The substrate of the quinol oxidase CymA is also a specific co-factor

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Introduction

An overwhelming number of chemical reactions in nature are redox reactions. In biology, these reactions are catalysed by redox enzymes, many of which reside in the lipid membrane. Redox enzymes play a major role in almost all metabolic processes, including photosynthesis and biochemical processes such as the nitrogen cycle. By electrically connecting redox enzymes to electrodes, a powerful sensing platform is constructed that is able to characterise details of the catalytic mechanism of these enzymes.

Little is known about enzymatic quinone-quinol interconversions in the lipid membrane when compared to our knowledge of substrate transformations by globular enzymes. In 2012 we studied the enzyme mechanism of a relatively simple quinone converting enzyme, CymA, belonging to the NapC/NirT superfamily. In contrast to most other quinone oxidoreductases, members of this family are monotopic membrane proteins with one globular head domain, facing the periplasm in Gram-negative bacteria. CymA is found in the inner membrane of the γ-proteobacterium *Shewanella oneidensis* MR-1, which are bestowed with a remarkably diverse multicomponent and branched electron transport chain. CymA plays a central role in this multi-branched respiratory chain where it couples the oxidation of menaquinone-7 (MK-7) to a number of multi-heme cytochromes, terminal reductases and soluble extracellular electron carriers (Figure 1).

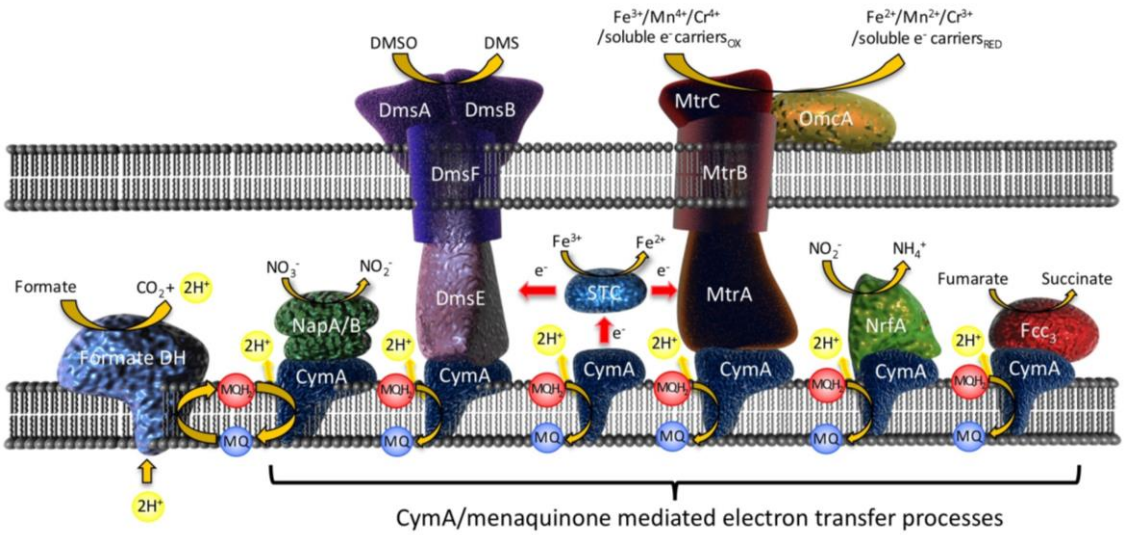

Figure 1: schematic of the role of CymA in Shewanella oneidensis MR-1 anaerobic respiration. Inner membrane-bound dehydrogenases pass electrons through the lipophilic menaquinone pool to CymA where they are passed to four functionally distinct periplasmic reductases. CymA also plays the key role in shuttling electrons to terminal electron acceptors outside the cell though either outer-membrane associated heme-proteins directly such as DmsE or MtrA or via STC as a periplasmic shuttle.

