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 ± 0.30, while the value was 2.76 ± 0.25 for the Δ*ctaD* mutant. In contrast, the value was 5.23 ± 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 ± 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