Biocatalytic approaches for the quantitative production of single stereoisomers from racemates

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Abstract

Strategies for the chemoenzymatic transformation of a racemate into a single stereoisomeric product in quantitative yield have been developed. A range of industrially relevant α-hydroxycarboxylic acids was deracemized in a stepwise fashion via lipase-catalysed enantioselective O-acylation, followed by mandelate racemase-catalysed racemization of the remaining non-reacted substrate enantiomer. Alternatively, aliphatic α-hydroxycarboxylic acids were enzymatically isomerized using whole resting cells of Lactobacillus spp. Enantioselective hydrolysis of rac-sec-alkyl sulphate esters was accomplished using novel alkyl sulphatases of microbial origin. The stereochromatic path of catalysis could be controlled by choice of the biocatalyst. Whereas Rhodococcus ruber DSM 44541 and Sulfolobus acidocaldarius DSM 639 act through inversion of configuration, stereo-complementary retaining sulphatase activity was detected in the marine planctomycete Rhodopirellula baltica DSM 10527.

Introduction

According to a recent survey, between 70 and 90% of all chemical processes on an industrial scale are performed in a catalytic way [1]. Among them, the vast majority of asymmetric variants can be classified into the following categories (Scheme 1). (i) Desymmetrization of a prochiral- or meso-compound M through ‘enantiomeric’ pathways leads to the formation of a single product in 100% theoretical yield. Its absolute configuration and enantiomeric composition are determined by the relative magnitude of rate constants kP and kQ [2,3]. (ii) In contrast, kinetic resolution of a racemate consisting of enantiomers A and B furnishes a pseudo-enantiomeric pair of product and remaining non-converted substrate (P/B or Q/A respectively) each in 50% theoretical yield [4–6]. Since in general only one enantiomer is needed for synthesis, and there is little (or no) use for the other, it has to be regarded as ‘waste’ and thus represents a considerable economic burden for the process.

On a first glimpse, the overall efficiency of desymmetrization appears to be far superior to that of kinetic resolution. However, due to the fact that the (theoretically possible) number of racemates will always be higher than that of prochiral or meso-compounds [whereas a racemic compound bearing a central element of chirality (e.g. located on an sp3-carbon atom) always possesses four functional groups, which may be altered, only three functional elements can be varied in prochiral and meso-compounds bearing an sp3-carbon atom due to the inherent symmetry of the molecule], the question is not how to avoid racemic starting materials by choosing prochiral or meso-compounds, but how to improve the economic balance of kinetic resolution by breaking the 50% yield threshold for a single enantiomer. In this context, several strategies, generally denoted as ‘deracemization’ [7,8], were recently proposed, which allow the quantitative transformation of a racemate into a single stereoisomeric product in 100% theoretical yield (Scheme 1).

This may be achieved in two ways (for an elegant deracemization method based on cyclic oxidation–reduction, see [9,10]) (Scheme 1). (iii) Interconversion of substrate enantiomers usually takes place in equilibrium and thus represents the classic process of racemization [11–13]. In situ combination of substrate racemization with kinetic resolution leads to the concept of dynamic (kinetic) resolution [14–19]. (iv) Alternatively, both substrate enantiomers can be transformed in an enantioselective fashion through independent stereo-convergent pathways into the desired enantiomeric product. In order to furnish a single stereoisomer, the two pathways must match each other by proceeding through inversion and retention of configuration respectively [20–24a].

Deracemization based on enzymatic racemization

Racemization has been generally considered as an unwanted side reaction rather than a synthetically useful transformation and thus the controlled racemization of organic compounds has scarcely been studied [11–13]. In view of the fact that approx. two-thirds of all racemization methods reported to...
date require harsh reaction conditions which are incompatible with the presence of a stereoselective (bio)catalyst, enzymatic racemization holds great potential [11–13]. One of the best studied racemases so far is mandelate racemase (EC 5.1.2.2) from Pseudomonas putida A.T.C.C. 12633 [25–27]. The cofactor-independent enzyme acts through a general ‘two-base-mechanism’, whereby two bases (Lys166 and His297) positioned on opposite sides of the substrate within the active site act through deprotonation–protonation of the α-H of both substrate enantiomers (the pKₐ value of the α-H of isolated mandelate anion has been estimated to be approx. 29; see [28]).

