membrane mechanistic/thermodynamic coupling between the quinone-binding sites of this enzyme [1–3].

**References**
[1] A.Y. Mulkidjanian, Biochim. Biophys. Acta 1709 (2005) 5–34.
[2] A.Y. Mulkidjanian, W. Junge, in: P. Mathis (Ed.), Photosynthesis: from Light to Biosphere, vol. II, Kluwer Academic Publishers, Dordrecht, 1995, pp. 547–550.
[3] A.Y. Mulkidjanian, Biochim. Biophys. Acta 1757 (2006) 415–427.

doi:10.1016/j.bbabio.2010.04.068

### 1P.21 Correlation between proton translocation and growth on *Corynebacterium glutamicum*

Yoshiki Kabashima[1], Junshi Sakamoto
*Kyushu Institute of Technology, Department of Bioscience and Bioinformatics, Japan*
*E-mail:* yoshiki.kabashima@gmail.com

*Corynebacterium glutamicum* is not only industrially important but also useful as a model organism of pathogenic Gram-positive bacteria, such as *C. diphtheria* and *Mycobacterium tuberculosis*. This actinobacterium contains at least two terminal oxidases in the respiratory chain; cytochrome $aa_3$-type cytochrome *c* oxidase [1] and *bd*-type menaquinol oxidase [2]. Thus, the chain has two branches of electron flow. The *bcc*-$aa_3$ branch translocates three protons per electron transferred, while the *bd* branch translocates only one. Here, we constructed two mutant strains, lacking of either the cytochrome $aa_3$ (Δ*ctaD*) or cytochrome *bd* oxidase (Δ*cydAB*), and also plasmids for complementing the deficient genes to investigate their effects on energy conservation and cell growth [3]. The amount of cytochrome *bd* oxidase was very low even in the Δ*ctaD* mutant, because the expression of the oxidase may be tightly limited with a regulation system. Therefore, we also constructed the mutant overexpressing cytochrome *bd* to investigate the cytochrome *bd* branch in more detail. First, we measured $H^+/O$ ratios of wild-type and mutant cells to evaluate the efficiency of the respiratory chain. The $H^+/O$ ratio of the wild-type cells grown in the semi-synthetic medium was $3.94 \pm 0.30$, while the value was $2.76 \pm 0.25$ for the Δ*ctaD* mutant. In contrast, the value was $5.23 \pm 0.36$ for the Δ*cydAB* mutant. The overexpression of cytochrome *bd* in the Δ*ctaD* mutant caused further reduction of the value, $2.29 \pm 0.29$ for the cytochrome *bd* overexpression mutant. Interestingly, the cells grown in the LB medium showed about 25% higher value compared to that of cells grown in the semi-synthetic medium except for the Δ*ctaD* mutant. Secondly, we investigated the growth rate and cell yield with different nutrients; semi-synthetic medium containing 1% (w/v) glucose and LB medium. The Δ*ctaD* and cytochrome *bd* overexpression mutants grew less than the wild-type in LB, while they grew about equally insemi-synthetic medium. In contrast, the lack of cytochrome *bd* oxidase did not largely affect to cell growth in both medium. These findings suggest that correlation between bioenergetics and cell growth is significantly affected by nutritional condition for the growth.

[1] Present address: Kyorin University School of Medicine, Department of Chemistry, Japan.

**References**
[1] J. Sakamoto, et al., Microbiology 147 (2001) 2865–2871.
[2] K. Kusumoto, et al., Arch. Microbiol. 173 (2000) 390–397.
[3] Y. Kabashima, et al., J. Biochem. 146 (2009) 845–855.

doi:10.1016/j.bbabio.2010.04.069

### 1P.22 NADH:ubiquinone oxidoreductase (complex I) of brain mitochondria

Denis S. Kalashnikov, Andrei D. Vinogradov
*Moscow State University, School of Biology, Department of Biochemistry, Russian Federation*
*E-mail:* adv@biochem.bio.msu.su

