have measured CD and absorption spectra of ba_3-type cytochrome *c* oxidase in the fully reduced ($b^{2+}a_3^{2+}$), fully oxidized ($b^{3+}a_3^{3+}$), reduced CO-bound ($b^{2+}a_3^{2+}$-CO) and mixed-valence, CN-bound ($b^{3+}a_3^{2+}$-CN) states. CD spectra in the Soret absorption band of both enzymes are very non-conservative, with the area under positive lobe of the spectra strongly prevailing over the area under the negative part. Knowing the crystal structure of the enzyme, we attempted at modeling the CD spectra of the fully reduced and fully oxidized forms of bovine CcO using classical polarizability theory. With this approach, we can explain the non-conservative character of the CD spectra in the Soret band and reproduce reasonably well the magnitudes and lineshapes of the CD signals. The analysis suggests that optical activity of the enzyme in the Soret band originates in the interaction of the hemes with each other as well as with the aromatic amino-acid residues around.

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11P.5 Role of membrane potential on the control of cytochrome *c* oxidase over respiration in intact hepatoma HepG2 cells

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Metabolic control analysis (MCA) [1] has been largely applied to the analysis of oxidative phosphorylation, in order to investigate the control exerted by each individual reaction step on the whole pathway [2]. Using this approach, the control exerted by the electron transport chain complexes was shown to be higher in experimental systems closer to *in vivo* conditions than in mitochondria. To study the effect of the mitochondrial transmembrane proton electrochemical gradient ($\Delta\mu_{H+}$) on the control of respiration by cytochrome *c* oxidase (CcOX) in intact cells, we applied MCA to mitochondrial respiration of HepG2 cells. Both the overall O_2 consumption and specific CcOX activity of actively phosphorylating cells were progressively inhibited by cyanide titration under conditions in which the electrical ($\Delta\psi$) and/or the chemical (ΔpH) component of $\Delta\mu_{H+}$ was selectively modulated by addition of ionophores. Under endogenous conditions, i.e., in the absence of ionophores, CcOX displayed a high control coefficient value, thus representing an important site of regulation of mitochondrial oxidative phosphorylation. A high CcOX control coefficient value was also measured in the presence of nigericin, when $\Delta\psi$ is maximal, and in the presence of nigericin and valinomycin, when $\Delta\mu_{H+}$ is abolished. On the contrary and interestingly CcOX displayed a markedly lower control coefficient in the presence valinomycin converting $\Delta\psi$ into ΔpH. These results show that CcOX activity and its control over oxidative phosphorylation critically depend on $\Delta\psi$ in actively phosphorylating cells [3].

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11P.6 Comparison of the recombinant wildtype with the ATCC wildtype of cytochrome *c* oxidase from *Paracoccus denitrificans*

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The cytochrome *c* oxidase from the soil bacterium *Paracoccus denitrificans* consists of four subunits and is the terminal member of the respiratory chain. It pumps four protons from the negative (N) to the positive (P) side of the membrane and catalyses the reduction of molecular oxygen to water using four more protons.

$$4\ \text{cyt}\ c^{+\,\text{II}} + O_2 + 8\ H^+_{(N)} \rightarrow 4\ \text{cyt}\ c^{+\,\text{III}} + 2\ H_2O + 4\ H^+_{(P)}$$

This process takes place in the protein's active binuclear center, housing a haem a_3 and a copper ion (Cu_B). In order to compare variants and the wild type (WT) in a more controlled way a recombinant WT was constructed carrying the SU I gene on a low copy number plasmid. Comparing the rec WT to the ATCC WT no differences were expected, yet a significant increase in the reactivity towards hydrogen peroxide was found for the rec WT. This observation raises the question: Why do these two differently expressed wild type forms of the enzyme vary in this reactivity? Presumably a somehow misplaced Cu_B leads to an increase in hydrogen peroxide reactivity. To test this hypothesis we used a His-tagged variant in order to complex copper during assembly of the protein. Therefore the His-tag could be used as a copper source for copper inserting chaperones during assembly. Additionally extra copies of both bacterial chaperones *ctaG* and *surf1c* required for metal insertion into SU I were cloned on the same plasmid along with the SU I gene to compensate the slight overproduction of SU I from the plasmid. We constructed variants H276R and H276K, and tested them for their activity. These variants presumably lack Cu_B but maintain the overall electrostatic properties. Additionally the variants H276D and H276E were expressed to potentially incorporate a Fe(III) ion instead of the Cu (II) ion. The negative charge for the side chain was chosen to maintain the overall electrostatic properties in this site when incorporating a ferric iron. The results of metal content measurements as well as the results of activity assays will be presented.

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11P.7 Structural studies of catalytic intermediates in cytochrome *c* oxidase

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Cytochrome *c* oxidase (CytcO) is the final electron acceptor in the electron-transport chain in the inner membrane of mitochondria and bacteria. This enzyme catalyzes the reduction of molecular oxygen to water and energetically couples this reaction to proton transfer across the biological membrane. In this project we use X-ray crystallography together with time-resolved spectroscopic techniques to study the specific intermediates of cytochrome *c* oxidase formed during catalysis. By solving the three dimensional crystal structures of these short-lived intermediates we aim to obtain a better understanding for how CytcO accomplishes the coupling between electron- and proton transfers during the reduction of molecular oxygen to water. In our attempts to trap these intermediates we use structural variants of the enzyme that have been shown to stop the catalytic reaction at specific steps in the reaction cycle. A second approach we use is soaking