Asymmetric Synthesis of β-Hydroxy Esters and α-Alkyl-β-hydroxy Esters by Recombinant *Escherichia coli* Expressing Enzymes from Baker's Yeast

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Introduction

Optically pure β-hydroxy esters and α-alkyl-β-hydroxy esters provide very versatile building blocks for chiral synthesis,[1,2] and several methods for producing these synthons have been explored. Asymmetric reduction of β-keto esters, either by chemical or enzymatic methods, has been the most extensively investigated route. Chemical approaches have included the use of chirally modified hydride reagents,[3] transition metal catalysts,[4−7] and Lewis acid mediated borohydride reductions.[8] A number of biocatalytic reagents have also been investigated, encompassing both purified enzymes and whole microbial cells.[9,10] Whole cells provide a continuous source of enzymes and cofactors, which simplifies these reactions significantly. In one example, Sugiyama screened several strains of bacteria for the enantioselective reduction of ethyl α-methyl acetoacetate and identified a *Klebsiella pneumoniae* strain as a suitable reagent.[11] Although some other microorganisms have been shown to reduce β-keto esters,[9] the ease of use, broad substrate tolerance, and good stereoselectivities have focused most attention on baker's yeast (*Saccharomyces cerevisiae*). Unfortunately, yeast-catalyzed biotransformations sometimes result in low optical purities as a result of the presence of enzymes with overlapping substrate specificities but different stereoselectivities.[12,13] There have been extensive studies aimed at improving the stereoselectivities of baker's yeast mediated reductions,[14−16] and we have recently reported the rational design of genetically engineered yeast strains with predictable stereoselectivities.[17]

Biochemical[18] and genome analysis[19] studies of baker's yeast have shown that a number of enzymes may be involved in the reduction of β-keto esters. In some cases, the enzymes have been isolated and shown to possess very high stereoselectivities;[12,20] however, the need for enzyme isolation and cofactor regeneration has limited the practical utility of these catalysts.

Among the reductase enzymes present in baker's yeast, YKER I (Ypr1p) has been characterized most completely and has been reported as the major enzyme responsible for production of *syn* (2*R*,3*S*) β-hydroxy esters.[20] However, using gene knock-out technology, we have shown that at least one other yeast enzyme participates in producing these alcohols.[17] There are two other yeast aldo keto reductases, Gcy1p[21] and Gre3p,[22] that share high amino acid sequence identity with Ypr1p,[19] but their potential role in reducing β-keto esters has not been investigated. To learn whether these two enzymes might be useful in stereoselective reductions of β-keto esters, engineered *E. coli* strains expressing Gcy1p and Gre3p were created. These recombinant strains form the basis for simple methods of producing chiral β-hydroxy esters with high optical purities.

Results and Discussion

Engineered *E. coli* strains expressing Gcy1p and Gre3p were constructed by placing the appropriate yeast gene under control of a *tac* promoter. In these strains, adding IPTG (isopropyl-β-D-thiogalactoside) to the culture induces enzyme expression. Whole cells of the two strains were tested for the reductions of several β-keto esters (Scheme 1); the results are summarized in Table 1. The optical purities of the products were determined by chiral-phase GC, and optical rotations were used to establish the absolute configuration of each β-hydroxy ester product. ^{13}C NMR spectra confirmed that only a single diastereomer was produced in all cases. Both strains yielded the (3*S*) alcohols with high enantiomeric excess values and acceptable yields for all the substrates tested. When racemic α-substituted β-keto esters were employed as substrates, facile racemization at the α-position allowed for dynamic kinetic resolutions (Scheme

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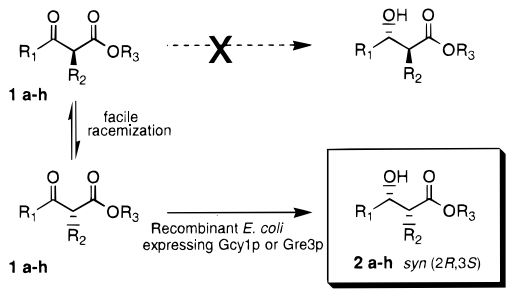
Table 1. Reduction of β-keto Esters by Recombinant *E. coli* Strains Expressing Gre3p and Gcy1p

	substituents			*E. coli* expressing Gcy1p		*E. coli* expressing Gre3p		product characterization	
substrate	R_1	R_2	R_3	isolated[a] yield	% ee, de[a]	isolated[a] yield	% ee, de	absolute config	$[\alpha]^{25}_{589}$ (*c*, $CHCl_3$)
1a	Me	H	Me	28%	>98, *c*	35%	>98, *c*	*S*	+28.5, (*c* 1.5)
1b	Et	H	Me	45%	91, *c*	40%	90, *c*	*S*	+20.5, (*c* 1.3)
1c	Me	H	Et	51%	>98, *c*	45%	>98, *c*	*S*	+44.6, (*c* 1.0)
1d	Et	H	Et	48%	96, *c*	40%	91, *c*	*S*	+23.3, (*c* 1.0)
1e	Me	Me	Et	68%	>98, >98	81%	>98, >98	2*R*,3*S*	+2.3, (*c* 5.4)
1f	Me	Et	Et	75%	>98, >98	41%	>98, >98	2*R*,3*S*	+6.8. (*c* 7.9)
1g	Me	allyl	Et	66%	>98, >98	72%	>98, >98	2*R*,3*S*	+3.7, (*c* 4.6)
1h	Me	propargyl	Et	56%	>98, >98	87%	>98, >98	2*R*,3*S*	+7.4, (*c* 4.1)

[a] Yields refer to chromatographically purified samples. [b] Values of enantiomeric and diasteromeric excess were determined by chiral-phase GC analysis on a Chirasil-Dex CB column. [c] Diastereoselectivity is not applicable in this case.

