Characterization of novel thermostable bacterial Laccase-like multi-copper oxidases

Laccase-like Multi-copper Oxidases (LMCOs) catalyze the oxidation of a broad range of substrates in a redox reaction coupled to the complete reduction of molecular oxygen to water. The reaction is catalyzed by four coupled copper-ions that are positioned in the LMCO in such a way, that the substrate bind to one side of the enzyme, while oxygen is recruited and water expelled on the other. This powerful mechanism makes LMCOs clean enzyme substitutions in all chemical processes that are traditionally driven by the addition of reactive oxygen species such as hydrogen peroxide. E.g. dye decolorization, bleaching of paper pulp, delignification of biomass and remediation of polluted water. In order to widen the applicability of LMCOs, it is important to establish the properties and substrate specificities of naturally occurring LMCOs. This is especially true for LMCOs from bacteria, whose role in nature is not well understood. If we want to change a LMCO, to specifically catalyze a man-made reaction, it becomes important to have a diverse and stable starting protein. In this regard bacterial LMCOs are of special interest, because they are intrinsically thermostable and distinct variants can be found in the rapidly increasing number of sequenced bacterial genomes. This dissertation describes our effort to identify and express novel LMCOs from bacterial origins. Some of these enzymes were also characterized, and special emphasis was put on revealing their substrate specificity and thermostability. Bacillus clausii KSM-16 is known to produce a potent alkalophilic and thermostable protease that is sometimes used in laundry detergent mixes. We have expressed and characterized the LMCO coded in the genome of the same bacterial strain, and found that it is a thermostable enzyme with substrate specificity similar to that of the well-characterized Bacillus subtilis CotA. Stability and catalytic reactivity were both slightly less than B. subtilis CotA, while the preferred pHs for both properties were shifted about 1 unit to the more alkaline. Thermobaculum terrenum is a thermophilic bacterium cultured from a hot dirt patch in Yellowstone National Park. It belongs to the evolutionary interesting phylum Chloroflexi that has been proposed to represent some of the earliest lifeforms on Earth. The genome of T. terrenum codes for a LMCO, and we have expressed and characterized the enzyme. It is the second most thermostable characterized LMCO, but was only able to selectively oxidize two out of 57 tested substrates. Of special interest to the characterization of bacterial LMCOs is the thermostability. Measurement of thermal inactivation of LMCOs is hampered by an often observed heat-induced increase in enzyme activity. We found that heat activation is accompanied by a change in the Electron Paramagnetic Spectroscopy (EPR) spectrum, and used this to characterize the mechanism behind the process. It is a redox transformation, and for the T. terrenum LMCO it was found to be controlled by temperature and NaCl, while the similar transformation in B. subtilis CotA also needed the reducing agent, ascorbate, in order to take place. The discovered mechanism can most likely be expanded to also encompass other LMCOs that have previously been shown to undergo heat-activation.

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