

P010 Oxygen reaction of D124N mutated cytochrome *c* oxidase monitored by time-resolved FTIR

Elena Gorbikova, Nikolay Belevich, Mårten Wikström, and Michael Verkhovsky

Finland, University of Helsinki, Institute of Biotechnology, HBG

Cytochrome *c* oxidase (CcO) is a terminal complex in respiratory chain that catalyses oxygen consumption and proton pumping across the membrane. In this work we present an FTIR flow-flash approach which allows measure oxygen reaction on CcO with time resolution of 46 ms. The oxygen reaction was followed by FTIR flow-flash on D124N mutant CcO where proton-conductive D-channel is blocked that result in a strong inhibition of the ferryl→oxidized transition. The FTIR spectrum of the CO-inhibited fully reduced→ferryl transition shows a trough at 1735 cm⁻¹ due to deprotonation of Glu 278, the spectrum of ferryl→oxidized transition, showed reprotonation of Glu 278 with a peak position at 1743 cm⁻¹. These observations confirm the proposal that the proton required for chemistry at the binuclear site is taken from Glu 278 in the peroxo→ferryl step, and that the rate of the next step (ferryl→oxidized) is limited by reprotonation of Glu 278 from the N-side of the membrane in the D124N mutant enzyme. The blockage of the D-pathway in this mutant for the first time allowed direct detection of deprotonation of Glu 278 and its reprotonation during oxidation of CcO by O₂.