

P031 Coupling of electron and proton transfer in haem copper oxidases studied by PELDOR spectroscopy

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Haem copper oxidases constitute the terminal complex of the respiratory chain and catalyse the reduction of oxygen to water. This exergonic redox reaction is coupled to proton pumping across the inner mitochondrial or bacterial membrane. O₂ reduction occurs at the binuclear haem-Cu_B centre. Despite high resolution X-ray crystallographic structures, the properties of the catalytic redox states of the metal centres and their relation to protonation states within this class of enzyme remain still poorly understood. Using a cysteine-free strain of quinol oxidase from *E. coli* (*bo*₃), cysteines were introduced at positions R134 and R309 and labelled with a spin label probe (MTSL). Using EPR these positions were probed as a function of different catalytic intermediate states. Pulsed ELDOR spectroscopy was used to resolve potentially subtle distance changes on the doubly labelled system (R134/309). This technique can accurately probe distances between spin labels in the range of 20 - 80 Å. Conditions for trapping intermediate states of the enzyme while maintaining the spin labels in their paramagnetic states were successfully developed permitting the study of local conformational changes in great detail. This allows us to probe the recent suggestions for electron/proton-transfer coupling, which may involve a conformational change within the proton uptake channels.