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FEMS Yeast Research 5 (2005) 513–525

FEMS Yeast Research

www.fems-microbiology.org

MiniReview

Strain engineering for stereoselective bioreduction of dicarbonyl compounds by yeast reductases

Ted Johanson, Michael Katz, Marie F. Gorwa-Grauslund *

Department of Applied Microbiology, Lund University, P.O. Box 124, SE-22100 Lund, Sweden

Received 30 July 2004; received in revised form 6 December 2004; accepted 7 December 2004

First published online 13 January 2005

Abstract

Pure chiral molecules are needed in the pharmaceutical and chemical industry as intermediates for the production of drugs or fine chemicals. Microorganisms represent an attractive alternative to chemical synthesis since they have the potential to generate single stereoisomers in high enantiomeric excess (*ee*). The baker's yeast *Saccharomyces cerevisiae* can notably reduce dicarbonyl compounds (in particular α- and β-diketones and keto esters) to chiral alcohols with high *ee*. However, products are formed at a low rate. Moreover, large amounts of co-substrate are required for the regeneration of NADPH that is the preferred co-factor in almost all the known dicarbonyl reductions. Traditionally, better *ee*, reduction rate and product titre have been achieved via process engineering. The advent of recombinant DNA technology provides an alternative strategy to improve productivity and yield by strain engineering. This review discusses two aspects of strain engineering: (i) the generation of strains with higher reductase activity towards dicarbonyl compounds and (ii) the optimisation of co-substrate utilisation for NADPH cofactor regeneration.

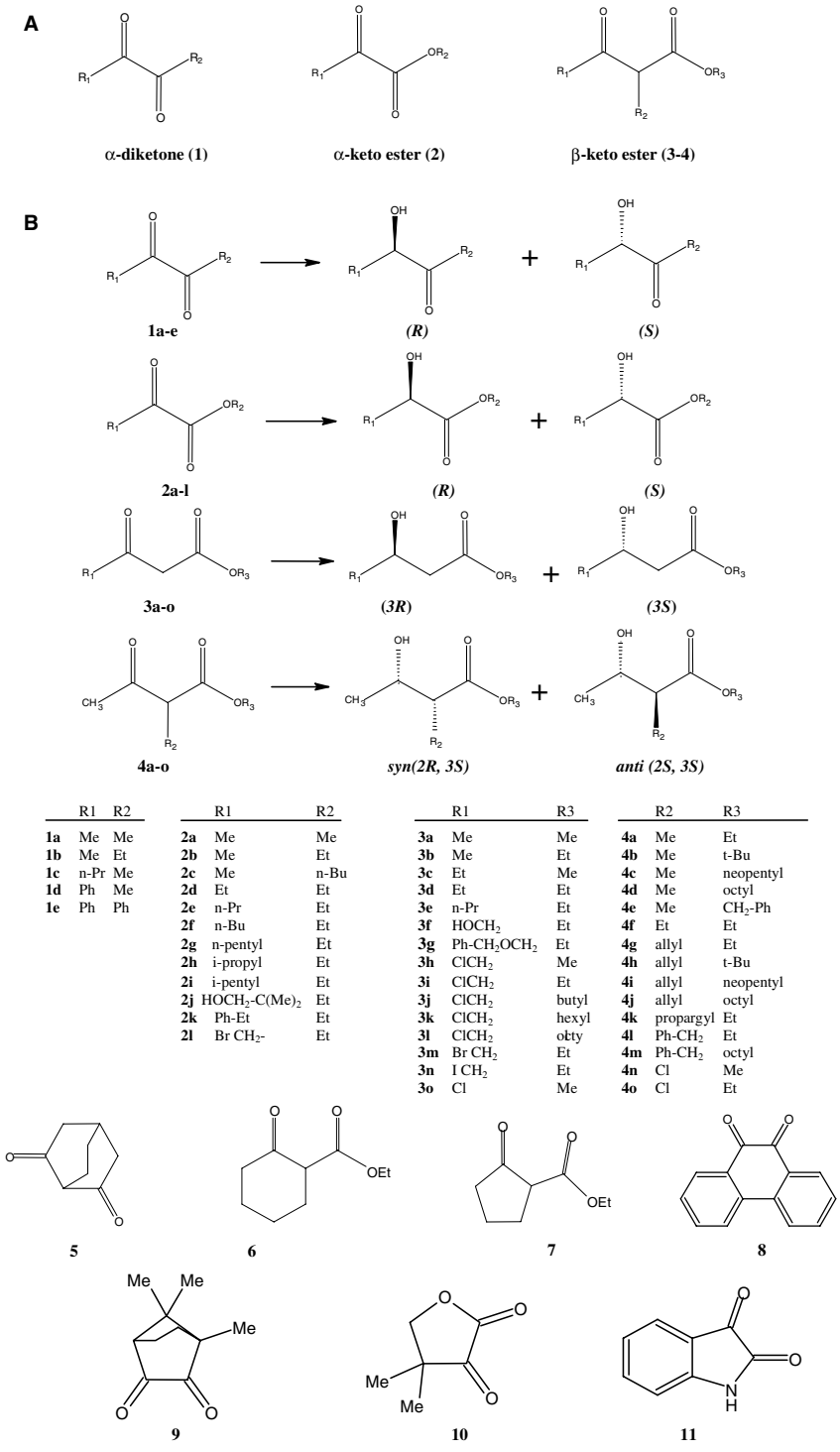
Keywords: Whole-cell bioreductions; Strain engineering; Reductase; Yeast; Dicarbonyl; NADPH availability

