# High Enantioselectivity and Broad Substrate Specificity of a Carbonyl Reductase: Toward a Versatile Biocatalyst

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*Received August 13, 2001*

## Introduction

The simultaneous achievement of high enantioselectivity and broad substrate specificity is one of the most important aspects of chiral catalysts. Recent experimental results[1] and mechanistic studies[2] indicate that even an enzyme can exert high enantioselectivity that is compatible with broad substrate specificity. A carbonyl reductase that we have purified from bakers' yeast (BY,[3] *Saccharomyces cerevisiae*) exhibits high enantioselectivity for a variety of ketones including α-chloro ketone, α-acetoxy ketone, α- and *β*-keto esters, and *β*-diketones.[4] This carbonyl reductase is a monomeric, NADPH-dependent enzyme with a molecular mass of ca. 37 kDa, although its natural substrate and function are unknown. BY is a useful biocatalyst,[5] partly because such an enzyme, capable of showing broad substrate specificity, is contained in the cells. Although many reductases have been isolated from BY[6] and other organisms,[7] little is known about the scope and limitation of substrate specificity of isolated enzymes. In this paper, we report that the potential capabilities of this enzyme as a versatile chiral biocatalyst are promising and even surprising.

## Results and Discussion

The purification of the enzyme and asymmetric reductions were carried out according to the procedures reported previously.[4] The glucose-6-phosphate (G6P)/glucose-6-phosphate dehydrogenase (G6PDH) system[8] was used to regenerate a catalytic amount of NADPH in the enzymatic reductions. Under the reaction conditions employed, the enzyme and NADPH make approximately 30 000 and 100 turnovers at 100% conversion, respectively.[4] The results of the asymmetric reductions using the purified enzyme or the BY whole cells are summarized in Table 1, where the previously reported results are also shown for comparison.

The enzymatic reductions showed higher enantioselectivity than the corresponding whole-cell reductions in most cases, and 13 out of 20 alcohols obtained in the former had the enantiomeric purity of more than 98% enantiomeric excess (ee) (Table 1). The selectivity was even inverted by using the purified enzyme as compared with the corresponding whole-cell reductions (entries 12−16 and 19). The isolated yields for some ester-containing alcohols were higher in the enzymatic reductions than in the corresponding whole-cell reductions (entries 5−8, 11, 13, 16−17, and 20), partly because hydrolases that cleave the ester bond of the substrates and/or the reduced products were not contained in the enzymatic reductions. The isolated yields for **32** and **35** in the enzymatic reductions were very low, which is due to the relatively low enzymatic activity for **12** and **15**.

Optically active alcohols useful for organic synthesis were obtained by the enzymatic reductions. Some of them have been used as chiral building blocks for natural products and biologically active compounds: e.g., (*R*)-**34** for fluoxetine,[9] (*S*)-**35** for dihydrokawain,[10] (*S*)-**36** for xestospongin A,[11] and (*S*)-**37** for pyrenophorin.[12] The antipodal enantiomer of (*S*)-**32** has been used in the syntheses of compactin analogues.[13] Compounds (*R*)-**22** and (*S*)-**26** are applicable to the syntheses of chiral host molecules.[14] As shown in Table 1, both aliphatic and aromatic ketones were successfully transformed. Not only α- and *β*-keto esters **9**−**16** but also *γ*-keto ester **17** were converted to the corresponding alcohols in high enantiomeric excesses. Entries 2, 6, and 12 indicate that olefin is inert for the enzyme. Previously, we have observed that the enzymatic reductions of α-chloro ketone **1** and α-acetoxy ketone **5** give (*R*)-**21** and (*S*)-**25**, respectively. In this study, α-chloro ketones **2**−**4** were reduced to the corresponding (*R*)-alcohols, while α-acetoxy ketones **6**−**8** were reduced to the corresponding (*S*)-alcohols. Thus, this single reductase can afford the derivatives of both enantiomers of 1,2-diols in a manner that is dependent on the α-substituent (Cl or OAc).

(1) For example: (a) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: Oxford, 1994. (b) *Enzyme Catalysis in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; VCH: New York, 1995. (c) Faber, K. *Biotransformations in Organic Chemistry*; Springer-Verlag: Berlin, 1995.

(2) Ema, T.; Kobayashi, J.; Maeno, S.; Sakai, T.; Utaka, M. *Bull. Chem. Soc. Jpn*. **1998**, *71*, 443.

