



Structure-based design of the FAD-dependent monocygenase StyA1 from Variovorax paradoxus J. Kratky¹, R. H. Weiße¹, T. Heine², D. Tischler², N. Sträter¹

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Introduction

Styrene monooxygenases (SMOs) catalyse the enantioselective epoxidation of styrene and structurally related compounds. They are of interest for the development of new biocatalytic production processes for pharmaceuticals and fine chemicals. Our aim is to improve the substrate specificity and enantioselectivity of the SMO StyA1 for such applications. Hence, we used protein crystallography to determine the binding modes of substrates and the redox cofactor FAD. Challenges on the road to obtain structural information on the substrate binding mode were overcome by mutation of an active site residue. Residues lining the active site pocket were mutated to generate variants with different substrate preference.



Fig. 1: Binding mode of indole within the substrate tunnel in presence of the cofactor FAD analyzed at a resolution of 1.8 Å.

Substrate binding mode -Anomalous data





Fig. 2: Binding mode of 6-bromoindole in presence of the cofactor FAD analyzed at a resolution of 1.41 Å. The $(2mF_{obs} - DF_{calc}, \alpha_{calc})$ electron density map is depicted at a contour level of 1.0 σ and the anomalous map at 4.0 σ . Anomalous data was collected at $\lambda = 2.214$ Å.

Initially, no catalytically competent substrate binding modes could be obtained (Fig. 1). We identified a residue that potentially blocks the entry of the substrate to the active site. Exchange of this residue for smaller side chains resulted in a more open binding pocket. Indeed, structures with catalytically competent substrate binding modes could be determined.

Fig. 4: Crystals of a StyA1 mutant. Crystals from the cocrystallization with methyl phenyl sulfide (A), styrene oxide (B), indene oxide (C), indene (D), indole (E), 6-bromoindole (F), benzyl phenyl sulfide (G), benzyl phenyl sulfoxide (H) and diphenyl sulfide (I) are shown.





Fig. 3: Selected mutations: Phe202, Ile303 and Thr304 line the substrate binding pocket and are likely to influence substrate specificity.



Expression, purification, co-crystallisation

Discussion and Outlook

A modification near the substrate binding pocket allowed for the first time crystallographic studies of the binding modes of different substrates to a SMO. This opens the door to structure-based design of StyA1 variants. We expect that further modifications of the binding pocket might lead to an increase in enzymatic activity or novel substrate turnover and will also give us the possibility to steer the enantiospecificity of the enzyme. This is a big step towards biocatalytic applications of this protein. So far over 20 different mutants were created and are currently studied via crystallographic and biochemical approaches regarding their catalytic properties.







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