NADH:ubiquinone oxidoreductase (complex I) is the largest component of the mitochondrial respiratory chain. Most of the current knowledge on the enzyme structure, its catalytic and regulatory properties have been accumulated from comprehensive studies of bovine heart enzyme and its prokaryotic homologues, NDH-1. Little is known about tissue specificity, if it exists, of mammalian complex I. In order to characterize catalytic and regulatory properties of complex I in brain mitochondria a large-scale procedure for preparation of coupled pig brain inside-out submitochondrial particles (B-SMP) was developed. B-SMP catalyzed rotenone sensitive NADH oxidase and NADH:quinone ($Q_1$) reductase reactions at the specific rates of 0.8 and 0.6 μmol/min per mg protein, respectively (30 °C, pH 8.0) and the activities corresponding to complex I turnover number to about 200 $s^{-1}$. Artificially coupled (by treatment with oligomycin), B-SMP showed a respiratory control ratio of about 3 and 5 with succinate and NADH as the respiratory substrates, respectively. The molar content of enzymatically active complex I (determined as piericidine, rotenone and the active site directed inhibitor, NADH–OH [1] titers) in B-SMP was 0.06 nmol per mg protein, the value, which is about 3-fold less than that of heme a (0.2 nmol per mg). Treatment of B-SMP with pore-forming antibiotic, alamethicin stimulated their NADH oxidase by about 30% thus showing that about 70% of the particles were inside-out. About 70% of the NADH oxidase activity of B-SMP (as prepared) was abolished by preincubation with N-ethylmaleimide thus showing that a substantial fraction of complex I was present as its de-activated form [2]. The activated NADH oxidase and NADH:quinone ($Q_1$) reductase reactions were sensitive to endogenous and exogenous free fatty acids (FA) with the highest inhibitory efficiency of palmitate. Inhibition of complex I activity by FA was time-dependent and greatly promoted by $Ca^{2+}$. The time dependency of FA-induced $Ca^{2+}$-promoted inhibition of complex I was not due to the enzyme active/de-active transition [2].

Supported by Russian Foundation for Fundamental Research grant 08-04-00594 and by NIH Fogarty grant 5R03TW007825.

**References**
[1] A. Kotlyar, FEBS Lett. 579 (2005) 4861–4866.
[2] A. Vinogradov, et al., IUBMB Life 52 (2001) 129–134.

doi:10.1016/j.bbabio.2010.04.070

### 1P.23 Purification and characterisation of native and recombinant complex II from *Thermus thermophilus* HB8

Olga Kolaj-Robin[1], Sarah R. O'Kane[1], Frauke Baymann[2], Wolfgang Nitschke[2], Tewfik Soulimane[1]
[1]*Chemical and Environmental Science Department, Materials and Surface Science Institute, University of Limerick, Ireland*
[2]*BIP/CNRS, 31, chemin Joseph Aiguier, 13402 Marseille cedex 20, France*
*E-mail:* tewfik.soulimane@ul.ie

Complex II is the only membrane-bound enzyme of the tricarboxylic acid cycle and functions also as a member of the electron transport chain. Complexes II belong to the succinate:quinone oxidoreductase (SQOR) superfamily which consists of succinate: quinone reductases (SQRs) and quinol:fumarate reductases (QFRs). SQORs are classified into 5 types of (A–E) depending on number of

anchor peptides and heme *b* composition. Delineation of mechanism of action of these enzymes is highly desirable due to the medical disorders caused by their dysfunctions in eukaryotic organisms. Although several 3D structures of complex II exist, the exact mechanism of action remains unclear. Here we present identification, isolation, purification and characterisation of SQR from an extreme thermophile *Thermus thermophilus,* analysis of which is additionally interesting from the evolutionary perspective and the aspect of protein thermostability. The *Thermus* SQR is constituted by flavoprotein (SdhA), iron–sulfur protein (SdhB) and two membrane anchor proteins (SdhC&D). It has been purified to homogeneity following series of chromatographic steps yielding 8 mg per 100 g of *Thermus* biomass. The EPR analysis revealed unusual influence of succinate on the properties of the [2Fe2S] center and presence of two heme *b* molecules in the protein. The latter places the *Thermus* SQR in the Type A SQOR, crystal structure and detailed characterisation of which are not available to date. The enzyme exists in trimeric form and unexpectedly, it is not stable at temperatures above 70 °C in the purified state as judged by Circular Dichroism analysis. Steady-state kinetic measurements show that the *Thermus* SQR displays standard Michaelis–Menten kinetics at low temperature (30 °C) but exhibits positive cooperativity for succinate oxidation at the optimum temperature of the enzyme (70 °C). To our knowledge, this is the first example of allosterically regulated complex II. To facilitate purification, increase the production yield and enable mutagenesis studies of the *Thermus* complex II, a homologous expression system has been developed. Recombinant form of the enzyme has been produced, purified and its biochemical and biophysical characterisation has also been performed.

doi:10.1016/j.bbabio.2010.04.071

### 1P.24 Structure of a soluble subcomplex of NADH:ubiquinone oxidoreductase from *Aquifex aeolicus*

Ramona Labatzke, Markus Kohlstädt, Leonhard Jakob, Thorsten Friedrich, Oliver Einsle
*Institute for Organic Chemistry and Biochemistry, Albert-Ludwigs-Universität, Albertstr. 21, D-79104 Freiburg, Germany*
*E-mail:* labatzke@bio.chemie.uni-freiburg.de

