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Study on the catalytic current in the cytochrome c oxidase from P. denitrificans
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Cytochrome c oxidase couples the reduction of oxygen to water to the translocation of 4 protons across the membrane. Oxygen reduction occurs at a binuclear center composed of a heme $a_3$ and a Cu$_A$ center. During oxygen reduction, chemical protons access the binuclear center through 2 proton pathways, namely the K- and the D-pathway. These pathways form a hydrogen bonding network facilitating proton diffusion [1]. Residues crucial for proton translocation have previously been reported by biochemical, structural and spectroscopic techniques [2,3].

We have developed an electrochemical approach that allows studying the electrochemical and catalytic properties of redox active membrane proteins. Efficient electron transfer is possible due to the immobilization of the membrane proteins on gold nanoparticle networks which provide a high surface to volume ratio and consequently allow to immobilize a significant amount of protein on the electrode surface [4,5]. These particles also act as a relay in the long-range electron transfer between the electron and the cofactors.

The catalytic current of wild type cytochrome c oxidase from Paracoccus denitrificans and of variants with mutations introduced into crucial positions of the D- and K-pathway was determined. Interestingly some activity remained even for the mutant enzymes that are reported to be inactive. These results are presented together with the effect of Zn inhibition.

Reference

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Cytochrome c oxidase heme and Cu centres: Redox and spectral interactions
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In beef heart cytochrome c oxidase turning over aerobically with ascorbate and cytochrome c, the three redox centres heme c, heme a and CuA are at near equilibrium in fully active and partially inhibited states [1]. In the presence of formate there is no reduction at the binuclear centre (heme $a_3$/CuB). $E_0$ values (pH 7.4, 30 °C) are +310 mV for heme a and +260 mV for CuA, if $E_0$ cyt. c is +255 mV. This standard redox potential difference between heme a and CuA permits determination of their separate difference spectra. Cupric CuA has positive absorbances in the 500–600 nm region plus the characteristic 835 nm band. The 605 nm reduced heme a alpha peak is red-shifted in the presence of oxidized CuA. Heme a and CuA titrations are close to simple Nernstian one-electron processes, indicating almost no redox interaction between the centres. But there are both redox and spectral interactions between the binuclear centre and the heme a/CuA system. Reduction and/or ligation of the binuclear centre decreases the redox potential of the haem a and induces shifts in the heme a spectrum. These interactions affect the kinetic analysis of the enzyme as well as the use of the CuA steady state in determining the functional status of the terminal oxidase in vivo.

Reference

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Cytochrome c oxidase signalling impact: Does the phosphorylation status really correspond to the enzyme kinetics or its enzymatic activity?
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Mammalian cytochrome c oxidase (CoO) is a dimeric multifunctional mitochondrial enzyme consisting of 13 subunits in each monomer. The study of kinetics and phosphorylation status of the enzyme provides an insight of its possible regulatory function and shed a light on its role in the network of mitochondrial respiratory chain. Using KinasePhos 2.0 web server, multiple phosphorylation sites are predicted in various subunits of CoO. These predicted sites are shown on the crystal structure of the enzyme with already identified phosphorylation sites. Although by default, several phosphorytosine sites are predicted but with 90% specificity, no subunit phosphorylation was suggested at the tyrosine residues in the whole enzyme. Interestingly, this is in contrast to the already known findings of Tyr304 at I [1], Tyr218 at II [2] or Tyr11 at IV – 1 [3]. Even the Thr35 at Va [4] was not predicted by this web server. Therefore, we conclude the dynamic changes in the phosphorylation patterns of CoO corresponding to the metabolic and respiratory status of mitochondria. In order to compare, BN PAGE isolated enzyme was incubated under the same