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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