Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 1075–1078

Asymmetric reduction of a variety of ketones with a recombinant carbonyl reductase: identification of the gene encoding a versatile biocatalyst

Tadashi Ema,* Hideo Yagasaki, Nobuyasu Okita, Kumiko Nishikawa, Toshinobu Korenaga and Takashi Sakai*

Department of Applied Chemistry, Faculty of Engineering, Okayama University, Tsushima, Okayama 700-8530, Japan

Received 19 January 2005; accepted 1 February 2005

Abstract—The gene encoding a versatile biocatalyst that shows high enantioselectivity for a variety of ketones, SCR (*Saccharomyces cerevisiae* carbonyl reductase), has been identified, cloned, and expressed in *E. coli*. Recombinant *E. coli* co-producing SCR and GDH (glucose dehydrogenase) is an easy-to-use, synthetically useful biocatalyst, and 8 out of the 16 alcohols obtained had enantiomeric purities of >98% ee.

1. Introduction

Asymmetric synthesis with biocatalysts attracts much attention from the viewpoint of green chemistry.[1] Among many kinds of biocatalysts, carbonyl reductases from various microorganisms have been used to prepare optically active alcohols from carbonyl compounds.[2] We have been interested in versatile biocatalysts capable of showing high enantioselectivity and broad substrate specificity simultaneously.[3] A *Saccharomyces cerevisiae* (bakers' yeast) carbonyl reductase (SCR) previously reported by us showed catalytic activity for various ketones, such as α-chloro ketones, α-acetoxy ketones, α-keto esters, β-keto esters, γ-keto ester, and β-diketones; 13 out of the 20 alcohols obtained had the enantiomeric purities of >98% ee.[4] Despite the potential of this enzyme as a versatile biocatalyst, its utility was restricted by its low expression level in *S. cerevisiae* and the laborious purification procedure. These drawbacks may be overcome by constructing an SCR gene expression system. The synthetic power of recombinant carbonyl reductases from various origins has recently been demonstrated.[5–13] Here we identified and cloned the gene coding for SCR, and investigated the capabilities of the recombinant SCR by conducting both enzymatic and whole-cell reductions of various ketones.

2. Results and discussion

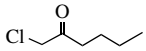
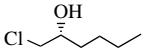
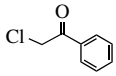
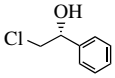
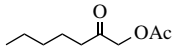
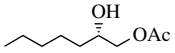
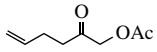
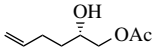
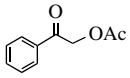
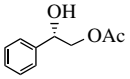
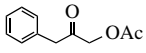
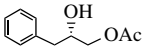
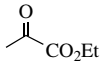
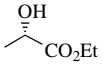
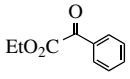
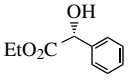
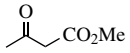
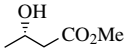
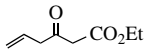
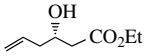
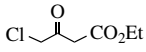
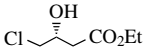
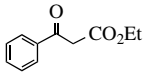
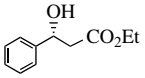
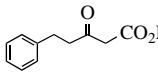
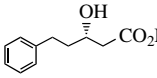
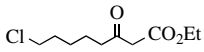
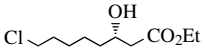
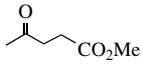
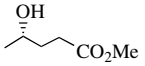
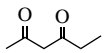
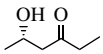
The amino-acid sequence analysis of purified SCR strongly suggested that the gene encoding SCR is *Gre2* (*YOL151w*). Although Stewart et al. have reported the asymmetric reduction of ketones using a recombinant Gre2 from a different strain of *S. cerevisiae*, the scope of its substrate specificity has not yet been investigated.[5c–f] Herein, the SCR gene was PCR-cloned from the genomic DNA of an *S. cerevisiae* strain that we used previously, and an expression plasmid, pESCR, was constructed. Enzymatic activity (90 U/1 g of wet cells) of SCR heterologously expressed in *E. coli* BL21(DE3) harboring pESCR was 800-fold higher than that (0.11 U/1 g of wet cells) of SCR in *S. cerevisiae*. The *E. coli* cell-free extract (CFE) containing SCR was used to reduce several ketones at 30 °C. To regenerate a catalytic amount of NADPH in situ, the glucose-6-phosphate (G6P)/glucose-6-phosphate dehydrogenase (G6PDH) system was employed as reported previously.[4] The results are shown in Table 1, where those previously obtained with SCR purified from *S. cerevisiae* are also shown for comparison.[4]