Most important in view of its application for the transformation of non-natural compounds, the substrate spectrum of mandelate racemase has been found to be remarkably wide (Scheme 2) [25–27]. (i) Aryl-substituted and heteroaryl mandelate analogues are freely racemized. The relative rates of racemization strongly depend on the electron density of the aryl moiety – electron-withdrawing and -donating substituents enhance and reduce racemization rates respectively – which clearly reflects the importance of resonance stabilization of the α-carbanion emerging during catalysis [29,30]. Any aryl moiety up to the size of a naphthyl residue can be accommodated in the hydrophobic binding pocket. (ii) Quite remarkably, the aromatic system can be drastically reduced to a minimum of a single C=C–π-bond in the β,γ-position of the substrate without significant loss of activity. (iii) However, disconnection of this system, e.g. by a –CH₂-unit from the α-C, would disrupt resonance stabilization and thus is not allowed. Thus all aliphatic and arylaliphatic α-hydroxyacrylic acids tested so far turned out to be non-substrates. (iv) Variations of the α-hydroxyacrylic acid moiety proved to be very limited. Whereas α-amino acids (and their N-acyl derivatives) were not accepted, a few α-hydroxyacrylamides were racemized at low rates [31].

In order to circumvent the limitation of mandelate racemase regarding its inability to accept saturated aliphatic and aryl-aliphatic α-hydroxyacrylic acids, a matching α-hydroxyacid racemase activity was sought. Based on early reports on the lactate racemase activity of halophilic Archaea, anaerobic rumen bacteria and Lactobacillus spp., a screening provided a set of lactic acid bacteria which were able to racemize a broad spectrum of aliphatic and aryl-aliphatic α-hydroxyacrylic acids at fair rates [32,33]. Whereas aliphatic α-hydroxyacrylic acids bearing structurally demanding branched side chains were accepted at moderate rates, straight-chain analogues and in particular aryl-aliphatic
derivatives, such as 3-phenyl-lactate and 2-hydroxy-4-phenyl-butanoate, were racemized at excellent rates. The latter compounds play an important role in the synthesis of rhinovirus and ACE (angiotensin-converting enzyme) inhibitors respectively.

Finally, a one-pot two-enzyme process was devised as follows (Scheme 2) [34]. Lipase-catalysed kinetic resolution of a range of (±)-α-hydroxycarboxylic acids using vinyl acetate as acyl donor gave the (S)-O-acetyl derivative at 50% conversion. Without separation of materials, the remaining non-reacted (R)-hydroxycacid enantiomer could be racemized in aqueous buffer; the latter proved to be essentially clean without occurrence of side reactions. When this lipase-racemase process was run in a sequence, the (S)-O-acetyl α-hydroxycarboxylic acid was obtained as the sole product in >80% yield and >97% enantiomeric excess. Any attempts to perform both reactions in situ have failed so far due to the inactivity of racemase preparations in organic solvents, such as di-isopropyl ether [35].

Since kinetic resolution provides both enantiomers, one can choose which of the pseudo-enantiomers P/B or A/Q (cf. Scheme 1) is harvested and which one is discarded. In contrast, this is impossible for deracemization processes, because only a single stereoisomer is formed. As a consequence, the availability of enantiocomplementary (bio)catalysts becomes a crucial point in order to direct the stereochemical outcome of a deracemization process. Making use of the apparent symmetry of lipase-catalysed ester hydrolysis and acyltransfer [36–39], enantiocomplementary deracemization of (±)-2-hydroxy-4-phenyl-3-butenic acid (a precursor for ACE inhibitors) was recently accomplished [40].

**Deracemization based on stereo-complementary sulphatases**

The molecular mechanism of the commonly employed hydrolyses involves nucleophilic attack on the carbonyl group of carboxylic acids or derivatives thereof, such as esters and amides [41]. As this group is a planar entity, any stereochemical alterations of the substrate (such as inversion of configuration) caused by enzymatic catalysis is impossible and as a consequence, the converted substrate(s) and product(s) are always homochiral, i.e. they possess the same absolute configuration. For an enantiocomplementary transformation, however, stereo-complementary (bio)catalysts acting with inversion and retention of configuration are required (Scheme 1, iv). Biocatalysts, which elicit the more complex potential to affect the stereochemistry of the substrate in a controlled fashion during catalysis, are rather rare and encompass haloalkane dehalogenases [42–44], epoxide hydrolases [45,46] and (alkyl) sulphatases [47,48]. In each case, an sp³-configured carbon atom is directly involved in catalysis, which opens the possibility for stereo-complementary pathways. These enzymes display not only enantioselectivity (by converting one enantiomer faster than the other), but also stereoselectivity with respect to retention or inversion of configuration.

