

P008 The Reversibility of P → F State Transition in Cytochrome c Oxidase from *Paracoccus denitrificans*

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Terminal oxidases such as the *Paracoccus denitrificans* aa₃ type cytochrome c oxidase (CcO) require four electrons for the O₂ reduction reaction. Stoichiometric and excess amounts of H₂O₂ induce P_H/F_H^{*} and F_H states, respectively, representing doubly and triply reduced intermediates of CcO. The P_H intermediate state is already an oxoferryl state, which implies the lack of an electron for the transition from the two-electron reduced R state to the P_H state, and Y167 has been found by electron paramagnetic resonance (EPR) studies to host a thermodynamically stable amino acid radical in this state. The wild type structure suggests a bound peroxide in the O state. Optical and EPR studies have given indications that also other intermediates could contain peroxide species. The excess of H₂O₂ used to induce the F_H state in wild type CcO can be eliminated using catalase. This treatment leads to formation of the F → P/F^{*} states reaction in both solubilised CcO and reconstituted CcO into proteoliposomes. The reformation of the P and F^{*} states is accompanied by appearance of the Y167 radical EPR signal. Here we present the *novel* P_{FH}/F_{FH}^{*} states. The F_H state may host a superoxide (or a peroxide) adduct in the active site. The consequences of these findings are discussed and a new model of the catalytic cycle is introduced. The central concept of the model is a complexed peroxide bound in the O state.