The cytochrome *c* nitrite reductase complex (NrfHA) from *Wolinella succinogenes*

S. L. A. Andrade, J. Simon[1] and O. Einsle

Dept. Molecular Structural Biology, University of Göttingen, Justus-von-Liebig-Weg 11,37077 Göttingen, Germany

[1]Inst. of Microbiology, University of Frankfurt, Marie-Curie-Str. 9, 60439 Frankfurt/Main, Germany

The six-electron reduction of nitrite to ammonia is a crucial step in the biogeochemical cycle of nitrogen. Within the pathway of dissimilatory nitrate ammonification (DNRA) it is catalyzed by the multiheme enzyme cytochrome *c* nitrite reductase. We have previously determined the structure of this enzyme from two members of the ε-group of proteobacteria, *Sulfurospirillum deleyianum* [1] and *Wolinella succinogenes* [2]. In both organisms the protein presents itself as a stable homodimer of the NrfA protein (**n***itrite* **r***eduction with* **f***ormate*), that folds into a single, α-helical domain of ca. 500 amino acid residues and contains five binding motives for covalently attached heme groups.

The active site of cytochrome *c* nitrite reductase is located on heme group one, which is distinguished by having the distal ligand of the heme iron – usually a histidine – replaced by a unique lysine residue. The proximal axial position of the heme iron is the substrate binding site. We have examined the interaction of various inhibitors [2], substrates and reaction intermediates with this site by X-ray crystallography. In conjunction with quantum chemical calculations, these structures allowed for the postulation of a concise reaction mechanism for the complex six-electron reduction carried out by the enzyme [3].

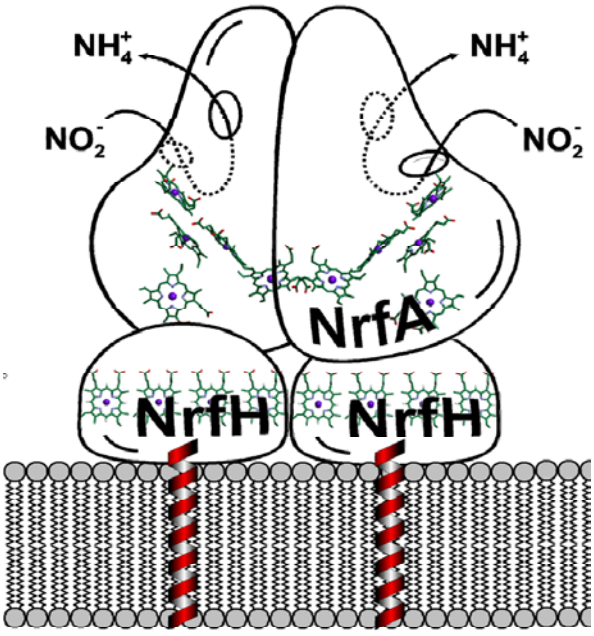

Figure 1: Schematic representation of the NrfHA complex from *W. succinogenes*

While it is possible to obtain the NrfA protein in a soluble form that shows enzymatic activity with benzyl viologen as an electron donor, it has been shown that *in vivo* NrfA is merely the catalytic subunit of a membraneous respiratory complex. In addition to NrfA, this complex contains a small subunit, NrfH, that is able to extract electrons from menaquinone in the membrane [4]. NrfH therefore functions as a quinol oxidase, and its primary sequence shows it to be a member of the NapC/NirT family of multiheme cytochromes. This is a family of small, tetra- or pentahemic cytochromes *c* which are found as links between the quinone pool and a considerable number of periplasmic redox enzymes. So far, no structural information is available for any of its members.

In contrast to many other systems containing a NapC/NirT-type quinol oxidase, the NrfHA complex from *W. succinogenes* is stable and can be purified from the membrane fraction by solubilization with detergent. We obtained large single crystals of the complex and confirmed the presence of both subunits by SDS-PAGE of dissolved crystals [5]. However, diffraction quality of the crystals was poor and further optimization was needed.

We intended to collect a dataset of highest possible quality from NrfHA crystals and use the model of *W. succinogenes* NrfA for phasing by partial molecular replacement. Data were collected for a full 360° rotation of a crystal, but it turned out that the accumulating radiation damage would be detrimental to data quality after ca. 250°. Thus we obtained a dataset to 4.3 Å, with a redundancy of 17.5.

Molecular replacement with the structure of NrfA was carried out using MOLREP from the CCP4 package and yielded a clear solution for the NrfA dimer that also creates a sensible packing in the unit cell. After phase improvement, the position of NrfH is identifiable and we hope that successful tracing of the chain will be possible.

References

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