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Discovery and rational mutagenesis of methionine sulfoxide reductase (MsrA) biocatalysts to expand the substrate scope of the kinetic resolution of chiral sulfoxides

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PURPOSE OF THE ABSTRACT

Chiral sulfoxides are an important class of organic compounds that are often used in organic synthesis as chiral auxiliaries, synthons for C–C bond forming reactions, directing groups in C–H bond functionalisation and can partake in numerous other functionalisation reactions. Moreover, sulfoxides are widely found in pharmaceutically active ingredients such as the blockbuster drug esomeprazole. The most common biocatalytic approach to the synthesis of chiral sulfoxides rely on the use of oxidative enzymes such as monooxygenases or peroxygenases.[1] However, these can present some disadvantages when it comes to their application on large scale industrial production, such as low-yielding profiles, the use of expensive cofactors and the need of effective oxygenation throughout the reaction mixture.[1] A less common route to obtain asymmetric sulfoxides is the use of reductive enzymes, which, starting from a racemic mixture, can perform kinetic resolutions to afford single enantiomers. For example, Methionine sulfoxide reductases (Msrs) are enzymes known to reduce (S)-methionine sulfoxide to methionine in order to repair cellular damage by reactive oxygen species and have proved to be capable of reducing non-natural sulfoxides.[2,3]

Therefore, following our interest in industrially applicable green methodologies for the synthesis of sulfur-containing compounds, herein we show how Methionine sulfoxide reductases A (MsrAs) were used for the development of a new biocatalytic protocol for the synthesis of (R)-sulfoxides at high substrate concentrations.[4] In this investigation, we identified the selective and robust scMsrA from Saccharomyces cerevisiae as hit enzyme from a panel of 15 MsrAs. A series of small alkyl aryl sulfoxides racemates were effectively reduced with excellent conversion and ee (>90%) using dithiothreitol (DTT) as the enzyme regenerating agent, a cheaper and low molecular weight dithiol substitute to thioredoxin. Moreover, with the aim to expand the substrate scope of MsrA biocatalysts, a library of mutant enzymes has been designed via rational mutagenesis utilising in silico docking, molecular dynamics, and structural NMR studies. The mutant enzyme MsrA33 was found able to catalyse the kinetic resolution of bulky sulfoxide substrates bearing non-methyl substituents on the sulfur atom with ees up to 99%, overcoming a significant limitation of the currently available MsrA biocatalysts.

FIGURES

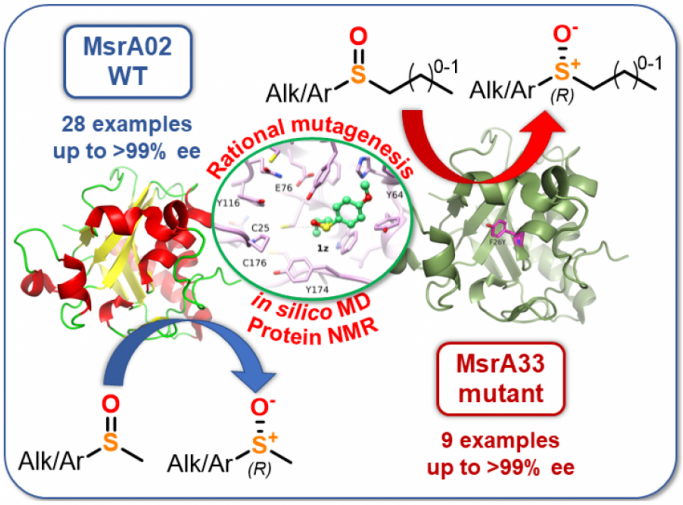

FIGURE 1

Graphical Abstract

Discovery and Rational Mutagenesis of Methionine Sulfoxide Reductase Biocatalysts To Expand the Substrate Scope of the Kinetic Resolution of Chiral Sulfoxides

FIGURE 2

KEYWORDS

sulfoxides | methionine reductase | mutagenesis | mutant enzyme

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