(3) Abbreviations used in this paper: BY, bakers' yeast; G6P, glucose-6-phosphate; G6PDH, glucose-6-phosphate dehydrogenase; $NADP^+$, *β*-nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of $NADP^+$; SDS−PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

(4) Ema, T.; Sugiyama, Y.; Fukumoto, M.; Moriya, H.; Cui, J.-N.; Sakai, T.; Utaka, M. *J. Org. Chem*. **1998**, *63*, 4996.

(5) For example, see: (a) Servi, S. *Synthesis* **1990**, 1. (b) Csuk, R.; Glänzer, B. I. *Chem. Rev*. **1991**, *91*, 49.

(6) (a) Shieh, W.-R.; Gopalan, A. S.; Sih, C. J. *J. Am. Chem. Soc.* **1985**, *107*, 2993. (b) Nakamura, K.; Kawai, Y.; Nakajima, N.; Ohno, A. *J. Org. Chem*. **1991**, *56*, 4778. (c) Shieh, W.-R.; Sih, C. J. *Tetrahedron: Asymmetry* **1993**, *4*, 1259. (d) Ishihara, K.; Nakajima, N.; Tsuboi, S.; Utaka, M. *Bull. Chem. Soc. Jpn*. **1994**, *67*, 3314. (e) Nakamura, K.; Kondo, S.; Kawai, Y.; Nakajima, N.; Ohno, A. *Biosci. Biotechnol. Biochem*. **1994**, *58*, 2236. (f) Nakamura, K.; Kondo, S.; Nakajima, N.; Ohno, A. *Tetrahedron* **1995**, *51*, 687.

(7) (a) Kim, M.-J.; Whitesides, G. M. *J. Am. Chem. Soc*. **1988**, *110*, 2959. (b) Casy, G. *Tetrahedron Lett.* **1992**, *33*, 8159. (c) St. Clair, N.; Wang, Y.-F.; Margolin, A. L. *Angew. Chem., Int. Ed*. **2000**, *39*, 380. The cell-free extracts are also used as biocatalysts: (d) Bradshaw, C. W.; Hummel, W.; Wong, C.-H. *J. Org. Chem*. **1992**, *57*, 1532.

(8) Wong, C.-H.; Whitesides, G. M. *J. Am. Chem. Soc*. **1981**, *103*, 4890.

(9) Chênevert, R.; Fortier, G.; Rhlid, R. B. *Tetrahedron* **1992**, *48*, 6769.

(10) Spino, C.; Mayes, N.; Desfossés, H. *Tetrahedron Lett*. **1996**, *37*, 6503.

(11) Baldwin, J. E.; Melman, A.; Lee, V.; Firkin, C. R.; Whitehead, R. C. *J. Am. Chem. Soc*. **1998**, *120*, 8559.

(12) Baldwin, J. E.; Adlington, R. M.; Ramcharitar, S. H. *Synlett* **1992**, 875.

(13) (a) Bennett, F.; Knight, D. W. *Tetrahedron Lett*. **1988**, *29*, 4865. (b) Bennett, F.; Knight, D. W.; Fenton, G. *J. Chem. Soc., Perkin Trans. 1* **1991**, 133.

(14) Bradshaw, J. S.; Huszthy, P.; McDaniel, C. W.; Zhu, C. Y.; Dalley, N. K.; Izatt, R. M.; Lifson, S. *J. Org. Chem*. **1990**, *55*, 3129.

10.1021/jo0108257 CCC: $20.00 © 2001 American Chemical Society
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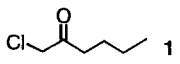
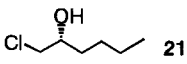
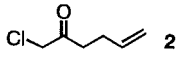
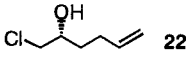
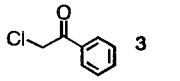
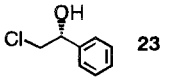
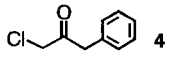
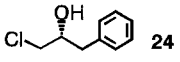
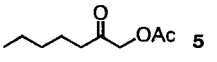
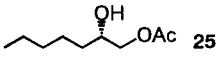
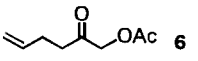
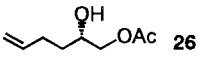
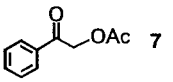
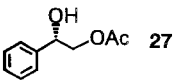
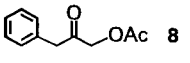
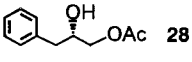
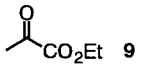
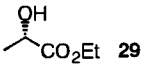
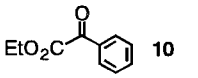
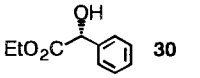
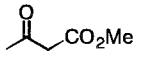
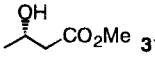
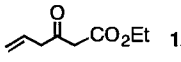
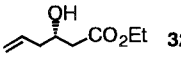
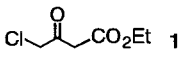
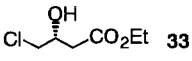
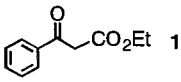
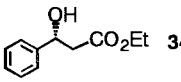
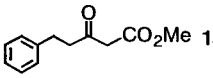
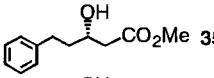
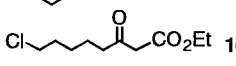
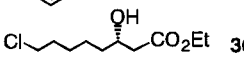
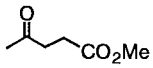
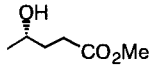
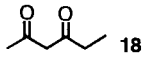
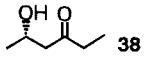
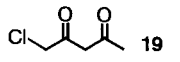
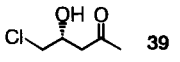
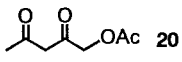
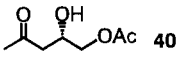
**Table 1. Asymmetric Reduction of Carbonyl Compounds Using the Purified Reductase and BY Whole Cells[a]**

