Identification two key residues at the intersection of subdomains of a thioether monooxygenase for improving its sulfoxidation performance

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Abstract

ABSTRACT: AcCHMO, a cyclohexanone monooxygenase from Acinetobacter calcoaceticus, is a typical Type I Baeyer-Villiger monooxygenase. AcCHMOM6 is a mutant of AcCHMO we obtained previously that could oxidase the omeprazole sulfide to chiral sulfoxide drug esomeprazole. Based on the structural characteristics of AcCHMO, focused mutagenesis strategy was adopted at the intersections of FAD binding domain, NADPH binding domain and ∝-helical domain. By the focused mutagenesis and subsequent global evolution, two key residues (55-Leu and 497-Pro) at the intersection of subdomains were identified, of which the L55Y mutagenesis accelerated the H- transfer from NADPH to FAD, while the P497S mutagenesis widened the bottleneck radius of the substrate tunnel and alleviated the substrate inhibition remarkably. By combination of the two mutagenesis, AcCHMOM7 (L55Y/P497S) increased its specific activity from 18.5 U/g to 108 U/g, and its Ki of the substrate sulfide was increased from 34 μM to 265 μM. These results indicated that the catalytic performance can be elevated by modification of the sensitive sites in the intersection of subdomains of AcCHMO, which also provided some insights for the engineering of other type I BVMOs or other multi-subdomain proteins.

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