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The road to large-scale production of enantiopure epoxides: kinetic resolution with the glutathione S-transferase StyI

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PURPOSE OF THE ABSTRACT

Epoxides are important platform chemicals but are also a common motive in nature. Especially chiral epoxides are of high interest in industry as they have a wide range of application like in drug or agrochemical synthesis [1]. One of the major obstacles in modern organic chemistry is to achieve a high yield production while maintaining both: enantio- and regioselectivities. Enzymatic epoxidation allows for both characteristics but is limited by the selectivity of the respective enzymes and thereby not universal applicable. Styrene monooxygenases (SMOs) for example, produce the (S)-epoxide with over 99% ee but no easy access to the (R)-epoxide was identified so far. Thus, kinetic resolution emerges as alternative solution. A few enzymes were described to convert epoxides with a certain selectivity like epoxide hydrolases (EHs), glutathione S-transferases (GSTs), CoM-transferases (CoMTs) or haloalcohol dehalogenases (HADHs) [2]. So far, a certain selectivity has only been reported for GSTs which brought these enzymes to our focus [2]. Until now, investigations on these enzymes have only been made for isoenzymes from rat liver and a restricted substrate amount. The overall function of GSTs in epoxide metabolism and their potential as enantioselective catalyst remain ambiguous [1].

Recently, a bacterial glutathione S-transferase (StyI) was found in the styrene degradation pathway of the actinobacterium Gordonia rubripertincta CWB2 [3]. Unlike other styrene degraders, strain CWB2 demonstrated its ability to metabolize a variety of styrene-related compounds, which was exploited biotechnologically to produce ibuprofen in a co-metabolic process [4]. The unusual presence of GSTs within bacterial styrene degradation appears to broaden the substrate spectrum of this pathway. We therefore produced StyI heterologously in E. coli for subsequent characterization of the substrate selectivity and enantiomeric preference. Our studies revealed a three times higher activity with (S)-styrene oxide compared to its (R)-enantiomer (Figure 1). This was also supported by a 16-times higher affinity and a 1.8 fold higher vmax for (S)-styrene oxide, giving a clear evidence of StyI's stereoselectivity. This observation is in agreement with the proposed natural role of the enzyme as StyI is the subsequent enzyme of the (S)-enantioselective styrene monooxygenase [5].

To investigate the potential of the GST StyI for enantioselective epoxide opening, chiral GC-FID analysis was performed. More than 10 substrates comprising different structural motives (aromatic and aliphatic) were tested within the substrate screening. For styrene oxide derivatives, like para-fluoro- or para-chlorostyrene oxide, also a high selectivity for the (S)-enantiomer was found. In contrast, the enantiomeric preference of more diverse structures such as phenylpropylene oxide, indene oxide, and aliphatic epoxides revealed a reduction in enantioselectivity. Despite this, the overall substrate scope for StyI was remarkably large.

Since StyI showed high enantioselectivity towards (S)-styrene oxide and therefore indicating its promising application in production of enantiopure (R)-epoxide, enzymatic kinetic resolution was performed. Subsequently, the reaction volume was increased to 150 mL scale to proof the feasibility of large-scale kinetic resolution. The

approach yielded about 18 mg pure (R)-styrene oxide which was validated by NMR spectroscopy.

In conclusion, we found the glutathione S-transferase StyI to be highly enantioselective for the native substrate (S)-styrene oxide and structural analogous. Further, a certain selectivity was observed for non-aromatic substrates which highlights the broad application range for this enzyme. This led to the subsequent upscaling of the process enriching pure (R)-styrene oxide. Hence, this study reveals StyI's potential within the road to large-scale production of enantiopure epoxides like styrene oxide.

FIGURES

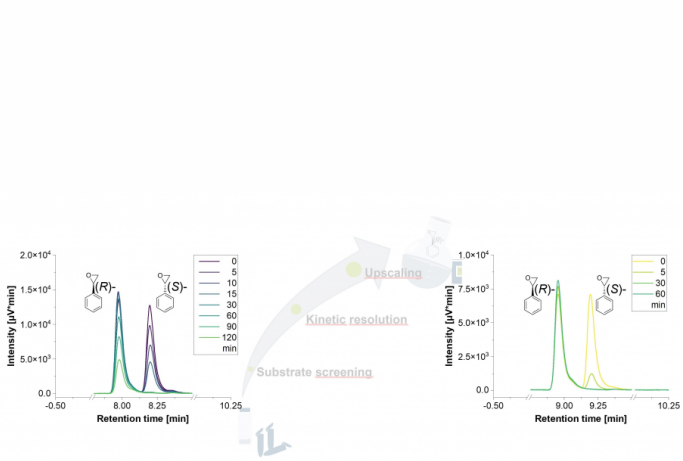

FIGURE 1

Workflow from enantioselective substrate consumption to the upscaling of kinetic resolution with StyI.

Left: Conversion of the chiral substrate styrene oxide by StyI determined by GC-FID. StyI shows a strong enantiomeric preference for (S)-styrene oxide, whereas the (R)-epoxide is consumed afterwards. Right: Kinetic resolution of racemic styrene oxide.

FIGURE 2

KEYWORDS

Kinetic resolution | Enantiopure epoxides | Glutathione s-transferase | Upscaling

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