| | reduction | | purified reductase | | whole cells | | |
|---|---|---|---|---|---|---|---|
| entry | substrate | product | % yield (% ee) | *R*/*S* | % yield (% ee) | *R*/*S* | ref |
| 1 |  **1** |  **21** | 64 (>99) | *R* | 74 (80) | *R* | 4 |
| 2 |  **2** |  **22** | 83 (>98) | *R* | 79 (51) | *R* | this |
| 3 |  **3** |  **23** | 49 (88) | *R* | 59 (66) | *R* | this |
| 4 |  **4** |  **24** | 80 (>99) | *R* | 61 (31) | *R* | this |
| 5 |  **5** |  **25** | 80 (98) | *S* | 52 (96) | *S* | 4 |
| 6 |  **6** |  **26** | 43 (>98) | *S* | 32 (35) | *S* | this |
| 7 |  **7** |  **27** | 84 (96) | *S* | 75 (94) | *S* | this |
| 8 |  **8** |  **28** | 96 (85) | *S* | 44 (83) | *S* | this |
| 9 |  **9** |  **29** | 41 (>99) | *S* | 65 (93) | *S* | 4 |
| 10 |  **10** |  **30** | 79 (95) | *R* | 99 (91) | *R* | this |
| 11 |  **11** |  **31** | 64 (>99) | *S* | 49 (97) | *S* | 4 |
| 12 |  **12** |  **32** | 22 (>99) | *S* | 70 (59) | *R* | this |
| 13 |  **13** |  **33** | 84 (98) | *R* | 52 (1) | *S* | this |
| 14 |  **14** |  **34** | 43 (72) | *R* | 51 (88) | *S* | this |
| 15 |  **15** |  **35** | 8 (>98) | *S* | 16 (64) | *R* | this |
| 16 |  **16** |  **36** | 52 (90) | *S* | 10 (62) | *R* | this |
| 17 |  **17** |  **37** | 39 (>99) | *S* | 36 (>99) | *S* | this |
| 18 |  **18** |  **38** | 69 (>99) | *S* | 35 (98) | *S* | 4 |
| 19 |  **19** |  **39** | 19 (>99) | *R* | 34 (54) | *S* | 4 |
| 20 |  **20** |  **40** | 65 (68) | *S* | 33 (65) | *S* | 4 |

[a] For the reaction conditions and procedures, see ref 4 or Supporting Information.

To understand the stereochemical trend in the enzymatic reductions, all the products in Table 1 are drawn in such a way that the $\beta$-face of the carbonyl group in the substrates is attacked by NADPH. The enzymatic reductions of **1**−**6**, **9**, **11**−**13**, and **17**−**20** yielded the Prelog-type alcohols; the right-hand moiety of the products is bulkier than the left-hand moiety.[15] The enantiomeric purity of these products was high in most cases. Although we have previously reported that the present reductase is one of the enzymes that contribute to Prelog's rule,[4] the anti-Prelog-type alcohols **7**−**8** and **14**−**16** were also obtained in this study; the left-hand moiety is bulkier than the right-hand moiety. It should be noted that all of the anti-Prelog-type alcohols possess an ester

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group. These results (Table 1) can be most simply explained by assuming a binding pocket that can accommodate, in order of affinity, either an ester group or a hydrophobic substituent. In the case of substrates **1**−**6**, **9**, **11**−**13**, and **17**−**20**, an ester group or a bulkier substituent (the right-hand moiety in each structure in Table 1) might be preferentially accommodated in the binding pocket, with the carbonyl group oriented as shown in Table 1. The $\beta$-face attack of the carbonyl group by NADPH would produce a Prelog-type alcohol. In the case of substrates **7**−**8**, **10**, and **14**−**16** having an ester group on one side and a bulkier substituent on the other side, the competitive binding of both substituents could occur, resulting in the ambiguous recognition of the enantioface of the carbonyl group; however, except for **10**, the binding of an ester group could predominate considerably over that of the bulkier substituent, giving an anti-Prelog-type alcohol. Table 1 suggests that enantioselectivity increases as the putative binding abilities of the two substituents flanking the carbonyl group are unbalanced.

