

Met-rich Domains and Cu⁺ Oxidation by Multicopper Oxidases: an Electrochemical Study

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Multicopper oxidases (MCOs) are redox enzymes present in all domains of life that contain a couple of Cu-centres: mononuclear T1 and trinuclear T2/T3. Although the coordination and environment of these copper centres are highly conserved, the overall protein structure may present significant disparities responsible notably for various physiological roles MCOs can play. Accordingly, electron donors accepted by MCOs vary from small organic molecules to metal ions, while electron acceptor is always oxygen [1]. Together with terminal cytochrome c oxidases, MCOs are the only enzymes capable of four-electron oxygen reduction. But, unlike the former, MCOs can perform this reaction at higher potentials reaching 0.78 V (NHE) which is beneficial for different enzymatic and biohybrid fuel cells.

Earlier, we showed that the MCO from *Thermus thermophilus*, which exhibits direct electron transfer of oxygen reduction, gives rise to a new catalytic wave upon Cu²⁺-addition in certain conditions [2,3]. We rationalised and ascribed this wave to a cuprous oxidase activity displayed by this enzyme thus allowing us to propose its physiological role in copper detoxification process [4]. The only other well-studied MCO with confirmed cuprous oxidase activity, CueO from *E.coli*, has a large methionine-rich domain proposed to play a role in the copper binding. This domain is significantly shorter in the MCO from *T.thermophilus* raising a question about its true role. Here we present the utility of electrochemistry and importance of electrode surface chemistry in study and quantification of the cuprous oxidase activity of MCOs related notably to the presence of an additional Met-rich domain.

[1] N. Mano, A. de Poulpiquet, Chem. Rev. 118 (2018) 2392–2468.

[2] V.P. Hitaishi et al., J. Am. Chem. Soc. 142 (2020) 1394–1405.

[3] R. Clément et al., Biochimie 182 (2021) 228–237.

[4] I. Mazurenko et al., Curr. Opin. Electrochem. 32 (2022) 100919.