S13.22 The mechanism of nitric oxide reduction in NOR from *Paracoccus denitrificans*

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The nitric oxide reductase (NOR) from *Paracoccus denitrificans* catalyses the reduction of NO to N_2O; $2NO + 2e^- + 2H^+ \rightarrow N_2O + H_2O$. The NOR is purified as a two-subunit (NorB and NorC) integral membrane protein where the NorB, the catalytic subunit, contains a low-spin heme *b*, a high-spin heme b_3, and a non-heme Fe_B where the two latter form the active site of NO reduction. NorC contains a low-spin heme *c* which is the initial acceptor of electrons. The detailed mechanism of NO reduction by this enzyme is unknown; different scenarios have been put forward where either the heme b_3 or the non-heme Fe binds one or both NO molecules. In order to elucidate this mechanism, we are studying rapid kinetics of the reaction between the fully reduced NOR and NO using flash-induced optical spectroscopy. Preliminary data indicate that the heme b_3 binds NO directly from bulk by-passing the Fe_B, and that the inhibition of catalytic turnover observed at high NO concentration can be explained by slow electron transfer from the low-spin hemes to the oxidised NO-bound active site.

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S13.23 Proton transfer along surfaces of membranes and membrane-proteins

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The energy metabolism of a living cell involves proton translocation by membrane-bound proton transporters, thereby maintaining an electrochemical proton gradient. The aim of our studies is to investigate the role of the membrane in facilitating proton uptake by the transport proteins and in providing a proton-transfer link between the components of the energy-conservation machinery. In an earlier study, using fluorescence correlation spectroscopy (FCS), we showed that the protonation kinetics of a fluorescein molecule was accelerated when it was anchored to the surface of a membrane. Further acceleration was observed upon introduction of high-pK_a protonable lipid head groups in the membrane, indicating that under these conditions the membrane acted as proton-collecting antenna (Brändén et al. (2006) *PNAS* 103, 19766). Here, we have extended these studies to include proton transfer along the surfaces of proteins. Fluorescein was covalently linked to the surface of *Rhodobacter sphaeroides* wild type cytochrome *c* oxidase, near the protein–membrane interface, and the protonation dynamics was monitored using FCS. Preliminary results indicate that proton transfer to the fluorescein is significantly slower for the detergent-solubilized than for the membrane-anchored enzyme, which indicates that the membrane facilitates proton transfer to the protein surface.

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S13.24 Monitoring promoter activity of the branched respiratory chain of *Corynebacterium glutamicum* using GFP reporter system

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Bacterial respiratory chains contain highly diverged oxidoreductases and are branched usually to two through six routes, which are switched depending on the environmental conditions, such as aeration. In order to monitor the promoter activity of each respiratory enzyme and to clarify the regulatory mechanism of the switching, here we constructed a reporter gene system by using Green Fluorescent Protein (GFP). *Corynebacterium gultamicum*, an amino-acid producing bacterium, has also branched respiratory chain. We have made seven plasmids, in which the promoter regions of each respiratory enzyme were combined with GFP gene. Transformed cells were cultured under various growth conditions, including several aeration conditions and carbon-source concentrations. At several growth stages, cells were harvested and were measured fluorescence to estimate the promoter activity. The cytochrome "*bcc*" complex had high promoter activity and decreased gradually. The two terminal oxidases, aa_3 complex and the cytochrome *bd*-type quinol oxidase, had similar tendency that there were no major changes through the exponential and steady stages. Only when the culture was extended to some extreme, difference between the aa_3 complex and the cytochrome *bd* became significant. The shift of the major terminal oxidase from aa_3 complex to cytochrome *bd* might be arisen under heavily exhausted air condition.

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S13.25 Succinate:menaquinone oxidoreductase (complex II) from *Corynebacterium glutamicum*

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The actinobacteria include many pathogenic ones, such as mycobacteria. We have been using *Corynebacterium glutamicum* as a model organism of this class of bacilli, which share conserved respiratory enzyme system. In the *C. glutamicum* cells, reducing equivalents are transferred to menaquinone (MK) via several dehydrogenases, i.e. succinate:MK oxidoreductase (SQR or complex II). From MKH_2, the electrons are passed either to cytochrome *bcc*-type MKH_2:cytochrome *c* oxidoreductase (complex III) and aa_3-type cytochrome *c* oxidase (complex IV), or to *bd*-type MKH_2 oxidase. Since the redox potential of MK/MKH_2 is much lower than UQ/UQH_2, the latter of which is utilized by many eukaryota and proteobacteria, the energetics of respiration are expected to be also different. To analyze the function of SQR, we purified and analyzed this enzyme from *C. glutamicum*. The enzyme activity of SQR was inhibited by low concentration of HQNO but little by *p*-benzoquinone (PBQ) when decylubiquinone was used as the electron acceptor (K_m=1.91 μM, V_{max}=22.2 S^{-1}), while the activity was inhibited by low concentration of PBQ but little by HQNO when PMS was used (K_m=8.77 μM, V_{max}=87.7 S^{-1}). This inhibition of PBQ was competitive with the K_i value of 5.7 μM. Purified SQR was reconstituted into proteoliposomes and it was suggested that the SQR reaction of *C. glutamicum* was driven by proton motive force.

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S13.26 Divergence in structure of mitochondrial respiratory complex II (succinate–ubiquinone reductase) revealed by protozoan enzymes

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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