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The aim of this study was to clarify structures and properties of protozoan parasite Complex II, which often plays a pivotal role in adaptation to hypoxic host environments. Generally, protist mitochondrial enzymes are difficult to purify and still remain uncharacterized. We isolated Complex II from epimastigotes of *Trypanosoma cruzi*, the causative agent of Chagas disease, and identified all genes coded for subunits. In contrast to the mammalian enzyme (SDH1–SDH4), *T. cruzi* Complex II is consisted of six each of hydrophilic (SDH1, $SDH2_N$, $SDH2_C$, and SDH5~7) and hydrophobic subunits (SDH3, SDH4, and SDH8~11). Notably, an iron–sulfur subunit is heterodimeric; $SDH2_N$ and $SDH2_C$ contain the plant-type ferredoxin domain in the *N*-terminal half and the bacterial ferredoxin domain in the *C*-terminal half, respectively. This is a first direct evidence for the splitted SDH2 in Complex II. Sequence analysis indicates that trypanosomatid-specific subunits have evolved by gene duplication of canonical subunits followed by degeneration of one copy. Catalytic subunits contain all key residues for binding of substrates but the enzyme showed the lower affinity for substrates and inhibitors than mammalian enzymes. Further, we characterized Complex IIs from other parasites including malaria parasites, *Ascaris suum* and *Echinococcus multilocularis*. Unusual features of parasite enzymes make Complex II a target for new chemotherapeutic agents.

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S13.27 Fidelity of water-gated mechanism in cytochrome *c* oxidase

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Cytochrome *c* oxidase utilizes the energy released by oxygen reduction to drive proton pumping across the mitochondrial or bacterial membrane. Proton-pumping must therefore be controlled to transfer protons both to a pump site, and to the site for oxygen reduction. Previously, we suggested a mechanism in which water molecules in the non-polar cavity above Glu-242 orientate in a redox state-dependent way to connect Glu-242 either with the D-propionate of heme a_3, or with the oxygenous ligand of Cu_B. To control proton pumping in this way, the energy for the "wrong" orientation of the water molecules must be considerable. We have studied the fidelity of the water-gated mechanism by classical free-energy calculations and molecular dynamics simulations, and explored the cause and energetics of the redox-state dependent orientation of the water molecules.

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S13.28 *c*-type cytochromes coupled to chlorate reduction in *Ideonella dechloratans*

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The present work is part of an investigation of the enzyme chemistry of the chlorate reduction in *Ideonella dechloratans,* a bacterium capable of using chlorate as the sole electron acceptor under anaerobic conditions. The aim of the present work is to investigate the electron transfer route for the chlorate reduction, and to isolate the electron carrier, responsible for the delivery of electrons from the membrane-bound electron transport chain to the periplasmic chlorate reductase. Optical difference spectrum of dithionite reduced periplasm show that at least one of the soluble *c*-type cytochrome can deliver electrons to the periplasmic chlorate reductase. Five native heme-containing proteins, with molecular weights in the range 4.5–20 kDa have been isolated from the periplasm of *I. dechloratans* and identified by SDS-PAGE with heme staining. Two of these (6- and 10-kDa) were purified by IEX and tested as electron donors by optical spectroscopy analysis. After reduction with dithionite, the 6-kDa *c*-cytochrome could be reoxidized by addition of chlorate, in presence of a catalytic amount of chlorate reductase and in absent of oxygen. The 10-kDa cytochrome could not deliver electrons in anaerobic respiration. Both can donate electrons to the terminal cytochrome *c* oxidase when oxygen is present. It is not clear whether the unpurified *c*-cytochromes participate in the electron transfer between the membrane-bound respiratory chain and the periplasmic chlorate reductase.

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S13.29 Purification, characterisation and crystallisation of *Thermus thermophilus* succinate dehydrogenase

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Succinate-ubiquinone oxidoreductase (SQR, Complex II) is unique in being a member of the Krebs TCA cycle as well as the respiratory chain, catalysing the oxidation of succinate to fumarate in prokaryotic and eukaryotic organisms. Recently, we have succeeded in identifying, purifying and characterising succinate dehydrogenase from the extreme thermophilic bacterium *Thermus thermophilus*. *Thermus* complex II differs from mitochondrial complex II as it contains two hemes bound to its transmembrane subunits. The purity and integrity of the enzyme were determined by biochemical and biophysical methods including: UV–Vis spectroscopy, SDS-PAGE, N-terminal sequencing and mass spectrometry. Activity assay and EPR measurements were also performed to measure activity of the purified enzyme and to analyse its redox centres. Crystals of the enzyme have been produced by both the *in surfo* sitting drop and *in meso* crystallisation methods. Optimisation of the crystallisation conditions is underway with a final outlook towards high quality diffracting crystals for X-ray analysis.

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S13.30 The *Escherichia coli* hydrogenase activity under glycerol fermentation

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Escherichia coli catalyses oxidation of formate to H_2 and CO_2 under anaerobic conditions upon fermentation of glucose by formate hydrogen lyase; the latter is suggested to be composed of hydrogenase 4 at neutral or alkaline pH and hydrogenase 3 at acidic pH. *E. coli* has been shown to be also able to ferment glycerol and to produce H_2 at pH 6.5, however terminal pathways and the end products including