Probing the conformational changes of the yeast mitochondrial ADP/ATP carrier

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This dissertation is submitted for the degree of Doctor of Philosophy.
August 2012
Declaration

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text. None of this work has been submitted for any other qualification.

Valerie Lauren Ashton
August 2012
Acknowledgments

First, I thank the Medical Research Council Mitochondrial Biology Unit (MRC MBU) for providing me with seemingly unlimited access to equipment and supplies for my research. I thank Professor Sir John Walker, the MRC and Poland for the partial stipend. I would especially like to thank the Higher Education Funding Council for England for the Overseas Research Students Awards Scheme Studentship, Cambridge Overseas Trusts for the Overseas Research Studentship and the Lundgren Fund for a Hardship Award.

I thank my graduate tutor Dr Penny Barton for her impartial advice and continuing support. I also thank my external collaborators Dr Chris Tate, Dr Fraser Macmillan and Dr Jess van Wonderen, and my internal collaborators Dr Ian Fearnely and Dr Kamburapola Jayawardena for their experimental expertise and assistance. I am very grateful to all Kunji lab members past and present. Dr Christof Bös, Dr Alex Hellawell and Dr John Mifsud provided essential training. Liz Cerson always had time to listen and be supportive through any 'life crisis'. In addition I enjoyed mentoring two summer students, Lisa Görs and Janina Tiedemann, who also helped collect some crucial data. Last but certainly not least, I would like to thank Dr Edmund Kunji for his endless supervision, advice and support. The supervisor is one of the most important determinants of a successful PhD, and having an excellent supervisor helped make my time in the MBU worthwhile.

Outside of lab, I would like to thank the Hillwalking Club for helping me to relax and for facilitating the formation of many lasting friendships. I would especially like to thank my mom, Diane, and dad, Roland, who always support me in whatever I do (even when I run off to other countries!). My husband, Tom, provided love and support and cooked us superb dinners whilst I was busy in the lab. This dissertation is dedicated to the memory of my best friend, Ashley Serola, who always provided a listening ear. Without her, I would not be the person I am today.
Summary

The mitochondrial ADP/ATP carrier in the inner mitochondrial membrane imports ADP and exports ATP by switching between two conformational states. In the cytoplasmic state, which can be locked by carboxy-atriactyloside, the substrate binding site is accessible to the cytoplasm, whereas in the matrix state, which can be locked by bongkrekic acid, the substrate binding site is open to the mitochondrial matrix. Access to the substrate binding site is regulated by salt bridge networks on either side of the central cavity, called the matrix and cytoplasmic salt bridge network. It has been proposed that during transport the salt bridge networks disrupt and form in an alternating way, opening and closing the binding site to opposite sides of the membrane, but experimental evidence has not been obtained for this mechanism.

Single cysteine mutations were introduced at the cytoplasmic side of the yeast mitochondrial ADP/ATP carrier, and the mutant carriers were expressed in the cytoplasmic membrane of *Lactococcus lactis*. They were capable of ADP transport and they could be inhibited by carboxy-atriactyloside and bongkrekic acid. The complete inhibition by carboxy-atriactyloside demonstrated that the carriers were oriented with the cytoplasmic side to the outside of the cells. To probe the accessibility of the single cysteines, the mutant carriers were locked in either the cytoplasmic or matrix state with the two inhibitors and labelled with the membrane-impermeable sulphydryl reagent eosin-5-maleimide. Specific cysteines that were accessible in the cytoplasmic state had become inaccessible in the matrix state. Subsequent experiments showed that ADP and ATP, but not AMP, led to the occlusion of single cysteines, demonstrating that the cytoplasmic side of the ADP/ATP carrier closes as part of the transport cycle. In addition, cross-linking studies combined with mass spectrometry and electron paramagnetic resonance spectroscopy were tried to probe the closure of the cytoplasmic salt bridge network.
Abbreviations and definitions

Abbreviations and definitions of genes and proteins used in this dissertation are listed below. The canonical one letter abbreviations for deoxyribonucleic acid bases are used. Likewise, the canonical one and three letter abbreviations for amino acids are utilised. ‘X’ denotes any amino acid.

