

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

Submitted by

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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, cytc7 and cytc4, and cytc4 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 cytc4 to the cytochrome *c*₄ family of dihaem cytochromes. Redox potentiometry combined with UV-visible and electron paramagnetic spectroscopy showed that cytc4 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- <i>bis</i> 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	millamps
MES	2-Morpholinoethanesulfonic acid
MOPS	3-(N-Morpholino) propanesulfonic acid
mT	millitesla

mW	microwave
MW	molecular weight
MWCO	molecular weight cut-off
μ_{\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- <i>bis</i> (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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