60	49 (*R*)

[a] Synthetic seawater (20 ml) α-keto ester (**1f**) (7.5 mM), and 1.5 mmol of additive (75 mM) were added to the wet cells (0.5 g) and the reaction mixtures were incubated at 30°C for 24 h under light (1000 lx).
[b] The conversion was measured by GLC with a capillary column.
[c] The e.e. and configuration were determined by GLC with an optically active capillary column.

of the product which was reduced under the acidic conditions (pH 3 and 5) was not changed (data not shown). In the presence of D-lactate ion, the substrate was not reduced. These results suggest that D-lactate ion is an inhibitor for both (*R*)- and (*S*)-α-hydroxy ester producing enzyme(s), while L-lactate ion inhibits only the activity of the (*S*)-enzyme(s). The stereocontrolled reduction of ethyl 3-methyl-2-oxobutanoate using *C. gracilis* was accomplished by the introduction of L-lactic acid as an additive. Thus, marine microalga was a useful tool for the stereoselective reduction of keto esters.

To gain insight into the mechanistic interpretation of the marine algal reduction, further detailed studies including purification of the oxidoreductases, which contribute to the reduction of α- and β-keto esters, are currently under investigation.

Acknowledgements

The authors would like to acknowledge the financial support of Æon Group Environment Foundation (2000) and the financial support of The Foundation of Sanyo Broadcasting (2000).

References

[1] S. Servi, Synthesis (1990) 1.
[2] R. Csuk, B.I. Gränzer, Chem. Rev. 91 (1991) 49.
[3] S.M. Roberts, J. Chem. Soc., Perkin Trans. I (1998) 157.
[4] T. Sugai, Curr. Org. Chem. 3 (1999) 373.
[5] K. Nakamura, Y. Kawai, T. Kitayama, T. Miyai, M. Ogawa, Y. Mikata, M. Higaki, A. Ohno, Bull. Inst. Chem. Res. Kyoto Univ. 67 (1989) 157.
[6] K. Nakamura, A. Ohno, J. Synth. Org. Chem. Jpn. 49 (1991) 110.
[7] D. Buisson, D.R. Azerad, Biocatalysis 5 (1992) 249.
[8] Y. Kawai, K. Takanobe, A. Ohno, Bull. Chem. Soc. Jpn. 68 (1995) 285.
[9] K. Nakamura, J. Mol. Catal. B: Enzym. 5 (1998) 129.
[10] Y. Yasohara, N. Kizaki, J. Hasegawa, S. Takahashi, M. Wada, M. Kataoka, S. Shimizu, Appl. Microbiol. Biotechnol. 51 (1999) 847.
[11] T. Kuramoto, K. Iwamoto, M. Izumi, M. Kirihara, F. Yoshizako, Biosci. Biotechnol. Biochem. 63 (1999) 598.
[12] K. Ishihara, K. Iwai, H. Yamaguchi, N. Nakajima, K. Nakamura, T. Ohshima, Biosci. Biotechnol. Biochem. 60 (1996) 1896.
[13] K. Ishihara, M. Nishitani, H. Yamaguchi, N. Nakajima, T. Ohshima, K. Nakamura, J. Ferment. Bioeng. 84 (1997) 268.
[14] K. Ishihara, H. Yamaguchi, H. Hamada, N. Nakajima, K. Nakamura, J. Mol. Catal. B: Enzym. 10 (1998) 429.
[15] K. Ishihara, H. Yamaguchi, N. Adachi, H. Hamada, N. Nakajima, Biosci. Biotechnol. Biochem. 64 (2000) 2099.
[16] K. Nakamura, K. Inoue, K. Ushio, S. Oka, A. Ohno, J. Org. Chem. 53 (1988) 2589.
[17] K. Nakamura, Y. Kawai, T. Miyai, A. Ohno, Tetrahedron Lett. 31 (1990) 3631.
[18] H. Hamada, Y. Miyamoto, N. Nakajima, T. Furuya, J. Mol. Catal. B: Enzym. 5 (1998) 187.