small copper protein called sulfocyanin. This complex is enzymatically functional, reducing oxygen in the presence of the electron donor ferrous iron, at low pH. A second putative cytochrome ba–Rieske protein complex, reminiscent of archaeal complexes analogous to the bc1 complex, may be involved in regeneration of reducing equivalents by reverse electron flow. A model of energy ferrous iron metabolism of F. acidophilum is proposed [1]. This study constitutes the first detailed biochemical investigation of iron metabolism in acidophilic Archaea, and confirms that iron respiratory chains are clearly different from an organism to another even among Archaea.

Reference

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S8.P14
Structure and function of the Rnf complex from Acetobacterium woodii
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The anaerobic acetogenic bacterium Acetobacterium woodii has a novel Na\(^+\)-translocating electron transport chain that couples electron transfer from reduced ferredoxin to NAD\(^+\) with the generation of a primary electrochemical Na\(^+\) potential across its cytoplasmic membrane [1]. The enzyme was identified to be encoded by the rnf genes previously identified to be essential for nitrogenase function in Rhodobacter [2]. The Rnf genes are widely distributed in Bacteria, indicating that this novel ion-translocating electron transport chain is widely distributed [3]. In most anaerobic bacteria, the Rnf complex functions in ferredoxin-driven NAD\(^+\) reduction coupled to the generation of a transmembrane ion gradient, but we will show here that it also catalyzes the reverse function in vivo, ferredoxin reduction with NADH as reductant at the expense of the electrochemical sodium ion gradient. This reaction is important for many anaerobes, but also the most likely in vivo function for aerobes or facultative aerobes in which the Rnf complex provides reduced ferredoxin for biosynthetic (N\(_2\) fixation) or regulatory functions. The Rnf complex is encoded by six genes whose products are involved in NAD\(^+\) binding, binding of reduced ferredoxin, electron flow from reduced ferredoxin to NAD\(^+\) and coupling of the electron transfer reaction to Na\(^+\) transport but the function of the individual subunits remains elusive. Here, we present a structure-function analysis of single subunits of the Rnf complex of A. woodii.

References

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S8.P16
Phylogenomic analysis of membrane Na\(^+\)-translocating decarboxylases
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NADH type II dehydrogenase (Ndh) in bacteria oxidizes NAD(P)H, without contributing to the generation of proton motive force. For the facultatively anaerobic ethanol-producing bacterium Zymomonas mobilis this is the sole functional respiratory NAD(P)H dehydrogenase, able to oxidize both cofactors, although with a preference for NADH. Rapid, yet energetically uncoupled respiratory chain of this bacterium might be useful for biotechnological application in bioconversions, where redox balancing is needed, but synthesis of excess ATP is unwanted. However, the redox cofactor specificity is important for such applications. Z. mobilis is a perspective producer of bioethanol. Its both alcohol dehydrogenase (ADH) isoenzymes are NAD(H)-specific. If novel substrate pathways would involve generation of excess reducing equivalents, NADP(H) should preferably be the redox cofactor for recycling, to avoid competition between the respiratory chain and ADH reaction for NADH, leading to accumulation of acetaldehyde and fall of ethanol yield. To increase the NAD(P)H specificity of Ndh, we replaced the two amino acid residues located at the end of the second beta-sheet of the NAD(P)H-binding domain of Ndh by site-directed mutagenesis, as previously demonstrated for Agrobacterium tumefaciens [1]. Glutamate in the position 219 of the Ndh protein was replaced by glutamine, and alanine in the position 220 — by serine. Mutated variants of ndh (ZMO1113) (including their own promoter regions) were inserted in the shuttle vector pBBR1MCS-2 and transformed into the strain with an Ndh-deficient background (strain Zm6-ndh, derived from Zm6 ATCC29191) [2]. The mutant strains showed a decrease of NAD(P)H-dehydrogenase activity by more than an order of magnitude, yet, in contrast to the wild type Ndh, Vmax for NADPH exceeded the respective value for NADH. Kinetic properties of the mutant Ndh, and potential implications of these mutations for aerobic growth energetics of the recombinant Z. mobilis strains are discussed.

References

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Some archaea and bacteria possess the Na$^+$-dependent membrane bioenergetics. Since this type of bioenergetics could apparently precede the H$^+$-dependent membrane bioenergetics in evolution [1, 2, 3], the reconstruction of the evolutionary histories of the involved enzymes is particularly challenging. Membrane Na$^+$-translocating decarboxylases, found both in archaea and bacteria, couple the biotin-dependent decarboxylation of carboxylic acids with the transfer of Na$^+$-ions out of the cell; thereby the sodium-motive force that can be used for the ATP synthesis is generated. To investigate the evolution of these enzymes, we have performed the phylogenomic analysis. This type of analysis, which demands completely deciphered genomes, is particularly helpful upon reconstructing the evolutionary histories of individual proteins in those cases, when these histories differ from the phylogenies of their host organisms [4]. We have analysed membrane Na$^+$-translocating decarboxylases with different substrate specificities (oxaloacetate decarboxylase, glutaronyl-CoA decarboxylase, methylnalony-CoA decarboxylase and malonate decarboxylase system). A representative sample of bacteria and archaea with completely sequenced genomes was produced; similar sequences of the two main subunits of membrane Na$^+$-decarboxylases ($\alpha$ and $\beta$ subunits) were found by using BLAST within complete proteomes of selected bacteria and archaea; the phylogenetic tree of each subunit was reconstructed according to the domain structure. The evolutionary analysis of the reconstructed trees indicates, that the genes of membrane Na$^+$-translocating decarboxylases were horizontally transferred from bacteria to archaea and then were accommodated within archaean genomes as effective utilizers of carboxylic acids.

References


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S8.P18

Comparative temperature-dependent activity and structural stability of the succinate:quinone oxidoreductases from Escherichia coli and Thermus thermophilus

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Succinate:quinone oxidoreductases (SQORs) (also known as complex II) are membrane proteins which couple the oxidation of succinate and the reduction of quinones in the respiratory chain of prokaryotes and eukaryotes [1–3]. Recently, an extensive characterization of the SQOR from the extremophilic bacterium Thermus thermophilus, was described for the first time [4]. This enzyme is found in a trimeric state and it exhibits atypical features, including high thermostability and an optimum of activity at 70 °C. A recombinant form of the enzyme bearing a His-tag protruding into the trimerization contact point preventing oligomerization has been also prepared [5]. Interestingly, this monomeric SQOR complex is still functional and it only shows a slightly lower thermostability. We have studied the temperature-dependent electrocatalytic activity of the monomeric SQOR complex from the thermophilic bacterium T. thermophilus and its counterpart from the mesophilic bacterium Escherichia coli by protein film voltammetry. To optimize the protein