In conclusion, the present enzyme, showing high enantioselectivity and broad substrate specificity simultaneously, is promising as a versatile biocatalyst for the asymmetric reduction of carbonyl compounds. The enzymatic reductions afforded useful alcohols and exhibited the interesting stereochemical trend.

## Experimental Section

**General.** $^1$H and $^{13}$C NMR spectra were measured in $CDCl_3$ at 200 and 50 MHz, respectively. Silica gel column chromatography was performed using Fuji Silysia BW-127 ZH (100−270 mesh), and TLC was performed on Merck silica gel 60 $F_{254}$. G6PDH (from BY, lyophilized powder) and the molecular-weight marker for SDS−PAGE (lysozyme (14 kDa), $\beta$-lactoglobulin (18 kDa), trypsinogen (24 kDa), ovalbumin (45 kDa), albumin (66 kDa)) were purchased from Sigma. The pressed BY, NADPH, $NADP^+$, and G6P were purchased from Oriental Yeast Company. Purification of the enzyme and asymmetric reductions were performed as described previously.[4] The purity of the enzyme was examined by SDS−PAGE. All compounds have been reported elsewhere. $\beta$-Keto esters **12**,[13] **15**,[16] and **16**[11] were prepared according to the literature. Although synthetic methods for α-chloro ketones **2**[17] and **4**[18] and α-acetoxy ketones **6**,[19] **7**,[20] and **8**[21] have been reported, α-chloro ketones **2** and **4** were prepared by a different method,[22] and α-acetoxy ketones **6**, **7**, and **8** were prepared from **2**, **3**, and **4**, respectively, as described previously.[4] Ketones **3**, **10**, **13**, **14**, and **17** were purchased. Alcohols **22**,[23] **23**,[24] **24**,[25] **26**,[26] **27**,[20] **28**,[21] **30**,[27] **32**,[13] **33**,[28] **34**,[29] **35**,[10] **36**,[11] and **37**[30] were characterized according to the literature. Bakers' yeast reductions of **3** (90% ee, *R*),[24] **7** (94% ee, *S*),[20] **8** (72% ee, *S*),[21] **10** (82% ee, *R*),[31] **12** (43% ee, *R*),[13] **13** (55% ee, *S*),[28] **14** (87−93% ee, *S*),[9] and **15** (80% ee, *R*)[10] have been reported by other researchers.

**Determination of Enantiomeric Purities and Absolute Configurations.** The enantiomeric purities of alcohols **23**, **24**, **32**, and **37** (acetate derivative) were determined by GC with a CP-cyclodextrin-$\beta$-2,3,6-M-19 capillary column (Chrompack). The enantiomeric purities of alcohols **30**, **34**, and **35** were determined by HPLC without derivatization. For the determination of the enantiomeric purities of other alcohols, derivatization was necessary. Alcohols **22** and **26** were converted to the corresponding tosylates. Alcohols **27** and **28** were converted to the corresponding diol and diacetate derivatives, respectively. Alcohols **33** and **36** were converted to the corresponding MTPA esters. They were then analyzed by HPLC with the following chiral columns (Daicel Chemical Industries): Chiralpak AD-H (**22**), Chiralcel OB−H (**26**, **27**, **30**, **34**), and Chiralcel OD-H (**28**, **33**, **35**, **36**). The absolute configurations of all the optically active alcohols except **22** were determined by comparing the sign of their specific rotations with that of the reported specific rotations. The absolute configuration of **22** was determined to be (*R*) by the stereochemical trend of the reductase as discussed above. Details are given in the Supporting Information.

**Acknowledgment.** We are grateful to the SC-NMR Laboratory of Okayama University for the measurement of NMR spectra. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

**Supporting Information Available:** Spectroscopic data, HPLC or GC data for the determination of the enantiomeric excesses, copies of $^1$H and $^{13}$C NMR spectra for **22**−**24**, **26**−**28**, **30**, and **32**−**37**, and the general procedures for enzymatic or BY whole-cell reductions along with the reaction times. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0108257

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