Scheme 1



1).[23] Reduction of **1d**−**g** with either recombinant strain resulted in almost complete conversion of the substrate to the *syn* (2*R*,3S) diasteromer.

E. coli provides an ideal microorganism for expression of these yeast enzymes since the level of intrinsic reductase(s) is low. Unmodified *E. coli* has been shown to reduce **1e**,[11] and our results indicated that it can also reduce several other β-keto esters to a small degree. Fortunately, this low background activity did not compromise the stereoselectivity of the recombinant strains, since control experiments using the unmodified *E. coli* strain showed that it resulted in the same stereoisomer in all cases examined.

These two new biocatalysts complement the existing methodologies for reduction of β-keto esters by providing simple access to *syn* (2*R*,3*S*) α-alkyl-β-hydroxy esters in high optical purities. Catalytic hydrogenation of α-substituted β-keto esters has been limited to formation of the (2*R*,3*R*) and (2*S*,3*R*) diasteromers.[5,6] The (2*S*,3*S*) *anti* diasteromer has been obtained through stereoselective alkylation of ethyl 3(*S*)-hydroxybutanoate.[24] Lewis acid mediated reductions have produced the *syn* diasteromer; however, the enantioselectivities of these reagents were not reported.[8] A further advantage of these new biocatalytic reagents is their ability to provide good stereoselectivities in the absence of bulky substituents, which is not always the case for chemical methods of β-keto ester reduction. Using whole cells of the engineered *E. coli* strains, two stereocenters can be set in one reaction from simple, commercially available starting materials. In addition, the reaction proceeds under mild conditions, and the process is environmentally friendly.

In summary, two yeast enzymes (Gcy1p and Gre3p) that catalyze stereoselective reductions of β-keto esters have been identified and shown to be useful biocatalysts for chiral synthesis. Furthermore, by expressing the enzymes in *E. coli*, we have created simple and inexpensive biological reagents that can be used by nonspecialists. This approach eliminates the need for enzyme isolation and cofactor regeneration, and these new biocatalysts broaden the spectrum of reactions that can be catalyzed with recombinant *E. coli* cells.

Experimental Section

Materials. β-Keto esters **1a**−**f** were purchased from Aldrich and used without further purification. Substrates **1g** and **1h** were synthesized by slight modifications of the method reported by Dauben et al.[25] Racemic β-hydroxy esters used as standards in GC analysis were obtained by $NaBH_4$ reduction of the corresponding β-keto ester.[26]

General Procedure for Biotransformation with Recombinant *E. coli* Strains. Fresh plates of engineered *E. coli* strains were streaked from the appropriate frozen stock, and a single colony was used to inoculate 10 mL of LB-ampicillin[27] in a 125 mL Erlenmeyer flask. The culture was incubated in a rotary shaker overnight (200 rpm, 37 °C), and 1 mL of this culture was used to inoculate 100 mL of fresh LB-ampicillin in a 500 mL Erlenmeyer flask. The fresh culture was grown under the same conditions until it reached an OD_{600} of 0.5 (approximately 2.5 h), and then IPTG was added (final concentration of 1mM) along with the substrate (final concentration of 10 mM). The culture was shaken (200 rpm) at room temperature and sampled periodically for GC analysis. After maximum conversion was achieved (24−50 h), the cells were removed by centrifuging at 4,000 × *g* for 15 min at 4 °C. The supernatant was extracted with CH_2Cl_2 (4 × 50 mL),[28] and the combined organic extracts were washed with brine, dried with Na_2SO_4, filtered, and evaporated. The product was purified by flash chromatography with $CHCl_3$/ether (9:1) as the solvent system. The products were characterized by IR, NMR, GC−MS, and optical rotations. Enantiomeric and diasteromeric excess values were determined by chiral GC (Chrompack 0.25 mm × 25 m CP Chirasil-Dex CB). Alcohols **2a** and **2f** required acetylation for complete resolution on the chiral column. Analytical conditions appropriate for each substrate, as well as copies of the chromatograms, can be found in the Supporting Information.

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Supporting Information Available: Detailed experimental procedures and GC analysis of the reaction products. This material is available free of charge via the Internet at http://pubs.acs.org.

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(27) LB (Luria−Bertani medium)−ampicillin: per liter, Bacto-Tryptone (10 g), Bacto-Yeast extract (5 g), NaCl (10 g), ampicillin (0.2 g; added after cooling), Bacto-Agar (15 g; included for solid media).
(28) Centrifugation of the extraction mixture (4000 × *g*, 10 min) was useful to break the emulsions formed during some extractions.