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Role of a flexible loop in the substrate selectivity of an engineered variant of hyperthermophilic multicopper oxidase McoP

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PURPOSE OF THE ABSTRACT

Multicopper oxidases (MCOs) oxidize a range of aromatic substrates such as polyphenols, methoxy-substituted phenols, and amines, concomitantly with reducing dioxygen to water. Some MCOs, denominated metallo-oxidases, exhibit catalytic activity toward metal ions, such as Cu(I) and Fe(II). In this work, we have used directed evolution to improve substrate efficiency of the metalloxidase McoP from the Pyrobaculum aerophilum for organic substrates. Random mutagenesis and DNA shuffling followed by high-throughput screening led to the generation of variant 3F3, showing only six mutations and a 100-fold higher catalytic efficiency for ABTS than wild-type enzyme.
The X-ray crystallography was used to solve the crystal structure of the 3F3 variant, using the wild-type structure as a search model (PDB 3AW5, Sakuraba et al). Two mutations (V206I and S331P) are distal to the T1Cu, at ~26 Å and ~33 Å, mutations P292H and F361S are at ~13 Å and ~12 Å, whereas mutations M393V and P390T are at ~ 6 Å to T1Cu; P390T is adjacent to the T1Cu ligand H391, and M393V is adjacent to the T2Cu ligand H394. An analysis of the solvent access in the crystal structures allowed us to define two main pathways from the solvent media to the T1Cu, one tunnel (Path 1) and one cavity (Path 2) parallel to each other. The tunnel is surrounded by a flexible loop (288-310) that contains one of the six mutations, P292H, that together with a short α-helix is covering the T1Cu access in the wild-type. This loop becomes more flexible in the 3F3 variant, resulting in an enlarged diameter (4.0 Å) of the tunnel, when compared with the wild-type (2.8 Å), which is expected to facilitate electron transfer from substrates to T1Cu. The cavity shows the H465 ligand and the nearby residue W355 (at 3 Å) exposed through a pocket (P1). Interestingly, the W355 is approximately three times more solvent-exposed in 3F3 due to the appearance of a new pocket (P2) as a result of the higher flexibility of the loop 288-310. The enlarged tunnel and the extra-pocket P2 in the cavity of 3F3 can explain the significantly higher activity of this variant towards ABTS. This work allows to investigate the molecular mechanisms involved in the evolution of enzyme fitness, which are important for engineering new proteins for biotechnological applications.

References:
Sakuraba, H., et al, Acta Crystallogr Sect F Struct Biol Cryst Commun, 2011, 67, 753-757.

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FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Multicopper oxidases | Directed evolution | X-ray crystallography | Biotechnology

BIBLIOGRAPHY

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