- **aac2**: *S. cerevisiae* ADP/ATP carrier isoform 2 gene
- **Δ2-19 cys-less aac2**: gene encoding for *S. cerevisiae* ADP/ATP carrier protein isoform 2 with amino acids 2-19 removed and cysteine residues substituted with alanines
- **AAC**: ADP/ATP carrier protein (no isoform or species specified)
- **AAC1**: Metazoan ADP/ATP carrier protein isoform 1
- **AAC2**: Metazoan ADP/ATP carrier protein isoform 2
- **AAC3**: Metazoan ADP/ATP carrier protein isoform 3
- **AAC4**: Metazoan ADP/ATP carrier protein isoform 4
- **Aac1p**: *S. cerevisiae* ADP/ATP carrier protein isoform 1
- **Aac2p**: *S. cerevisiae* ADP/ATP carrier protein isoform 2
- **Aac3p**: *S. cerevisiae* ADP/ATP carrier protein isoform 3
- **Aac4p**: *S. cerevisiae* ADP/ATP carrier protein isoform 4
- **Δ2-19 Aac2p**: *S. cerevisiae* ADP/ATP carrier protein isoform 2 with amino acids 2-19 removed
- **Δ2-19 cys-less Aac2p**: *S. cerevisiae* ADP/ATP carrier protein isoform 2 with amino acids 2-19 removed and cysteine residues substituted with alanines
- **hANT**: human adenine nucleotide translocase protein
Δp  protonmotive force
ΔpH  transmembrane proton concentration difference
ΔΨ  transmembrane electrical potential difference
Ω  ohm
Å  Angstrom(s) (1 Å = 0.1 nm)
Alexa  alexa fluor 488
ATR  atractyloside
APS  ammonium peroxodisulphate
AU  absorbance unit
BCA assay  bicinchoninic acid assay
BKA  bongkrekic acid
bp  base pair
c-state  cytoplasmic state (substrate binding site is open to the
cytoplasmic side)
CATR  carboxy-atractyloside
cw  continuous wave
DTT  dithiothreitol
e−  electron
EM  electron microscopy
$E_{m,7}$  midpoint potential at pH 7.0
EMA  eosin-5-maleimide
EPR  electron paramagnetic resonance
ETF-QO  electron-transferring flavoprotein:ubiquinone oxidoreductase
FMA  fluorescein-5-maleimide
G  gauss
GHz  gigahertz
kDa  kiloDalton
kHz  kilohertz
$K_i$  dissociation constant for inhibitor binding
LY  lucifer yellow iodoacetamide
M-2-M  1,2-ethanediy1 bismethanethiosulphonate
m-state  matrix state (substrate binding site is open to the matrix side)
MAL-6  (1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyl) maleimide
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Term</th>
</tr>
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<tbody>
<tr>
<td>MALDI</td>
<td>matrix-assisted laser desorption/ionization</td>
</tr>
<tr>
<td>mtDNA</td>
<td>mitochondrial deoxyribonucleic acid</td>
</tr>
<tr>
<td>mm</td>
<td>millimetre</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>mT</td>
<td>millitesla</td>
</tr>
<tr>
<td>MTSL</td>
<td>(1-Oxy-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl) methanethiosulphonate</td>
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<tr>
<td>mW</td>
<td>microwave</td>
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<td>m/z</td>
<td>mass-to-charge ratio</td>
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<tr>
<td>NEM</td>
<td>N-ethyl maleimide</td>
</tr>
<tr>
<td>nm</td>
<td>nanometre</td>
</tr>
<tr>
<td>OD</td>
<td>optical density</td>
</tr>
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<td>OSCP</td>
<td>oligomycin sensitivity conferring protein</td>
</tr>
<tr>
<td>P</td>
<td>P-value</td>
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<td>PAGE</td>
<td>polyacrylamide gel electrophoresis</td>
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<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>PC</td>
<td>phosphatidylcholine</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
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<td>PELDOR</td>
<td>pulsed double electron resonance</td>
</tr>
<tr>
<td>PMT</td>
<td>photonmultiplier tube</td>
</tr>
<tr>
<td>Psi</td>
<td>pounds per square inch</td>
</tr>
<tr>
<td>PVDF</td>
<td>polyvinylidene fluoride</td>
</tr>
<tr>
<td>Q</td>
<td>ubiquinone</td>
</tr>
<tr>
<td>QH₂</td>
<td>ubiquinol</td>
</tr>
<tr>
<td>r²</td>
<td>coefficient of determination</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>sarkosyl</td>
<td>N-Lauroylsarcosine sodium salt</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>TBS</td>
<td>tris-buffered saline</td>
</tr>
<tr>
<td>TCA cycle</td>
<td>tricarboxylic acid cycle</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>TEMED</td>
<td>N, N, N', N'-tetramethylethylene-diamine</td>
</tr>
<tr>
<td>TIM</td>
<td>translocase of the inner membrane</td>
</tr>
<tr>
<td>TOF</td>
<td>time of flight</td>
</tr>
<tr>
<td>TOM</td>
<td>translocase of the outer membrane</td>
</tr>
<tr>
<td>VDAC</td>
<td>voltage-dependent anion channel</td>
</tr>
</tbody>
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