The proton-pumping NADH:ubiquinone oxidoreductase (complex I) catalyzes the transfer of two electrons from NADH to ubiquinone via one FMN and a series of iron–sulfur (Fe–S) clusters. This process is coupled with the translocation of four protons across the membrane. Electron microscopy revealed that complex I is made up of a peripheral and a membrane arm. The peripheral arm contains all known cofactors and the binding site for NADH. We have obtained a subcomplex consisting of subunits NuoE and NuoF by heterologous expression of the corresponding genes from *A. aeolicus* in *E. coli*. This subcomplex comprises the NADH- and FMN-binding sites and harbours two Fe–S clusters. The NuoEF subcomplex was purified to homogeneity by chromatographic methods. The cofactors of the preparation were characterized by EPR- and UV/vis-spectroscopy [1]. The structure of the subcomplex was determined at 1.9 Å resolution. To define the substrate–protein interactions the structure of the subcomplex with nucleotides was examined.

**Reference**

[1] M. Kohlstädt, Biochemistry 47 (2008) 13036–13045.

doi:10.1016/j.bbabio.2010.04.072

### 1P.25 The study of $cbb_3$-type oxidase

Hyun Ju Lee, Pia Ädelroth
*Stockholm University, Biochemistry and Biophysics Department, Stockholm, Sweden*
*E-mail:* hjlee@dbb.su.se

Heme-copper superfamily are terminal respiratory oxidases in mitochondria and in many bacteria, coupling the oxygen reduction to water in order to generate proton-motive force across the membrane. The most established heme-copper oxidases are $aa_3$-type oxidases followed by the second abundant $cbb_3$-type oxidases. However, the sequence homology between $aa_3$- and $cbb_3$-type oxidases is low and they are highly different within the heme-copper superfamily. $aa_3$-type oxidases utilize two proton uptake pathways known as K-pathway and D-pathways for both catalytic and pumped protons. Whereas, the pattern of conserved polar residues supports the one proton uptake pathway in $cbb_3$-type oxidases. $cbb_3$-type oxidases express under low oxygen tension and have a lower $K_M$ for oxygen than $aa_3$-type oxidases. Moreover, $cbb_3$-type oxidases are the closest to the bacterial nitric oxide reductases (NOR), which reduce NO to $N_2O$. This reaction is not electrogenic and do not pump protons or generate proton-motive force. In this study, we investigate the molecular details of the reasons for the differences between the subfamilies of heme-copper oxidases by focusing on $cbb_3$-type oxidases.

doi:10.1016/j.bbabio.2010.04.073

### 1P.26 Kinetic and structural analysis of succinate:ubiquinone oxidoreductase (complex II) inhibition by thiapronil

Domenik Lisse[1], Li-shar Huang[2], Thorsten Gasteiger[1], Edward A. Berry[2], Peter Lümmen[1]
[1]*Bayer CropScience AG, D-40789 Monheim, Germany*
[2]*Upstate Medical University, Biochemistry & Molecular Biology, Syracuse, NY 13210, USA*
*E-mail:* peter.luemmen@bayercropscience.com

Mitochondrial succinate:ubiquinone oxidoreductase (complex II, SQR, EC 1.3.5.1) catalyzes the oxidation of succinate to fumarate as part of the tricarboxylic acid cycle. With ubiquinone (Q) as its terminal electron acceptor the SQR is also an entry point for electrons into the respiratory chain. The catalytic core of complex II consists of a flavoprotein (FP) with covalently bound FAD and an iron–sulfur protein (IP) carrying three Fe–S clusters. These two subunits are anchored to the inner membrane by two small transmembrane subunits [1,2]. The precise structural requirements for ubiquinone binding and the mechanistic details of its reduction remain to be elucidated. X-ray crystallographic studies of Q-site inhibitors bound to SQR have indicated that amino acid residues of the IP and of the transmembrane subunits constitute the putative Q-site [3,4]. Under these circumstances, chemically diverse specific inhibitors may be valuable tools for functional and structural studies of complex II. Evidence is presented here that the cyanoketone insecticide thiapronil [3-(2-chlorophenyl)-3-oxo-2-(4-phenyl-1,3-thiazol-2-yl)propanenitrile] blocks quinone reduction by purified housefly (*Musca domestica*) SQR at low nanomolar concentrations. Non-competitive inhibition is observed with respect to the electron acceptor decylubiquinone. However, Yonetani–Theorell analysis indicates that the established Q site inhibitor atpenin A5 and thiapronil bind in a mutually exclusive fashion. The structure of thiapronil bound to the chicken complex II has been solved at 2.2 Å resolution. A number of interactions contribute to the high binding affinity of thiapronil. Two H-bonds are formed between the carbonyl oxygen and the residues TyrD58 (transmembrane subunit D) and TrpB173 (iron–sulfur protein). The 3.9 Å distance between TrpB173 and the thiazole sulfur