The absolute configurations of all eight alcohols **1b**, **4b**, **7b–9b**, **11b**, and **15b–16b** obtained with the CFE containing the recombinant SCR were found to be the same as those obtained with SCR purified from *S. cerevisiae*. The enantiomeric purities in the former are comparable to those in the latter. These results, suggesting

* Corresponding authors. Tel.: +81 86 251 8091; fax: +81 86 251 8092; e-mail: ema@cc.okayama-u.ac.jp

0957-4166/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2005.02.004

Table 1. Asymmetric reduction of ketones with recombinant SCR overproduced in *E. coli*

Reduction				Recombinant SCR[a]		Purified SCR[b]	
Substrate		Product		% Yield[c] (% ee)	*R*/*S*	% Yield[c] (% ee)	*R*/*S*
	**1a**		**1b**	15 (92) [21 (97)	*R* *R*][d]	64 (>99)	*R*
	**2a**		**2b**	5 (92)	*R*	49 (88)	*R*
	**3a**		**3b**	58 (>98)	*S*	80 (98)	*S*
	**4a**		**4b**	81 (99) [44 (95)	*S* *S*][d]	43 (>98)	*S*
	**5a**		**5b**	78 (98)	*S*	84 (96)	*S*
	**6a**		**6b**	71 (90)	*S*	96 (85)	*S*
	**7a**		**7b**	64 (94) [21 (97)	*S* *S*][d]	41 (>99)	*S*
	**8a**		**8b**	77 (92) [72 (92)	*R* *R*][d]	79 (95)	*R*
	**9a**		**9b**	57 (>99) [56 (>99)	*S* *S*][d]	64 (>99)	*S*
	**10a**		**10b**	69 (93)	*S*	22 (>99)	*S*
	**11a**		**11b**	56 (97) [74 (94)	*R* *R*][d]	84 (98)	*R*
	**12a**		**12b**	33 (70)	*R*	43 (72)	*R*
	**13a**		**13b**	68 (>98)	*S*	8 (>98)	*S*
	**14a**		**14b**	41 (98)	*S*	52 (90)	*S*
	**15a**		**15b**	65 (>99) [54 (>99)	*S* *S*][d]	39 (>99)	*S*
	**16a**		**16b**	70 (>99) [29 (>99)	*S* *S*][d]	69 (>99)	*S*

[a] Conditions: substrate (3.0 mmol for **1a**–**6a**, **8a**–**9a**, **11a**, and **15a**–**16a**, 6.0 mmol for **7a**, 1.0 mmol for **10a** and **12a**–**14a**), wet cells of *E. coli* BL21(DE3) harboring pACRGD (2 g), glucose (6.0 mmol), $NADP^+$ (12 μmol), 0.1 M phosphate buffer (pH 7.0, 50 mL), 30 °C.
[b] Results obtained with SCR purified from bakers' yeast. Data taken from Ref. 4.
[c] Isolated yield.
[d] Results obtained with the CFE of *E. coli* BL21(DE3) harboring pESCR.

that the target gene encoding SCR was successfully cloned, encouraged us to construct a more elaborate but easy-to-use biocatalyst, a recombinant whole-cell reduction system.

A number of recombinant whole-cell reductions with high efficiency have been reported.[6–8] To regenerate NADPH in vivo, Kataoka and co-workers have successfully employed a glucose dehydrogenase (GDH) from

Bacillus megaterium.[6] To improve productivity and efficiency, we constructed an expression plasmid having the SCR and GDH genes, pACRGD (Fig. 1). pACYCDuet-1 (Novagen) was selected as an expression vector because it has two multiple cloning sites, each preceded by a T7 promoter, which is suitable for co-expression of two genes. Using *E. coli* BL21(DE3) harboring pACRGD, a high level of production of SCR and GDH was confirmed by SDS-PAGE, with the SCR and GDH activities being 60 and 15,000 U/1 g of wet cells, respectively. The results of the whole-cell reductions of various ketones with this recombinant *E. coli* are summarized in Table 1.

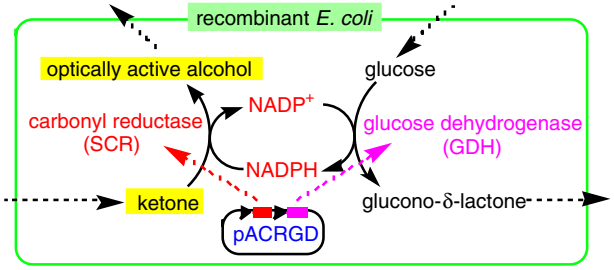


Figure 1. Recombinant *E. coli* coexpressing SCR and GDH genes to produce optically active alcohols.

