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Complex I (NADH:quinone oxidoreductase) proteins NuoL, NuoM and NuoN are homologous to one type of Na^+/H^+ antiporters. Thus, these subunits are prime candidates for harbouring important parts of the proton pumping machinery. If they also retain antiporter function and/or the ability to pump sodium has been a matter of debate. The aim of this study was to investigate the sodium binding properties of the NuoL, NuoM and NuoN subunits from *E. coli* complex I and compare them to those of the *bona fide* antiporters MrpA and MrpD from *Bacillus subtilis* using ^{23}Na NMR spectroscopy. This technique has been demonstrated to be particularly suitable to monitor ion binding properties of macromolecules under conditions of very fast chemical exchange [1, 2], as expected from a transporter protein. High amounts of the individual proteins were obtained by expressing them as cytochrome c fusion proteins in *E. coli*. Purification was facilitated by a C-terminal histidine tag fused to the cytochrome c domain. The mobility of Na^+ in the presence of the antiporter proteins was measured at gradually increasing concentrations. Subsequently, the concentration of another, non-interacting cation, NH_4^+, was increased, while sodium was kept constant. Cytochrome c alone was used as negative control protein. From this data, specific binding constants for Na^+ could be estimated for each of the five proteins. The Na^+ interaction was then assessed under different conditions and pH, in the presence of quinone and in the presence of the sodium-hydrogen exchange inhibitor 5-ethylisopropyl amiloride (EIPA). The sodium interaction was compared to the real antiporters MrpA and MrpD.

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doi:10.1016/j.bbabio.2010.04.066

1P.19 Infrared spectroscopic analysis on the substrate induced conformational flexibility of the NADH:ubiquinone oxidoreductase

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Protein dynamics play an important role in the catalytic efficiency of enzymes and conformational changes may take place during the substrate binding of the NADH:ubiquinone reductase, the respiratory complex I. A coupled FTIR spectroscopic and perfusion induced approach was applied that provides the possibility recording $^1H/^2H$ exchange kinetics at the level of the amide proton in the mid infrared (170–1500 cm^{-1}). This approach is extremely sensitive to tertiary structure changes [1–3]. In general the exchange rates depend on hydrogen bonding and solvent accessibility. It was suggested that protein structure can be divided in three types of structure characterized by their particular ($^1H/^2H$) exchange dynamics. These three domain types are clearly distinguishable for complex I and the soluble NADH binding fragment and their relative ratios depend on the presence of bound substrate. Furthermore the spectral signature of the overall internal hydrogen bonding was probed in the far infrared (300 to 30 cm^{-1}). The interest of this spectral range is based on the observation that the far-infrared contribution of a wide range of molecules is dominated by vibrations involving a substantial fraction of the atoms forming the molecule and motion associated with intermolecular hydrogen bond vibrations [4,5]. Due to their collective nature, such modes are highly sensitive to the intra- and intermolecular structure and thus provide a unique fingerprint of the conformational state of the molecule and effects of its environment. We use these two infrared spectroscopic approaches to learn about the conformational flexibility of the respiratory NADH:ubiquinone oxidoreductase (complex I) induced by various substrates and present evidence for a different effect of NADH and of NADPH. The role of the quinone for the conformational flexibility is discussed.

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doi:10.1016/j.bbabio.2010.04.067

1P.20 Tempering of the cytochrome bc_1 complex of *Rhodobacter capsulatus* by pH

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The cytochrome bc_1 complex is a dimeric membrane ubiquinol: cytochrome *c* oxidoreductase. After an ubiquinol molecule is oxidized in the catalytic center *P* close to the positively charged side of the coupling membrane, the two released electrons going to different acceptors. One is taken by the mobile domain of the [2Fe–2S] iron–sulphur Rieske protein to be passed further to the *c*-type cytochromes. The other electron crosses the membrane, via the low- and high-potential hemes of cytochrome *b*, to reduce a stable semiquinone molecule which is steadily maintained in one of the two centers *N* from the opposite membrane side [1]. The kinetics of flash-induced generation of membrane voltage by the cytochrome bc_1 complex can be traced via spectral shifts of native carotenoid pigments and correlated with the kinetics of electron transfer as measured in the same samples. Earlier we have shown, at neutral pH values, that small amounts of Zn^{2+} ions could make the flash-induced redox-reactions of cytochrome *b* visible, apparently, by retarding the oxidation of heme b_h. Binding of a Zn^{2+} close to the center *P* not only retarded the proton release from this center and the movement of the FeS domain towards cytochrome c_1, but also slowed down the oxidation of heme b_h and the formation of ubiquinol in center *N*. This correlation was attributed to the earlier postulated mechanistic coupling between the two quinone-binding centers [2]. In the case of such coupling, however, one could expect that the retardation of events in center *N* should, reciprocally, affect the events in center *P*. Here we show that the same kinetic behaviour of the cytochrome bc_1 complex could be observed at high pH, when the protonation of ubiquinol in center *N* is retarded. These observations support our suggestion of a cross-

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

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doi:10.1016/j.bbabio.2010.04.068

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

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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.

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doi:10.1016/j.bbabio.2010.04.069

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

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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.

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doi:10.1016/j.bbabio.2010.04.070

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

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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