MS09-P05

Crystal structures of *Serratia marcescens* reveal a homotetramer and insight into a flexible catalytic cleft

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The short-chain dehydrogenase/reductase (SDR) from erratia marcescens BCRC 10948 (SmSDR) that catalyzes an asymmetric reduction of alkyl ketones to the corresponding chiral alcohols is capable of converting 1-(3-hydroxyphenyl)-2-(methylamino) ethanone (HPMAE) into (R)-phenylephrine, which is marketed medically as a nasal decongestant agent. Here, we report the crystal structure of the apo-form SmSDR solved to 1.47 Å. The SmSDR structure shows a homotetramer of which each subunit consists a nucleotide-binding Rossmann domain and a presumed binding pocket surrounded by five loops and an helix (α7). Phe98 and Phe202 stand on the α7 helix and loop b4-a4) and form hydrophobic contacts with nearby residues. Site-directed mutagenesis characterization (WT, F98Y, F98YF202Y, and F98YF202L) revealed that F98YF202L exhibited a higher transformation activity toward HPMAE. Crystal structures of F98AF202L SmSDR, F98LF202L·NADPH, and F98YF202Y variants show an overall homologous structure. Substitution of F98 with alanine leads to the loss of the hydrophobic contacts between two arms, whereas F98YF202Y creates a strong H bond. In addition, the whole-cell conversion of F202A had an increased yield (1.9-fold) than that of WT. Together, our results suggest a robust structure-guided approach to stabilize the binding pocket that can be used to generate a valuable SmSDR variant for pharmaceutical applications.

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Keywords: short-chain dehydrogenase/reductase), Serratia marcescens BCRC 10948, (R)-phenylephrine

MS09-P06

Crystal structure of PigA, a proline-oxidizing enzyme in prodigiosin biosynthesis

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Prodigiosin is an intensely red pigment comprised of three pyrroles. The biosynthetic pathway includes a two-step proline oxidation catalyzed by PigA, using FAD as its cofactor. Here the enzyme is crystallized in apo and FAD-bound forms. The protein folds into two α-helical domains (I and III) separated by a β-sheet domain (II) and it belongs to the acyl-CoA dehydrogenase (ACAD) family. In the tetrameric enzyme, which comprises two dimers associated via domain III, FAD is located in a cleft surrounded by all three domains of one monomer and domain III of another. The overall structure as well as the FAD-binding mode is similar to those of other ACAD-family enzymes. Alternate backbone conformations in the N-terminal part of helix αG correlate well with the expected location of substrate to the Re side of FAD. Complex modeling with PigG, the acyl carrier protein, suggests a plausible binding mode. The structure helps explain the proline oxidation mechanism, in which Glu244 plays a central role in the double-bond formations. It also reveals a plausible pocket for oxygen binding and reduction on the Si side of FAD.

Keywords: Prodigiosin, proline-oxidizing, PigA