cases, the bovine C*c*O spectra are more complex than those of bacterial C*c*Os in that they involve more than one carboxyl group. For all C*c*Os, it is thought that the highly conserved Glu242 contributes an IR signature, consistent with its proposed involvement in an internal proton transfer that is crucial for interhaem electron transfer. In bovine C*c*O, crystallographic data suggest that Asp51 and Asp91, residues located in possible proton transfer pathways undergo redox-linked changes. Hence, they might be expected to provide additional carboxyl IR changes. To investigate this further, FTIR difference spectra of bovine C*c*O were recorded in the presence of Ca^{2+}/Na^{+} or Zn^{2+}/Cd^{2+}, since these metals have been shown to bind closely to Asp51 and Asp91, respectively, and might be expected to affect any ligand- or redox-linked structural changes that they might undergo. We will report the effects of these metals on CO photolysis- and redox-induced difference spectra, with particular attention to the 1800–1700 cm^{-1} carboxylic region. These results will be discussed in terms of carboxyl group functions in the proton pathway(s) of C*c*Os.

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S11.12 What can thermodynamics tells us about haem-copper oxygen reductases: A comparison between families

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Haem-copper oxygen reductases are enzymes that catalyze the reduction of oxygen to water coupled to the translocation of up to four additional protons across the membrane. Based on differences in the amino acid residue composition of their proton conducting channels and on the ligand proprieties of their binuclear centres (a high-spin haem and a copper ion, Cu_B) the existence of three families was proposed (one of them was further divided into 2 subfamilies). Despite these differences, the similarity between their overall structure fold, the redox metal centres composition of the catalytic subunit and the common function suggests the existence of a similar mechanism. In the pursuit of the existence of a common denominator, the study of the thermodynamic redox behaviour of the haems of enzymes representative of each family and subfamily of haem-copper oxygen reductases was performed at different pH values. By using the same methodology, a direct comparison between the obtained results could be done. It was observed that in solution, neither the haems redox pH dependency nor the overall order of their midpoint redox potentials is common to the members of the different subfamilies. This questions how the redox proprieties of these redox-driven pumps influence their general operation mode.

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S11.13 High resolution X-ray diffraction experiment of bovine cytochrome *c* oxidase

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Cytochrome *c* Oxidase (C*c*O) is a large membrane protein with a molecular weight of 200 kDa which couples proton pumping and oxidation-reduction reaction. It is essential to directly observe the protonation/deprotonation states of carboxyl groups participating in the proton pumping to make the mechanism clear at atomic level. We have determined the three dimensional structures in the oxidized state and the reduced state at 1.8 Å and 1.9 Å, respectively which were not sufficient to observe hydrogen atoms in the electron density. In general, higher than 1.2 Å resolution data is needed to observe hydrogen electron density in an (Fo-Fc) difference Fourier map, because atomic parameters should be accurately determined at higher ratio of number of Fo data to number of parameters. We have developed a new annealing method to improve the quality of crystals and have collected two datasets at 1.6 Å resolution on beamline BL44XU at SPring-8 and at 1.5 Å resolution on beamline X06SA at Swiss Light Source. Devising structural refinement, we are trying to determine protonation or deprotonation states of carboxyl groups with 1.5 Å and 1.6 Å resolution data.

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S11.14 Isolation and characterization of cytochrome *c* oxidase and its supercomplex from the hyperthermophilic eubacterium *Aquifex aeolicus*

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Cytochrome *c* oxidase was purified from native membranes of *Aquifex aeolicus*. Subunit I (coxA2) and subunit II (coxB2) were identified from current preparation by mass spectrometry. Interestingly, no transmembrane spanning segments predicted in subunit II by program TMHMM 2.0. The activity of cytochrome *c* oxidase was measured spectrometrically monitoring the oxidation of reduced horse-heart cytochrome *c* at 550 nm. The specific activity is 0.35 U/mg at 80° and it remains constant at 60° for 15 h. Meanwhile, a supercomplex containing at least cytochrome *c* oxidase and ubiquinol-cytochrome *c* oxidoreductase was also isolated in a highly stable form. This project focuses on investigating structural and functional relationships of cytochrome *c* oxidase and its related protein complexes.

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S11.15 Mutations within the k-channel of cytochrome *c* oxidase lead to rapid destabilization of the O_H state

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We have shown earlier that the freshly formed O_H state of *Paracoccus denitrificans* aa_3-type cytochrome *c* oxidase (C*c*O) is capable to pump protons, as opposed to the "relaxed" state O (Bloch et al., 2004, PNAS *101*, 529–533; Belevich et al., 2007, PNAS *104*, 2685–2690). The nature of the difference between these two oxidized states remains unclear. Recent data on mutants of C*c*O, where the T351 residue in the K-channel is replaced (T⇒S, T⇒A, T⇒N), show that (a) there is no appreciable effect on the oxidative phase, except slightly slower rates (as with the K354⇒M mutant); (b) flash-induced, $Ru(bpy)_3$-mediated