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Structure and function of *Aspergillus niger* laccase McoG

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Abstract: The ascomycete *Aspergillus niger* produces several multicopper oxidases, but their biocatalytic properties remain largely unknown. Elucidation of the crystal structure of *A. niger* laccase McoG at 1.7 Å resolution revealed that the C-terminal tail of this glycoprotein blocks the T3 solvent channel and that a peroxide ion bridges the two T3 copper atoms. Remarkably, McoG contains a histidine (His253) instead of the common aspartate or glutamate expected to be involved in catalytic proton transfer with phenolic compounds. The crystal structure of H253D at 1.5 Å resolution resembles the wild type structure. McoG and the H253D, H253A and H253N variants have similar activities with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid or *N,N*-dimethyl-*p*-phenylenediamine sulphate. However, the activities of H253A and H253N with 2-amino-4-methylphenol and 2-amino-4-methoxyphenol are strongly reduced compared to that of wild type. The redox potentials and electron transfer rates (k_s) of wild type and variants were determined (McoG wt E°' is +453 mV), and especially the reduced k_s values of H253A and H253N show strong correlation with their low activity on phenolic compounds. In summary, our results suggest that the His253 adaptation of McoG can be beneficial for the conversion of phenolic compounds.

Keywords: *Aspergillus niger*; crystal structure; laccase; redox potential; metalloprotein; multicopper oxidase

Data deposition: Coordinates have been deposited with the Protein Data Bank. Accession codes are 5LM8, 5LWW and 5LWX.

Abbreviations: CV, cyclic voltammetry; DT, decane-1-thiol; MCO, multicopper oxidase; *Ma*L, laccase from *Melanocarpus albomyces*; *Ta*L, laccase from *Thielavia arenaria*; SAM, self-assembled monolayer; SCE, saturated calomel electrode; SHE, standard hydrogen electrode; DMPPDA, *N,N*-dimethyl-*p*-phenylenediamine; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid).

1 Introduction

Multicopper oxidases (MCOs) form a family of redox enzymes that catalyze the reduction of molecular oxygen into water by a four-electron transfer process. It includes laccases (EC 1.10.3.2), ascorbate oxidases (EC 1.10.3.3), bilirubin oxidases (EC 1.3.3.5) and ferroxidases (EC 1.16.3.1), which are key enzymes in many biological processes of prokaryotic and eukaryotic organisms [1, 2]. Their ability to catalyze the oxidation of various aromatic substrates with the concomitant reduction of molecular oxygen to water as sole byproduct makes them interesting green biocatalysts. The MCO catalyzed redox process is mediated by two copper centers, which contain

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