MS07-P06 | COVALENT BOND BETWEEN SIDE CHAINS OF TRYPTOPHAN AND HISTIDINE IN BILIRUBIN OXIDASE IS ESSENTIAL FOR SUBSTRATE BINDING

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Bilirubin oxidase (EC 1.3.3.5) from *Myrothecium verrucaria* is a blue multicopper oxidase capable of oxidation of organic and inorganic substrates, including bilirubin. It utilizes two active sites with one and three copper ions to transfer electrons from substrates from site T1 to the trinuclear cluster and to reduce molecular oxygen to water [1,2]. The enzyme is used in a number of fields, including medicine and biotechnologies. The substrate oxidation site has an exceptional feature - a covalent link between His398, coordinating T1 Cu, and Trp396, forming the substrate binding site [3]. Here we show that the covalent modification, verified by mass spectrometry and crystallography, alters the T1 copper coordination and is crucial for formation of the properly organized substrate binding site. Mutagenesis of Trp396 leads to conclusions that the adduct is crucial for oxidation of substituted phenols and substantially influences the rate of bilirubin oxidation. Even if the link likely participates in the actual electron transfer, its importance lies rather in formation of the substrate binding site. Our crystallographic data for substrate binding uncover important amino acid residues of the substrate binding site and explain why in the absence of the Trp396 side chain the activity of the enzyme remains unchanged for some substrates. This work was supported by ERDF (CZ.02.1.01/0.0/0.0/15_003/0000447, CZ.02.1.01/0.0/0.0/16_013/0001776) and MEYS CR (LM2015043).

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