# RESOLVING ELECTRON TRANSPORT PATHWAYS IN THE SELENATE RESPIRING BACTERIUM *THAUERA SELENATIS.*

Submitted by

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Elisabeth Clare Lowe

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# Abstract

The Gram negative bacterium *Thauera selenatis* is able to respire with selenate as the sole terminal electron acceptor, utilising a periplasmic selenate reductase enzyme to reduce selenate to selenite. Previous characterisation of this enzyme has shown that it is a heterotrimeric molybdo-enzyme (SerABC) of the dimethylsulfoxide reductase family, containing a Mo-*bis* molybdopterin guanine dinucleotide co-factor, Fe-S clusters and a *b*-type haem (Schroder *et al*., 1997, J Biol Chem, **272**: 23765-68, Dridge *et al*., 2007, Biochem J, **408**: 19-28). In order to elucidate the electron transport pathway to selenate reductase, and how it can generate a proton motive force, detailed study was required. Firstly, the redox potential of the *b*-haem of SerC was determined by optical redox titration to be +234 mV. The *serC* gene was cloned and expressed heterologously in *E. coli*, but the protein was incorrectly folded into inclusion bodies, and attempts to refold and reconstitute SerC with haem were unsuccessful. A profile of *c*-type cytochromes in *T. selenatis* was undertaken, and characterisation of a number of cytochromes was carried out. Two cytochromes were purified, cyt*c*7 and cyt*c*4, and cyt*c*4 was shown to be able to donate electrons to SerABC *in vitro*. Protein sequence was obtained by N-terminal sequencing and LC-MS/MS, and assigned cyt*c*4 to the cytochrome $c_4$ family of dihaem cytochromes. Redox potentiometry combined with UV-visible and electron paramagnetic spectroscopy showed that cyt*c*4 is a dihaem cytochrome with a redox potential of +282 mV and both haems are predicted to have His-Met ligation. To investigate the role of membrane bound cytochromes in selenate respiration, PCR with degenerate primers amplified a partial gene coding for quinol: cytochrome c oxidoreductase (QCR). A microplate growth method was developed to monitor growth of *T. selenatis* under reproducible conditions, and used to analyse the effect of respiratory chain inhibitors on growth under different conditions. Aerobic metabolism was unaffected by QCR inhibitors, while nitrite reduction was totally inhibited, linking nitrite reduction to the generation of a proton motive force by the QCR. The QCR inhibitor myxothiazol partially inhibited selenate respiration, showing that some electron flux is via the QCR, but total inhibition of selenate respiration was achieved by combining myxothiazol with the more general inhibitor 2-n-heptyl-4-hydroxyquinoline N-oxide (HQNO). These data suggest that electron transfer to selenate reductase occurs via a branched pathway, in which one route is inhibited by myxothiazol and the other by HQNO. Electron transfer via a QCR and a dihaem cytochrome $c_4$ is a novel route for a member of the dimethylsulfoxide reductase family of molybdo-enzymes.

# List of abbreviations

Standard abbreviations are used without definition in accordance to the instructions for authors for submission to the Biochemical Journal (www.biochemj.org). The following abbreviations are also provided for the reader.

| | |
|---|---|
| Å | Angstrom ($10^{-10}$ m) |
| ANAMMOX | anaerobic ammonium oxidation |
| Mo-*bis*MGD | molybdopterin guanine dinucleotide |
| BLAST | basic local alignment search tool |
| CAPS | N-Cyclohexyl-3-aminopropanesulfonic acid |
| Clr | chlorate reductase |
| Ddh | dimethylsulfide dehydrogenase |
| Dor | DMSO reductase |
| Ebd | ethylbenzene dehydrogenase |
| EPR | electron paramagnetic resonance |
| ETC | electron transport chain |
| Fdh | formate dehydrogenase |
| GF | gel filtration |
| GHz | gigahertz |
| HCl | hydrochloric acid |
| HIC | hydrophobic interaction chromatography |
| HQNO | 2-n-Heptyl-4-hydroxyquinoline N-oxide |
| IPTG | isopropyl β-D-galactopyranoside |
| K | Kelvin |
| kDa | kilo Dalton |
| $K_m$ | Michaelis constant |
| $K_s$ | substrate affinity constant |
| LB | Luria-Bertani |
| M | molar concentration |
| ma | milliamps |
| MES | 2-Morpholinoethanesulfonic acid |
| MOPS | 3-(N-Morpholino) propanesulfonic acid |
| mT | millitesla |

| | |
|---|---|
| mW | microwave |
| MW | molecular weight |
| MWCO | molecular weight cut-off |
| $\mu_{max}$ | maximum specific growth rate |
| Nap | periplasmic nitrate reductase |
| Nar | membrane bound nitrate reductase |
| nm | nanometre |
| OD | optical density |
| PIC | protease inhibitor cocktail |
| PIPES | piperazine-1,4-*bis*(2-ethanesulfonic acid) |
| PMF | proton-motive force |
| rpm | revolutions per minute |
| S | substrate |
| Tat | twin-arginine translocase |
| TBE | Tris-borate-EDTA |
| TFB | transformation buffer |
| TMAO | trimethylamine N-oxide |
| Tor | TMAO reductase |
| Tris | Tris (hydroxymethyl) aminoethane |
| V | Volts |
| $V_{max}$ | maximum velocity |
| v/v | volume by volume |
| w/v | weight by volume |
| w/w | weight by weight |
| X-gal | 5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside |

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