Although the reaction did proceed without addition of $NADP^+$, the addition of a catalytic amount (0.4 mol %) of $NADP^+$ increased the conversion (not optimized). Although 3 mmol of ketone was typically used, the amount of a very reactive one, **7a**, was increased to 6 mmol, and that of some ketones with modest reactivity was decreased to 1 mmol. Table 1 clearly shows that the inherent characteristics of SCR are retained in the recombinant SCR. The absolute configurations of all 16 alcohols obtained herein were the same as those obtained with SCR purified from *S. cerevisiae*. The enantiomeric purities in the former are as high as those in the latter in most cases, and 8 out of 16 alcohols had enantiomeric purities of >98% ee. Both aliphatic and aromatic ketones were successfully reduced. Not only enantioselectivity but also regioselectivity for β-diketone **16a** were complete, with the less hindered carbonyl group being reduced exclusively, as observed previously.[4] Some of the obtained alcohols have been used in total synthesis of natural products and biologically active compounds, for example, (*R*)-**11b** for carnitine,[14] (*R*)-**12b** for fluoxetine,[15] (*S*)-**13b** for dihydrokawain,[16] (*S*)-**14b** for xestospongin A,[17] and (*S*)-**15b** for pyrenophorin.[18]

As a control, *E. coli* BL21(DE3) harboring pACYCDuet-1 was used for **1a**–**16a**, with very low conversions (0% conversion in many cases) confirmed by ^1H NMR in all cases. This suggests that although *E. coli* produces its own enzymes, its ketone-reducing and/or cofactor-regenerating capacity for 1–6 mmol of ketone is very low. Furthermore, *E. coli* BL21(DE3) strains harboring either pESCR or pABGD, which lack either GDH or SCR gene, were also examined for **5a** under the same reaction conditions, which resulted in very low conversions (4% and 0%). Table 1 shows that the difference in the NADPH-regenerating enzymes, G6PDH in the CFE reductions and GDH in the whole-cell reductions, did not affect the enantioselectivities. All these results indicate that SCR is responsible for the reductions of all the ketones and that both SCR and GDH (or G6PDH) are essential for the high-turnover catalysis.

3. Conclusion

In summary, the gene encoding the versatile biocatalyst that shows activity for a variety of ketones, SCR, has been identified, cloned, and expressed in *E. coli*. Recombinant SCR could be obtained efficiently, and showed simultaneously high enantioselectivity and broad substrate specificity as SCR purified from bakers' yeast did. Recombinant *E. coli* coexpressing SCR and GDH is an easy-to-use, synthetically useful biocatalyst. We have set up the basis to develop further the capabilities of the biocatalyst beyond the well-known paradigm of the lock-and-key concept by applying a variety of substrates and to evolve it for the purpose of environmentally benign organic synthesis.

Acknowledgements

We thank Dr. Hiroaki Yamamoto (Daicel Chemical Industries) and Prof. Tetsuo Toraya (Okayama University) for the determination of the internal amino-acid sequences of SCR and useful advice on gene expression, respectively. This work was supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science and by a grant from Venture Business Laboratory of Okayama University. We are grateful to the SC-NMR Laboratory of Okayama University for the measurement of NMR spectra.

References

1. Books: (a) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Elsevier: Oxford, 1994; (b) *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; Wiley-VCH: Weinheim, 2002; (c) Faber, K. *Biotransformations in Organic Chemistry*, 5th ed.; Springer: Berlin, 2004; (d) Bommarius, A. S.; Riebel, B. R. *Biocatalysis*; Wiley-VCH: Weinheim, 2004.
2. Recent reviews: (a) Sugai, T. *Curr. Org. Chem.* **1999**, *3*, 373; (b) Stewart, J. D. *Curr. Opin. Chem. Biol.* **2001**, *5*, 120; (c) Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, *14*, 2659; (d) Kataoka, M.; Kita, K.; Wada, M.; Yasohara, Y.; Hasegawa, J.; Shimizu, S. *Appl. Microbiol. Biotechnol.* **2003**, *62*, 437; (e) Kroutil, W.; Mang, H.; Edegger, K.; Faber, K. *Curr. Opin. Chem. Biol.* **2004**, *8*, 120.
3. (a) Ema, T.; Yamaguchi, K.; Wakasa, Y.; Yabe, A.; Okada, R.; Fukumoto, M.; Yano, F.; Korenaga, T.; Utaka, M.; Sakai, T. *J. Mol. Catal. B: Enzym.* **2003**, *22*, 181; (b) Ema, T. *Curr. Org. Chem.* **2004**, *8*, 1009; (c) Ema, T. *Tetrahedron: Asymmetry* **2004**, *15*, 2765.
4. (a) Ema, T.; Sugiyama, Y.; Fukumoto, M.; Moriya, H.; Cui, J.-N.; Sakai, T.; Utaka, M. *J. Org. Chem.* **1998**, *63*, 4996; (b) Ema, T.; Moriya, H.; Kofukuda, T.; Ishida, T.;

