Substrate and dioxygen binding to an endospore coat laccase from *Bacillus subtilis*, Francisco J. Enguita, David Marçal, Lígia O. Martins, Rosa Grenha, Isabel Bento, Gonçalo Gato, Peter F. Lindley and Maria Arménia Carrondo*, *Instituto de Tecnologia Química e Biológica, Oeiras, Portugal, Portugal*. E-mail: carrondo@itqb.unl.pt

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Endospores produced by the Gram positive soil bacterium *Bacillus subtilis* are shielded by a proteinaceous coat formed by over thirty structural components which self-assemble into a lamellar inner coat and a thicker striated electrodense outer coat. The 65 kDa CotA protein is an abundant component of the outer coat layer. CotA is a highly thermostable multicopper oxidase with laccase activity, whose assembly into the coat is required for spore resistance against hydrogen peroxide and UV light. We have reported previously the structure of the native CotA laccase solved by the MAD method using the copper anomalous signal [1,2]. The CotA laccase has been also crystallised in the presence of the non-catalytic co-oxidant, 2,2´-azinobis-(3-ethylbenzothiazoline-6-sulphonate) [ABTS] and the structure determined using synchrotron radiation [3]. The binding site for this adduct is well defined and indicates how ABTS in conjunction with laccases could act as an oxidative mediator towards non-phenolic moieties. In addition, a dioxygen moiety is clearly defined within the solvent channel oriented towards one of the T3 copper atoms in the trinuclear centre. The present study shows that cryo-freezing of crystals of the CotA laccase from *B. subtilis* soaked with ABTS for one hour induce the ABTS to form a stable complex with the laccase. Soaking times longer than this appear to cause dissociation of the ABTS and substantial loss of copper from the trinuclear centre. The enzyme has probably been trapped in a dormant form with an dioxygen molecule in the solvent channel awaiting binding to one of the T3 copper atoms. When the enzyme receives further electrons, the dioxygen will move closer to the copper and will be reduced to two molecules of water. Clearly more detailed studies are required to define the precise mechanisms of both dioxygen reduction and the role of oxidative mediators. We are currently investigating the reaction of CotA laccase crystals with other laccase activity mediators and substrates.

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