Alkyl sulphatases of microbial origin were intensely investigated during the 1970s in view of their role in the biodegradation of alkyl sulphate esters used as detergents which were introduced in large quantities into the environment. Only later on did interest in their enantioselectivity and the stereochemistry during catalysis arise [49]. Based on early reports on alkyl sulphatase activity in Gram-negative bacteria (Pseudomonas C12B/NCIMB 11753 and Comamonas terrigena NCIMB 8193), a screening among Actinomycetes indicated Rhodococcus ruber DSM 44541 as a promising candidate [50]. Further investigations revealed the existence of two alkyl sulphatases, one of which (termed ‘RS2’ for Rhodococcus sulphatase 2) proved to be stable enough to allow its characterization [51]. Although the monomeric, soluble and co-factor- and metal-independent protein proved to act through strict inversion of configuration, its enantioselectivity was less than perfect (E values up to 21) and its substrate tolerance was rather narrow, typically comprising sec-alkyl sulphate esters similar to 2-octyl sulphate [52]. Although these limitations could be circumvented by addition of Fe³+ as ‘selectivity enhancer’ [53], this enantioselective inhibition led to a significant decrease in activity.

The continued search for more selective alkyl sulphatases was guided by the assumption that organisms known to possess a rich inorganic sulphur metabolism (encompassing all oxidation states of sulphur from sulphide to sulphate) might also metabolize sulphated organic species such as alkyl sulphates. From a screening among anaerobic and aerobic Archaea, Sulfolobus acidocaldarius DSM 639 was identified as a top candidate, showing improved activities and significantly enhanced enantioselectivities compared with RS2, with E values ranging up to 200 or more [54,55]. Overall, the enantiopreference (i.e. R) and the stereochemical pathway (i.e. inversion of configuration) proved to be identical with those of RS2.

In order to exploit the full potential of the enzymatic hydrolysis of sulphate esters, stereo-complementary sulphatases that act with retention of configuration were needed. In contrast with inverting sulphatases, the mechanism of action of retaining sulphatases is well understood [56–58] and was shown to proceed through cleavage of the S–O bond by nucleophilic attack of an aldehyde hydrate (formed from a cysteine or serine residue by post-translational modification). The search for a retaining sulphatase was led by the fact that all known retaining sulphatases acting through the above-mentioned mechanism possess the canonical sulphatase consensus motif C/S–X–P–X–R–X–X–X–T–G [59], which can be found in sulphatases from mammals, lower eukaryotes and prokaryotes, but is missing in the inverting RS2 (L. Arbanas, T. Glieder, U. Wagner and W. Kroutil, unpublished work). Sequence comparison with genomic databases revealed the existence of an unexpectedly high number of genes putatively encoding sulphatases in the marine planctomycete Rhodopirellula baltica DSM 10527 [60]. Detailed investigation revealed that whole resting cells could indeed hydrolyse a series of sec-alkyl sulphate esters with high enantioselectivity, with E values ranging up to 200 or more.
As presumed, catalysis proceeded through strict retention of configuration [61].

On the basis of the enantioselective enzymatic hydrolysis of sec-sulphate esters described above, a chemoenzymatic deracemization process for sec-alcohols was designed as follows [24a]. In a first step, kinetic resolution of a rac-sec-alkyl sulphate ester catalysed by the inverting RS2 furnished a homochiral mixture of formed sec-alcohol and remaining sec-sulphate ester. The latter was treated with an optimized mixture of aqueous t-butyl methyl ether and dioxan in the presence of p-toluenesulphonic acid as catalyst to effect clean hydrolysis of the remaining non-converted sulphate ester with retention of configuration. Overall, this two-step sequence furnished a single stereoisomeric sec-alcohol as the sole product (Scheme 3) [62].

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References

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