Maehara, K.; Utaka, M.; Sakai, T. *J. Org. Chem.* **2001**, *66*, 8682.
5. (a) Rodríguez, S.; Kayser, M.; Stewart, J. D. *Org. Lett.* **1999**, *1*, 1153; (b) Rodríguez, S.; Schroeder, K. T.; Kayser, M. M.; Stewart, J. D. *J. Org. Chem.* **2000**, *65*, 2586; (c) Rodríguez, S.; Kayser, M. M.; Stewart, J. D. *J. Am. Chem. Soc.* **2001**, *123*, 1547; (d) Kaluzna, I.; Andrew, A. A.; Bonilla, M.; Martzen, M. R.; Stewart, J. D. *J. Mol. Catal. B: Enzym.* **2002**, *17*, 101; (e) Kaluzna, I. A.; Matsuda, T.; Sewell, A. K.; Stewart, J. D. *J. Am. Chem. Soc.* **2004**, *126*, 12827; (f) Walton, A. Z.; Stewart, J. D. *Biotechnol. Prog.* **2004**, *20*, 403.
6. (a) Kataoka, M.; Yamamoto, K.; Kawabata, H.; Wada, M.; Kita, K.; Yanase, H.; Shimizu, S. *Appl. Microbiol. Biotechnol.* **1999**, *51*, 486; (b) Kizaki, N.; Yasohara, Y.; Hasegawa, J.; Wada, M.; Kataoka, M.; Shimizu, S. *Appl. Microbiol. Biotechnol.* **2001**, *55*, 590; (c) Yasohara, Y.; Kizaki, N.; Hasegawa, J.; Wada, M.; Kataoka, M.; Shimizu, S. *Tetrahedron: Asymmetry* **2001**, *12*, 1713.
7. (a) Yamamoto, H.; Matsuyama, A.; Kobayashi, Y. *Biosci. Biotechnol. Biochem.* **2002**, *66*, 481; (b) Yamamoto, H.; Matsuyama, A.; Kobayashi, Y. *Appl. Microbiol. Biotechnol.* **2003**, *61*, 133; (c) Yamamoto, H.; Mitsuhashi, K.; Kimoto, N.; Matsuyama, A.; Esaki, N.; Kobayashi, Y. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 638.
8. Itoh, N.; Matsuda, M.; Mabuchi, M.; Dairi, T.; Wang, J. *Eur. J. Biochem.* **2002**, *269*, 2394.
9. Wada, M.; Yoshizumi, A.; Furukawa, Y.; Kawabata, H.; Ueda, M.; Takagi, H.; Nakamori, S. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 1481.
10. Shimada, H.; Fujiki, S.; Oginuma, M.; Asakawa, M.; Okawara, T.; Kato, K.; Yamamura, S.; Akita, H.; Hara, A.; Imamura, Y. *J. Mol. Catal. B: Enzym.* **2003**, *23*, 29.
11. Costello, C. A.; Payson, R. A.; Menke, M. A.; Larson, J. L.; Brown, K. A.; Tanner, J. E.; Kaiser, R. E.; Hershberger, C. L.; Zmijewski, M. J. *Eur. J. Biochem.* **2000**, *267*, 5493.
12. Kao, C. M.; McPherson, M.; McDaniel, R. N.; Fu, H.; Cane, D. E.; Khosla, C. *J. Am. Chem. Soc.* **1998**, *120*, 2478.
13. Anson, C. E.; Bibb, M. J.; Booker-Milburn, K. I.; Clissold, C.; Haley, P. J.; Hopwood, D. A.; Ichinose, K.; Revill, W. P.; Stephenson, G. R.; Surti, C. M. *Angew. Chem., Int. Ed.* **2000**, *39*, 224.
14. (a) Zhou, B.; Gopalan, A. S.; VanMiddlesworth, F.; Shieh, W.-R.; Sih, C. J. *J. Am. Chem. Soc.* **1983**, *105*, 5925; (b) Kitamura, M.; Ohkuma, T.; Takaya, H.; Noyori, R. *Tetrahedron Lett.* **1988**, *29*, 1555.
15. Chênevert, R.; Fortier, G.; Rhlid, R. B. *Tetrahedron* **1992**, *48*, 6769.
16. Spino, C.; Mayes, N.; Desfossés, H. *Tetrahedron Lett.* **1996**, *37*, 6503.
17. Baldwin, J. E.; Melman, A.; Lee, V.; Firkin, C. R.; Whitehead, R. C. *J. Am. Chem. Soc.* **1998**, *120*, 8559.
18. Baldwin, J. E.; Adlington, R. M.; Ramcharitar, S. H. *Synlett* **1992**, 875.