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Styrene oxide isomerase performing both epoxidation and isomerization of styrene in presence of hydrogen peroxide

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PURPOSE OF THE ABSTRACT

Styrene oxide isomerase (SOI), a membrane bound enzyme in the side chain oxygenation of styrene degradation pathway, believed to be co-factor independent. SOI catalyses the isomerization of styrene oxide (SO) into phenylacetaldehyde (PA). SOIs are approximately 20kDa in size as a monomer. During protein enrichment the membrane fraction comprising SOI was always reddish1, but this had never been explained. The mechanism of SOI was recently proved to be 1,2 hydride/methyl shift, but the active site remained as an enigma2. It was also known that SOI are highly stable at high temperature as well as wide range of pH1 potentially making this enzyme a candidate for various reaction in biotechnological application.

We discovered the presence of styrene degrading gene cluster from an alphaproteobacteria Zavarzinia compransoris Z-1155 by in silico genome mining. The ZcSOI-gene from Z. compransoris Z-1155 was cloned into pET vector and heterologously expressed in E. coli (yield: 6.1 mg/mL). A purification protocol for the membrane protein was established. Hence, this provided the chance to characterize ZcSOI in more detail which we did in comparison to related enzymes from Rhodococcus and Pseudomonas. SOIs are functional as trimer possessing an iron containing heme B at the interface of two protomers, comprising three active sites. This explains the previously observed red colour of the enriched protein. We confirmed the presence of the heme B cofactor by several methods (ICP-MS, UV/vis spectroscopy, EPR) and believe it is key for epoxide conversion. The direct kinetics of SOI for its natural substrate showed a turnover of around 500s-1 and the catalytic efficiency with just one order of magnitude short of catalase. The stopped-flow measurement of heme reduction with sodium dithionite showed the presence of reducible heme. Biochemically the enzyme behaved similar to that of other SOIs from previous studies1,3. Owing to the fact that SOI has reducible heme, performs the isomerization of SO, we aimed at exploring the biochemical potential of ZcSOI for complete conversion of styrene to phenylacetaldehyde (Fig. 1). Preliminary test of enzyme with styrene and hydrogen peroxide showed the production of both SO and PA. The extended incubation resulted in only PA as the final product. In order to optimize, the kinetic of H2O2 for the enzyme ZcSOI was tested spectrophotometrically using ABTS as substrate. The ZcSOI showed a Vmax of about 1568.9 µmoles/min with Km 1.6 mM. This assay proves that the enzyme acts like a peroxidase while the earlier assay with H2O2 mediated combined conversion of styrene to phenylacetaldehyde provides some hint that the enzyme could possibly play the role of peroxygenase in epoxidation while the produced epoxides being the natural substrate for ZcSOI instantly converted into phenylacetaldehyde. Both the products having significance in industrial applications, a single enzyme performing the combined reaction, possessing redox potential heme which accepts H2O2 makes this enzyme remarkably a trend setter in various peroxygenase, peroxidase and many more combined biotechnological reactions.

FIGURES

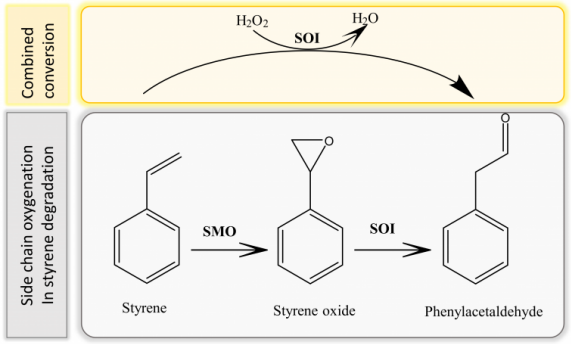

FIGURE 1

Combined conversion of styrene to phenylacetaldehyde by SOI

Schematic diagram showing the classical side chain oxygenation of styrene biodegradation in gray and the combined conversion of Styrene to PA by SOI in yellow (this study)

FIGURE 2

KEYWORDS

SOI | heme | epoxidation and isomerization | biotransformation

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