The alternative oxidase is a terminal respiratory chain protein found in plants, fungi and some parasites that still remains physically uncharacterised. Current model of the AOX, predicts that the enzyme is a monotopic integral membrane protein associating with one leaflet of the lipid bilayer. Although it is generally accepted that AOX is a non-haem diiron carboxylate protein in which the metal atoms are ligated by amino acid residues that all reside within a 4-helix bundle there is little biophysical experimental evidence in favour of this notion. We present EPR evidence from parallel mode experiments which reveal signals at approximately $g=16$ in both purified plant alternative oxidase protein ($g=16.9$), isolated plant mitochondrial membranes ($g=16.1$), and in the trypanosomal AOX expressed in *Escherichia coli* membranes ($g=16.4$). Of particular importance is the finding that such signals disappear in the presence of inhibitors of the AOX. Such signals are indicative of a dicarboxylate diiron centre at the active site of the enzyme. To our knowledge these data represent the first EPR signals from AOX present in its native environment.

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S13.15 Far infrared spectroscopic studies on hydrogen bonding features in proteins from the respiratory chain

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In order to understand the molecular basis of energy transduction, we have extensive interest in experiments which reveal at the molecular level how protons are drawn through proteins. It is crucial to determine the structural, dynamic and energetic requirements for the proton transferring groups in the proton pumping enzymes and the cofactor sites that rule them. A significant part of the proton conduction is made by channels that orient specifically bound water molecules. These water molecules can be monitored by X ray crystallography. However, high resolution structures of membrane proteins are difficult to obtain. Interestingly, water molecules and their hydrogen bonding interactions are expected to contribute in the far infrared spectral range ($<400\ cm^{-1}$). Experiments at synchrotron far infrared beamlines have been made with large membrane proteins from the respiratory chain and their models, clearly demonstrating the presence of this hydrogen bonding signature signal and, importantly, that it can be manipulated.

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S13.16 The role of the cross-linked Tyr in the catalytic cycle of cytochrome *c* oxidase

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Tyr-280 in cytochrome *c* oxidase (CcO) from *Paracoccus denitrificans* undergoes posttranslational modification that results in a covalent cross-link to histidine residue 276 that is a ligand of Cu_B. The Tyr-280 has been predicted to be a proton (or a proton and electron) donor for oxygen activation. The aim of our studies was to test the role of the cross-linked Tyr-280 as a proton donor for oxygen reduction and to resolve the stage in catalysis when it becomes reprotonated. The combination of three time-resolved techniques: visible spectroscopy, electrometry and FTIR spectroscopy were used. Electrometry showed positive charge transfer across a distance consistent with that from Tyr-280 to oxygen in the reaction site, and FTIR spectroscopy revealed a band at 1308 cm^{-1} that was assigned to deprotonated Tyr-280. The obtained results strongly indicate that Tyr-280 provides a proton for oxygen activation in fully reduced enzyme. According to the FTIR spectra it is fully deprotonated at the next step after O–O bond splitting (ferryl) and partly reprotonated in the fully-oxidized state, depending on pH. We showed that full reprotonation of Tyr-280 takes place in the one-electron reduced state when Cu_B becomes reduced.

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S13.17 Reverse redox loop enzymes for driving endergonic reactions in bacterial electron transport

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The study deals with enzymes that drive endergonic reactions of electron transport by a reverse redox loop mechanism and the proton potential. Succinate dehydrogenase (succinate: menaquinone (MK) reductase) from MK-containing bacteria catalyzes an endergonic reaction ($\Delta E_0' = +110$ mV). The soluble subunits (SdhAB) of the enzyme are similar in composition and function to succinate: ubiquinone reductase from mitochondria and ubiquinone containing bacteria. The quinone reactive subunit SdhC is a membrane integral diheme protein which allows electron transfer from the cytoplasmic to the extracellular side of the membrane. Function of succinate: MK reduction depends on the membrane potential and is inhibited by protonophores and ionophores. Bioenergetic studies and membrane topology of SdhC suggest that the active site for MK reduction is close the extracellular side of the membrane, resulting in the uptake of H^+ from the outside, whereas succinate oxidation and the accompanying proton release take place in the cytoplasm. In this way the enzyme consumes a proton potential and uses a reverse redox loop ($2\ H^+/2e$) for driving the redox reaction. The same type of enzyme is found in anaerobic electron transport of sulphate reducing bacteria. Database screening demonstrated that a reverse redox loop mechanism is predicted for other bacterial respiratory enzymes catalyzing endergonic reactions. Reverse redox loop enzymes therefore are of general significance for driving endergonic redox reactions in bacteria.

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S13.18 Light-activating the respiratory chain: Toward the time-resolved studies of the electron transfer chain in vivo

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Although the structure and function of most of the individual complexes involved in the respiratory chain are known with great details, the accurate understanding of the function of the chain as a whole is still missing. Yet, an increasing number of evidence suggests it may significantly differ from the simple combination of the functions of the individual complexes. Indeed, the supramecular association of complexes within identified super-complexes or the ultrastructure of the inner membrane which possibly restricts the diffusion