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Deciphering the complex paramagnetic NMR spectra of small laccase

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Citation

Dasgupta, R. (2021, June 15). *Deciphering the complex paramagnetic NMR spectra of small laccase*. Retrieved from <https://hdl.handle.net/1887/3188356>

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Issue Date: 2021-06-15

Summary

Enzymatic biofuel cells (EBCs) promise a sustainable future. They use enzymes to oxidize substrates at the anode and reduce oxygen to water at the cathode. Laccases are preferred enzymes for the oxygen reduction reaction but their stability is reduced when functionalized over an electrode. Artificial, bio-inspired catalysts were designed to overcome this challenge but come at a cost of losing catalytic efficiency. Motions within the active site of laccase might be one of the reasons that determine this efficiency. Specific motions can help attain a conformation that can reduce the energy barrier of a catalytic step by having favourable interactions with the transition state. For the complex oxygen reduction reaction, consisting of multiple steps, such motions seem unavoidable. This thesis provides the groundwork to study such motions at the active site of laccase using NMR spectroscopy.

Chapter 1 summarizes the properties of the tri-nuclear copper center (TNC) of several laccases, where oxygen reduction takes place, and compares the TNC complexity with the reported artificial catalysts. The two-domain trimeric small laccase from *Streptomyces coelicolor* (SLAC) is used in this study. Due to the paramagnetic nature of the copper ions in the TNC, application of standard NMR experiments is not possible to characterize the dynamics or assign the resonances. Paramagnetically tailored NMR experiments and second-shell mutagenesis aided in overcoming this challenge. The native intermediate (NI) state and the resting oxidized (RO) state of the TNC are studied in detail (Chapters 2 to 4). The NMR spectra of SLAC are shown to represent a mixture of these two states (Chapters 3 and 4). The NI state was studied using a type 1 site depleted mutant of small laccase (SLAC-T1D) because the relative intensities show it to be the major state in this mutant. Five chemical exchange processes were discovered and could be attributed to ligand histidine ring motions (Chapters 2 and 3). The second-shell mutation of Y108F aided in assigning two of these processes to two histidine ligands in the NI state (Chapter 3). The RO state was studied using SLAC wild type (wt) because this is the major state (Chapter 4). Interestingly, another second-shell mutation, Q291E of SLAC-T1D, is found to cause the enzyme to be exclusively in the RO state. This result suggests a subtle balance between the RO and the NI states (Chapter 4). Using SLAC-wt and SLAC-T1D/Q291E, resonances were assigned to two histidine ligands in the RO state (Chapter 4). In the NI and RO states, all eight (T3 + T2 sites) and six (T3 site only) histidine ligands, respectively, are observed.

Quantum calculation can be advantageous in assigning the resonances. This requires a proper description of the geometry of the TNC. Using previous studies as a benchmark, the geometry of the RO state was modelled and the electronic structure

was calculated. This work shows that the orientation of Y108 hydroxyl group is important for the spin density distribution at the TNC (Chapter 5).

Solid state NMR can provide information about the dipolar coupling between nuclei near the metal center. The dipolar coupling strength can be used to probe the presence of anisotropic motions at the active site of laccase. Chapter 6 shows the efficacy of rotational echo double resonance (REDOR) based pulse sequence in determining the dipolar coupling in a paramagnetic environment in a model compound Cu-DL-(Ala)₂.H₂O. It is shown that accurate distance information can be obtained from REDOR experiments (Chapter 6).