Biotechnology Advances 30 (2012) 1279–1288

Contents lists available at SciVerse ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Biocatalytic ketone reduction: A green and efficient access to enantiopure alcohols

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ARTICLE INFO

Available online 30 October 2011

Keywords:
Asymmetric reduction
Biocatalysis
Chiral alcohols
Genome mining
Reductases

ABSTRACT

Chiral secondary alcohols play an important role in pharmaceutical, agrochemical, and chemical industries. In recent years, impressive steps forward have been achieved towards biocatalytic ketone reduction as a green and useful access to enantiopure alcohols. An increasing number of novel and robust enzymes are now accessible as a result of the ongoing progress in genomics, screening and evolution technologies, while process engineering provides further success in areas of biocatalytic reduction in meeting synthetic challenges. The versatile platform of these techniques and strategies offers the possibility to apply high substrate loading and thus to overcome the limitation of low volumetric productivity of usual enzymatic processes which is the bottleneck for their practical application. In addition, the integration of bioreduction with other enzymatic or chemical steps allows the efficient synthesis of more complex chiral products.

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1. Introduction

Chiral secondary alcohols are frequently required as important intermediates for the introduction of chiral center into the pharmaceuticals, flavor, aroma and agricultural chemicals, and specialty materials. Enantioselective ketone reduction is a reliable, scalable and straightforward route to optically active alcohols. As compared to conventional chemical processing, biocatalytic asymmetric reductions can offer highly selective reactions, environmentally benign processes, and energy-effective operations and thus of great interest, but are still limited in their commercial application due in large part to the low volumetric productivity. Much attention has been paid to develop robust biocatalysts and subsequently to carry out biocatalytic reduction processes at a high substrate load to achieve economic feasibility and competitiveness for large-scale biotransformations. Recent enzyme discovery and engineering efforts have greatly increased the availability of ideal biocatalysts, rendering biocatalysis a much more versatile and powerful tool for organic synthesis, especially for chiral synthesis.

In the past few years, a considerable amount of literature on biocatalytic asymmetric reduction has been documented and reviewed

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doi:10.1016/j.biotechadv.2011.10.007

several times from various aspects. Matsuda et al. (2009) exhaustively discussed the asymmetric reductions and oxidations with biocatalysts, covering mechanism, substrate scope and many more. Hollmann et al. (2011) gave an overview over enzymatic reductions with a critical view on green chemistry aspects. Huisman et al. (2010) from Codexis emphasized the technical usage of ketoreductases, while another review by Carballeira et al. (2009) focused on the tailor-made whole-cell catalysts for redox reactions.

This review summarizes latest advances in reductase-catalyzed synthesis of chiral alcohols published mainly after 2008 and highlights the technologies that help to overcome the limitations of asymmetric bioreductions. In particular, focus will be on the rapid search for novel enzymes, the improvement of enzymes via protein engineering or immobilization, as well as the importance of medium optimization and continuous processes. Finally, combination of reductases with other enzymes or chemo-catalysts in cascade processes will be addressed.

2. Cofactor regeneration

Carbonyl reductases (CRs) and alcohol dehydrogenases (ADHs) used for ketone reduction require nicotinamide cofactors (NADH or NADPH) as hydride source. The high cost of cofactors makes efficient cofactor regeneration a prerequisite for preparative applications. Currently, there are several possibilities to circumvent the cofactor challenge: using whole cells, or via enzymatic, electrochemical, photochemical, and chemical approaches. The overview of these different cofactor regeneration concepts has been presented in some recent excellent reviews (de Wildeman et al., 2007; Goldberg, 2007; Hollmann et al., 2010). Among them, enzymatic methods including substrate-coupled and enzyme-coupled systems are presently preferred for cofactor regeneration. In the substrate-coupled system, the precondition is a chemostable dehydrogenase which can simultaneously transform substrate and cosubstrate such as 2-propanol. The latter must be applied in large excess to drive the equilibrium towards the desired direction. Enzyme-coupled system involves a second enzymatic irreversible reaction which frequently employs glucose dehydrogenase (GDH) or formate dehydrogenase (FDH) for the required cofactor recycling.

The efficient cofactor regeneration approaches can cut high cofactor costs significantly by increasing regeneration cycles (total turnover numbers, TTNs) so that they are not cost-determining anymore. A few examples can be found in the scientific literature wherein the TTN reaches a commercially interesting value of 10^3–10^5 (Zhao and van der Donk, 2003). In principle, enzymes involved in a reductive reaction of interest and in the regeneration of required cofactor can be provided to the host cell to achieve sufficient reduction efficiency, negligible side reaction and simple process. In few cases, bioreduction processes catalyzed by such designer cells could be performed at extremely high substrate concentration without external addition of any cofactor as usually done (Gröger et al., 2006; Ni et al., 2011c). However, it is the precondition that a robust reductase has been identified with exceptionally high activity and substrate tolerance.

