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Structure and function of the C-terminal end of MrpA — The evolutionary progenitor of the long, membrane parallel helix domain in Complex I

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MrpA is a subunit of the Mrp-antipporter, and the subunit that is evolutionary most closely related to the Complex I subunit NuoL. In addition to the 14 TM-helix protein part conserved in both MrpA and MrpD- type proteins in this family, both MrpA and NuoL contain a C-terminal extension. The recent structures of the Complex I membrane-spanning domain revealed this part of NuoL as one TM helix, followed by a long, membrane parallel helix and a final TM helix ending in the bacterial periplasm [1]. The long helix was immediately believed to have an important functional role for the coupling of the electron-transfer reactions to proton pumping. However, attractive, such a “piston” function has later been disowned [2], but an important structural role of the NuoL C-terminus seems indisputable.

In this work we have investigated the putative evolutionary progenitor of the long, membrane parallel (Imp) helix; the C-terminal domain of MrpA. Sequence alignments revealed that in MrpA, the putative Imp-helix is shorter, owing to the fact that the MrpA Imp-helix only needs to cover one partner protein (MrpD) whereas the NuoL Imp-helix is enclosing both NuoM and NuoN. Transmembrane topology studies showed that the remaining part of MrpA, that is absent from NuoL, instead seem to correspond to NuoJ in Complex I, although the primary sequence conservation is low in this part of the protein. We have previously demonstrated that the subunit NuoK, which is surrounded by NuoJ in the solved structure [1], is homologous to MrpC. The function of the MrpA C-terminus was subsequently assessed in a B. subtilis homologous to MrpC. The function of the MrpA C-terminus was assessed in a homologous production of NuoJ, and the truncated MrpA could still operate. But at pH 8.4, the C-terminal domain of MrpA was absolutely essential for function, demonstrating an important structural role for this domain also in the Mrp antiporter complex.

References

doi:10.1016/j.bbabio.2012.06.175

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The structure of the NADH:ubiquinone oxidoreductase from Vibrio cholerae

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The sodium pumping NADH:ubiquinone oxidoreductase (Na+-NQR) from the pathogenic Gram-negative bacterium Vibrio cholerae is an integral membrane protein complex [1,2]. The Na+-NQR consists of six different subunits NqrA-F [3] and contains a series of cofactors to transport electrons from NADH to ubiquinone. The energy that arises during this process is used to pump sodium ions from the cytoplasm to the periplasm to generate a sodium motif force (SMF). This SMF is essential for substrate uptake, motility, pathogenicity or efflux of antibiotics.

NQR was isolated and crystallized by Marco Casutt and Günter Fritz [4]. The structure was solved at a resolution of 3.7 Å, which provides the first detailed information about this respiratory enzyme. In some regions, especially in the flexible NqrA subunit, the quality of the electron density was too low to assign a distinct structure.

In order to obtain further detailed structural information, we aim to determine the structure single subunits at a high resolution. Recently, a C-terminal truncated version of NqrA (residues 1-377), was crystallized and the structure was determined at 1.95 Å.

References

doi:10.1016/j.bbabio.2012.06.176

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Overproduction of Aquifex aeolicus complex I in E. coli nuo-deletion strains

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The NADH:ubiquinone oxidoreductase, respiratory complex I, is the main entrance point of electrons into the respiratory chains. The complex couples the electron transfer from NADH to ubiquinone with proton translocation across the membrane. The enzyme comprises a noncovalently bound flavin mononucleotide and several iron-sulfur clusters as cofactors. In Aquifex aeolicus, a hyperthermophilic bacterium with an optimal growth temperature of 95 °C, 13 out of totally 24 nuo-genes coding for NuoA-N subunits of complex I, are organized in three different loci [1, 2]. Expression plasmids were constructed containing all 13 A. aeolicus nuo-genes and sequences for polyhistidine affinity tags were fused at several positions. Heterologous production of A. aeolicus complex I is attempted in an Escherichia coli strain missing the chromosomal nuo-operand (C43amp) [3, 4]. The entire A. aeolicus complex I was successfully produced and assembled into the bacterial membrane. However, only subcomplexes of the overproduced protein were isolated due to the instability of the overproduced protein.
The NuoEF subcomplex was crystallized and the structure was resolved at 1.7 Å and 1.9 Å when soaked with substrates. It is attempted to crystallize the other preparations containing subcomplexes in order to understand the structure and function of complex I.

References

doi:10.1016/j.bbabio.2012.06.177