Membrane-modified electrodes

In order to study CymA with electrochemistry, CymA was adsorbed on a surface (the electrode) and the quinone substrate is supplied within vesicles immobilised onto the CymA (Figure 2). It was found that during purification of CymA, its substrate (menaquinone-7; MQ7) was co-purified, indicating that CymA has a high affinity of its substrate. After immobilisation of CymA on the surface, turn-over of the co-purified substrate was detected electrochemically. However, the hydrophobic substrate MQ-7 could be removed from the protein by rinsing the protein-modified electrode surface with a low concentration of

detergent (0.01% n-Dodecyl β-D-Maltopyranoside). To our surprise, the catalytic activity towards substrate homologues like menadione (commonly used to study the activity of quinone-converting enzymes like CymA) was lost upon removal of the MQ-7. Without removal MQ-7, menadione in solution could be catalytically converted by CymA, indicating that menadione could not displace MQ-7 from CymA even though menadione concentrations where many times higher than the co-purified MQ-7. Given the requirement for MQ-7 presence, it seems unlikely that menadione or other homologues access the quinone binding pocket directly during their 'catalytic' turn-over. We therefore suggest that menadione conversion is mediated via quinone-quinone interactions and that MQ-7 may also function as a tightly bound co-factor.

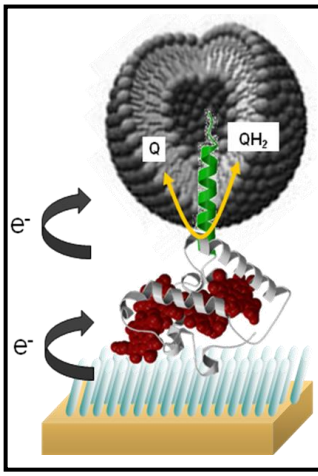

Figure 2: schematic showing a ribbon model of NrfH, a homologue of CymA, immobilised on a self-assembled monolayer modified template-stripped ultra-flat gold electrode with menaquinone-7 containing lipid vesicle. The SAM is shown as blue bars, the CymA polypeptide in white (globular head domain) or green (lipophilic tail domain) and the prosthetic heme groups in red.

Discussion

Several properties of lipophilic quinones and quinone oxidoreductases make this class of membrane enzymes unique in nature. Localisation of substrate and enzymes are restricted to the lipid membrane, where diffusion is an almost 2-dimensional property. Combined with the fact that quinones typically have concentrations of several pmol/cm^2 in the membrane, substrate-enzyme encounters will be much more frequent than typical encounters between globular enzymes and water-soluble substrates. We hypothesise that this has reduced the evolutionary pressure for a highly specific active site, which would only lower k_{off} rates and reduce the turn-over kinetics of the enzyme.

The traditional function of an enzyme is to lower the transition-state activation energy and thereby increasing the reaction rate. However, as has been noted before by others, this might not be required for quinone oxidation/reduction, which is an inherently fast reaction as long as a polar environment is provided to promote a rapid (de)protonation of the quinone. We hypothesize that the function of the quinone co-factors is to reduce the reactivity of the reaction intermediate, semiquinone, which are generally believed to be amenable to side-reactions, giving rise to damaging radical oxygen species.

Publications

Marritt, S., McMillan, D., Shi, L., Fredrickson, J., Zachara, J., Richardson, D., Jeuken, L. & Butt J. (2012) The roles of CymA in support of the respiratory flexibility of *Shewanella oneidensis* MR-1. *Biochem. Soc. Trans.* **40**: 2117-1221.

Marritt, S., Lowe, T., Bye, J., McMillan, D., Shi, L., Fredrickson, J., Zachara, J., Richardson, D., Cheesman, M., Jeuken, L. & Butt, J. (2012) A functional description of CymA, an electron-transfer hub supporting anaerobic respiratory flexibility in shewanella. *Biochem. J.* **444:** 465-474.

McMillan, D., Marritt, S., Butt, J. & Jeuken, L. (2012) Menaquinone-7 is a specific co-factor in the tetraheme quinol dehydrogenase CymA, *J. Biol. Chem.* **287**: 14215-14225.

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