3. Biocatalyst discovery and improvement of desired traits

3.1. Genome mining

Apart from conventional screening approaches to finding novel enzymes from soil samples or culture collections, the abundance of digital data in this post-genomic era brings about the significant development of biocatalysis research. As of April 2011, GenBank contains more than 135 million nucleotide sequence records representing over 428,000 species and 1760 complete microbial genomes. The rapid influx of new genomic information combined with continued development of predictive bioinformatics tools makes genome mining increasingly exploited, which has guided the discovery of a large number of reductases in recent years.

3.1.1. Genome hunting

A potential tool to search a biocatalyst for desired reactions is implicated by analysis of the sequenced whole genome of a certain microorganism. Based on such a "genome hunting" principle, one strategy is to screen a genome-wide expression library, which can be created by overexpressing the known or putative reductases from one genome-sequenced microorganism. One typical example of this strategy is the systematic investigation of bakers' yeast reductases catalyzing carbonyl reduction. After analysis of the yeast genome, 18 key reductases from bakers' yeast were overexpressed in *Escherichia coli* and tested for their abilities of reducing α- or β-keto esters. Many reductions with single purified reductases proceeded with ≥90% ee and ≥90% de (Kaluzna et al., 2004; Kaluzna et al., 2005). The utility of this library was further demonstrated by reducing 3-oxo-3-phenylpropanenitrile to both antipodes of corresponding alcohols with high enantiopurities (Hammond et al., 2007). Analogous screening method was used for the discovery of synthetically useful reductases from a ketone reductase-producing strain, *Bacillus* sp. ECU0013 (Xie et al., 2010). Among 11 recombinant oxidoreductases, three versatile reductases were identified and explored of their applications in efficient synthesis of optically active α- and β-hydroxyl esters (Ni et al., 2011a, 2011b, 2011c).

Homology-driven individual genome screening offers another useful strategy for exploring new reductases with special or desired traits. For example, analysis of the entire genome of a solventogentic bacterium, *Clostridium acetobutylicum*, revealed an ADH having 40% homology to an enantioselective carbonyl reductase from *Ralstonia eutropha*. This new ADH could enantioselectively reduce aromatic α-, β- and γ-keto esters to the corresponding D-hydroxy esters, affording ethyl (2*S*,3*R*)-2-chloro-3-hydroxy-3-phenylpropanoate, a building block for side chain of the taxoid chemotherapeutics with 95% de and 99% ee by dynamic reductive kinetic resolution (Applegate et al., 2011). Reductases with anti-Prelog stereopreference are of great demand because the majority of known reductases used for asymmetric reduction follow Prelog's rule. Bioinformatic analysis based on sequence-similarity with an anti-Prelog stereospecific ADH from *Candida parapsilosis* in its whole genome revealed three homologous carbonyl reductases (SCR1, SCR2 and SCR3) with anti-Prelog selectivity that converts 2-hydroxyacetophenone to (*S*)-1-phenyl-1,2-ethanediol (Nie et al., 2011). Genomes of thermophilic microbes are considerable fields for mining enzymes with high thermostability. An ADH mined from *Thermus thermophilus* genome has remarkable thermophilicity and thermal stability as well as good tolerance against organic solvents (Pennacchio et al., 2008). A taxonomic screening within the phylum of cyanobacteria to identify homologous enzymes with higher specific activity and enantioselectivity was performed on the basis of the identification of a 3-ketoacyl-(acyl-carrier-protein) reductase (KR) in *Synechococcus* sp. PCC 7942. A comparative study of the KRs from 16 cyanobacteria revealed a better KR from *Synechococcus* sp. RCC 307 that exhibited higher activities on all the four exemplary prochiral ketones than those of the original reductase (Hölsch and Weuster-Botz, 2010).

3.1.2. Data mining

Another effective and promising approach to rapidly identifying suitable biocatalysts is mining in the whole public databases for homologous protein sequences related to those of targeted enzymes discovered before. This "data mining" method is based on the search for matches in the database. Generally, a range of proteins with moderate sequence similarity to known enzymes are selected as candidates. For instance, a strategy of target reaction-oriented mining was applied to find new reductases with high catalytic activity towards ethyl 4-chloro-3-oxo-butanoate (COBE). Already known COBE reducatses were employed as probes to operate the BLAST program in the GenBank, resulting in the discovery of a novel *Streptomyces coelicolor* reductase (ScCR) from 10