1. Introduction

Chiral alcohols obtained from the stereo-selective reduction of carbonyl compounds are important building blocks in the synthesis of fine chemicals and pharmaceuticals [1]. The bioreduction of xenobiotic α- and β-ketoesters and diketones (see Fig. 1A for nomenclature) by yeast, which the review is focusing on, is of particular interest since the resulting chiral alcohols are used for the synthesis of many natural products, steroids and drugs [1]. Whereas organic synthesis requires several synthetic routes to produce a specific enantiomer, stereo-selective whole cell bioreduction is a cheap and simple way to introduce chirality using the stereospecificity of microbial enzymes. Biocatalysts offer the additional advantage over chemical asymmetric catalysts that they operate under mild conditions, such as physiological pH and low temperature. In whole-cell bioreduction of dicarbonyl compounds, the substrate is transported into the cell, where it is reduced to a chiral alcohol product, which is transported back to the surrounding medium (Fig. 2). This process requires two parallel reactions to occur simultaneously: (i) the stereo-selective enzymatic reduction of the dicarbonyl compound, catalysed by one or several reductases under the concomitant oxidation of NADPH co-factor – almost all the identified dicarbonyl reductases are NADPH-dependent [2] – and (ii) the regeneration of NADPH through the dissimilatory metabolism of a co-substrate, such as glucose, sucrose or ethanol (Fig. 2).

* Corresponding author. Tel.: + 46 46 222 0619; fax: +46 46 222 4203.
E-mail address: marie-francoise.gorwa@tmb.lth.se (M.F. Gorwa-Grauslund).

1567-1356/$22.00
doi:10.1016/j.femsyr.2004.12.006



	R1	R2
1a	Me	Me
1b	Me	Et
1c	n-Pr	Me
1d	Ph	Me
1e	Ph	Ph

	R1	R2
2a	Me	Me
2b	Me	Et
2c	Me	n-Bu
2d	Et	Et
2e	n-Pr	Et
2f	n-Bu	Et
2g	n-pentyl	Et
2h	i-propyl	Et
2i	i-pentyl	Et
2j	$HOCH_2$-C(Me)$_2$	Et
2k	Ph-Et	Et
2l	Br CH_2-	Et

	R1	R3
3a	Me	Me
3b	Me	Et
3c	Et	Me
3d	Et	Et
3e	n-Pr	Et
3f	$HOCH_2$	Et
3g	Ph-CH_2OCH_2	Et
3h	$ClCH_2$	Me
3i	$ClCH_2$	Et
3j	$ClCH_2$	butyl
3k	$ClCH_2$	hexyl
3l	$ClCH_2$	obty
3m	Br CH_2	Et
3n	I CH_2	Et
3o	Cl	Me

	R2	R3
4a	Me	Et
4b	Me	t-Bu
4c	Me	neopentyl
4d	Me	octyl
4e	Me	CH_2-Ph
4f	Et	Et
4g	allyl	Et
4h	allyl	t-Bu
4i	allyl	neopentyl
4j	allyl	octyl
4k	propargyl	Et
4l	Ph-CH_2	Et
4m	Ph-CH_2	octyl
4n	Cl	Me
4o	Cl	Et

Fig. 1. Dicarbonyl compounds discussed in this review. (A) Nomenclature of the dicarbonyl compounds (R_1, R_2 and R_3 being substitution groups): α-diketone (or type-**1** compound) in which the second ketone group is located adjacent to (i.e. in the α position of) the first ketone; α-ketoester (or type-**2** compound) in which the second ketone group is located adjacent to the ketoester (–$COOR_2$) group, and β-ketoester (type-**3** and type-**4** compounds) in which the second ketone group is located in the β position of the ketoester group. Type-**3** compounds comprise β-ketoesters that are not substituted in the α position (R_2 = H) whereas type-**4** compounds are substituted in the α position. (B) Specific type-**1**, type-**2**, type-**3**, type-**4** and other dicarbonyl compounds discussed in this review with their putative reduction products. The substitution groups R_1, R_2 and R_3 of each compound type are indicated in the corresponding enclosed tables.

Efficient bioreductions should combine high enantiomeric excess (*ee*), conversion yield, productivity and product titre [2,3]. An economically feasible process also requires easy product recovery, inexpensive co-substrate, a low co-substrate requirement, low by-product formation, good reproducibility and low investment costs [2].