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

References

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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_{ox}) 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_{ox}) 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].

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References


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 tri-carboxylic 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

References

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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 aa3-type cytochrome c oxidase [1] and bd-type menaquinol oxidase [2]. Thus, the chain has two branches of electron flow. The bcc-aa3 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 aa3 (Actd) or cytochrome bd oxidase (ΔcydAB), 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 Δctad 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 Δctad mutant. In contrast, the value was 5.23 ± 0.36 for the ΔcydAB mutant. The overexpression of cytochrome bd in the Δctad 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 Δctad 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 Actd and cytochrome bd overexpression mutants grew less than the wild-type in LB, while they grew about equally in semi-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.

References

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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 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.

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

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1P.25 The study of cbb3-type oxidase
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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 aa3-type oxidases followed by the second abundant cbb3-type oxidases. However, the sequence homology between aa3- and cbb3-type oxidases is low and they are highly different within the heme-copper superfamily. aa3-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 cbb3-type oxidases. cbb3-type oxidases express under low oxygen tension and have a lower KM for oxygen than aa3-type oxidases. Moreover, cbb3-type oxidases are the closest to the bacterial nitric oxide reductases (NOR), which reduce NO to N2O. This reaction is not electronic 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 cbb3-type oxidases.