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Improved apparent enantioselectivity of a hydrolase by sequential hydrolysis and racemization

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ABSTRACT

Further improvement of the enantioselectivity of hydrolases with moderate enantioselectivity is of important significance to fulfill the requirement in industrial application. Herein, a strategy based on sequential hydrolysis and racemization was adopted, using esterase BioH from *Escherichia coli* as an example. After coupling with a mandelate racemase, the *E* value of esterase BioH towards methyl (*S*)-*o*-choloromandelate was enhanced from 73 to 162, demonstrating the effectiveness of this strategy.

Enantioselectivity is one of the most attractive properties of enzyme, which makes biocatalyst the preferred choice in the pharmaceutical industries. However, most natural occurring enzymes exhibit low enantioselectivity, which is insufficient for industrial applications.[1] To date, many methods have been developed for improving enzyme enantioselectivity, such as substrate engineering,[2] solvent engineering[3] and protein engineering,[4] among which directed evolution and rational design have achieved great success.[5, 6] However, in some cases, only marginal success was achieved despite numerous efforts. In such cases, shall we keep trying or rather turn to an alternative solution?

Linking several enantioselective reactions in a sequential order has been reported to improve the enantiomeric purity of the target compound by reinforcing the catalytic results of each other.[7-9] Glycidyl acetate was successfully prepared with the maximum enantiomeric excess (*ee* value) of 89% by sequential enzymatic resolution, even though the enantioselectivity was as low as 7 for each reaction step when the enzymes were used separately,[9] demonstrating the remarkable synergetic effect of coupling enantioselective enzymes. However, the application of this strategy is limited by the requirement of two or more enzymes with at least a certain value of enantioselectivity. Moreover, the enhanced enantiomeric excess of the target compound is often achieved at the cost of lowered yield.

Here, a strategy of sequential hydrolysis and racemization by coupling a hydrolase with a racemase was adopted as an efficient alternative for simultaneous enhancement of the apparent enantiomeric excess and the yield of the target compound.

If the target compound is an enantiomer of the hydrolysis product (P^R), dynamic kinetic resolution could be realized by coupling the hydrolase with a suitable racemase (Fig. 1A), which has been proven to remarkably enhance the purity and yield of the target compound.[10, 11]

The situation would be more complicated, if the target compound is an enantiomer of the substrate (S^R) in the hydrolase-catalyzed reaction. With the proceeding of the coupled hydrolysis and racemization reactions, the hydrolysis rate of the preferred substrate (S^S) would be accelerated by the reduced concentration of the major product (P^S) due to racemization, while the hydrolysis of the unpreferred substrate (S^R) would be hindered by the accumulation of the minor product (P^R), leading to an improved apparent enantioselectivity (Fig. 1B). If the racemized product could be chemically converted back to the starting material, then a recycling process could be constructed, resulting in obviously improved yield of the target compound, with a theoretical maximum of 100%.

Methyl (*R*)-*o*-chloromandelate (*R*-CMM) is a key intermediate for the production of clopidogrel, a platelet