

Structure determination of a blue laccase from *Trametes Troglia* at 1.5 Å

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Laccase is an extracellular blue oxidase distributed in plants and fungi [1]. It is a multi-copper protein containing four copper atoms per molecule, which are organized into three types of copper sites that determine the characteristic spectral properties of the enzyme. Laccases oxidise a broad range of substrates such as polyphenols, methoxy-substituted phenols, aromatic amines, etc. The one-electron oxidation of these reducing substrates occurs concomitantly with a four-electron reduction of molecular oxygen to water. Laccase is considered to play a major role in the degradation of lignin by white rot fungi. Although the exact role of the laccase in lignin biodegradation is not known, these enzymes may have applications e.g. in pulp and paper industry and in removal of environmental pollutants from soil and water.

Here we report the crystal structure determination of a blue laccase from *Trametes Troglia* [2]. Crystals of laccase from *T. Troglia* grow after one week at 23 °C using the sitting drop vapour diffusion method. 1 µl of a 20 mg/ml protein solution were mixed with 1 µl of a solution containing 21% PEG 8000, calcium acetate 0.2 M, PEG 400 3%, Hepes 0.1 M pH 7.5. Crystals belong to the primitive orthorhombic space group $P2_12_12_1$ with unit cell dimension $a=84.4$, $b=85.1$, $c=108.6$. Assuming one molecule per asymmetric unit the solvent content 64% of the unit cell ($V_m = 3.5 \text{ \AA}^3/\text{Da}$). A complete native data set was collected at the BW7B beamline at the DORIS storage ring, Hamburg, Germany, using a MAR image plate detector and a wavelength of 0.8435 Å. Data were collected at 100 K adding 25% glycerol to the mother liquor as cryoprotectant at a maximum resolution of 1.58 Å. Data processing with Mosflm and Scala gave 107388 unique reflections, an overall completeness of 99.6% and an R_{sym} of 0.059. The structure was solved with molecular replacement using the structure of a laccase from *P. Tigrinus* (not yet deposited) as a starting model and the program MolRep from the CCP4 package. Model refinement is under progress, the current R-factor is 0.179 and R_{free} 0.193. The structure present all the four copper atoms and it contains several carbohydrates moieties.

It is aimed that the *T. Troglia* laccase structural model will assist in the elucidation of the catalytic mechanism and electron-transfer processes of this family of proteins.

References

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