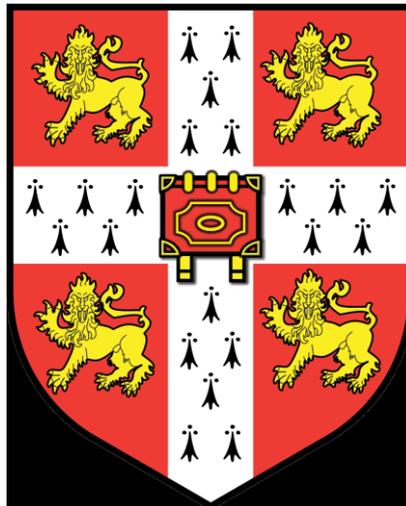


Probing the conformational changes of the yeast mitochondrial ADP/ATP carrier

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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

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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-atractyloside, the substrate binding site is accessible to the cytoplasm, whereas in the matrix state, which can be locked by bongkreikic 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-atractyloside and bongkreikic acid. The complete inhibition by carboxy-atractyloside 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.

<i>aac2</i>	<i>S. cerevisiae</i> ADP/ATP carrier isoform 2 gene
Δ 2-19 <i>cys-less aac2</i>	gene encoding for <i>S. cerevisiae</i> 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	<i>S. cerevisiae</i> ADP/ATP carrier protein isoform 1
Aac2p	<i>S. cerevisiae</i> ADP/ATP carrier protein isoform 2
Aac3p	<i>S. cerevisiae</i> ADP/ATP carrier protein isoform 3
Aac4p	<i>S. cerevisiae</i> ADP/ATP carrier protein isoform 4
Δ 2-19 Aac2p	<i>S. cerevisiae</i> ADP/ATP carrier protein isoform 2 with amino acids 2-19 removed
Δ 2-19 <i>cys-less Aac2p</i>	<i>S. cerevisiae</i> 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
$\Delta \Psi$	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	bongkrelic 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-ethanediyl bismethanethiosulphonate
m-state	matrix state (substrate binding site is open to the matrix side)
MAL-6	(1-Oxyl-2,2,6,6-tetramethyl-4-piperidiny) maleimide

MALDI	matrix-assisted laser desorption/ionization
mtDNA	mitochondrial deoxyribonucleic acid
mm	millimetre
MS	mass spectrometry
mT	millitesla
MTSL	(1-Oxyl-2,2,5,5-tetramethyl- Δ 3-pyrroline-3-methyl) methanethiosulphonate
mW	microwave
m/z	mass-to-charge ratio
<i>n</i>	sample size
NEM	<i>N</i> -ethyl maleimide
nm	nanometre
OD	optical density
OSCP	oligomycin sensitivity conferring protein
<i>P</i>	P-value
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PC	phosphatidylcholine
PCR	polymerase chain reaction
PELDOR	pulsed double electron resonance
PMT	photonmultiplier tube
Psi	pounds per square inch
PVDF	polyvinylidene fluoride
Q	ubiquinone
QH ₂	ubiquinol
<i>r</i> ²	coefficient of determination
RNA	ribonucleic acid
sarkosyl	<i>N</i> -Lauroylsarcosine sodium salt
SDS	sodium dodecyl sulphate
SEM	standard error of the mean
TBS	tris-buffered saline
TCA cycle	tricarboxylic acid cycle

TEMED	N, N, N', N'-tetramethylethylene-diamine
TIM	translocase of the inner membrane
TOF	time of flight
TOM	translocase of the outer membrane
VDAC	voltage-dependent anion channel

Table of contents

Title page	i
Declaration	iii
Acknowledgements	iv
Summary	v
Abbreviations and definitions	vi
Chapter 1 Introduction	1
1.1. The mitochondrion	1
1.1.1. Structural features	2
1.1.2. Oxidative phosphorylation	4
1.2. Mitochondrial Carrier Family	10
1.2.1. Initial discoveries and essential concepts	10
1.2.2. Mitochondrial carriers as uniporters	13
1.2.3. Mitochondrial carriers as proton couplers	13
1.2.4. Mitochondrial carriers as strict exchangers	14
1.3. The ADP/ATP carrier	15
1.3.1. Initial discoveries and essential concepts	15
1.3.2. Amino acid sequence	16
1.3.3. Substrates and inhibitors	16
1.4. Structural features of the ADP/ATP carrier	18
1.4.1. Inhibited structures	18
1.4.2. Carboxy-atractyloside binding site	21
1.5. The ADP/ATP carrier functions as a monomer	23
1.6. Substrate binding site of the ADP/ATP carrier	23
1.6.1. Sequence analysis	23
1.6.2. Molecular dynamics simulations	25
1.7. Transport mechanism of mitochondrial carriers	26
1.7.1. Inhibitor studies	26
1.7.2. Kinetic studies	28
1.7.3. Mutagenesis studies supporting common substrate binding site	30
1.7.4. Mutagenesis studies supporting mechanism	32
1.7.5. Genetic studies	33
1.7.6. Structural analysis	34
1.7.7. Sequence analysis	34
1.7.8. Disease models	39
1.8. Expression of mitochondrial carriers	40
1.8.1. Expression in <i>Lactococcus lactis</i> versus other systems	40
1.8.2. Truncation of yeast Aac2p	42
1.9. Techniques for probing conformational changes	43
1.9.1. Accessibility of cysteine thiols to probes	43
1.9.2. Thiol-specific cross-linking	48

1.9.3. Site-directed spin labelling for electron paramagnetic resonance spectroscopy	50
1.9.4. Antibody accessibility studies	53
1.9.5. Lysine accessibility studies	54
1.9.6. Hydrogen/deuterium exchange coupled to mass spectrometry	54
1.10. Aims and objectives	55
Chapter 2 Materials and Methods	57
2.1. Chemicals	57
2.2. Growth media and plasmid strains	57
2.2.1. Growth media for <i>Escherichia coli</i>	57
2.2.2. <i>Escherichia coli</i> strains and vectors	58
2.2.3. Growth media for <i>Lactococcus lactis</i>	59
2.2.4. <i>Lactococcus lactis</i> strains and vectors	60
2.3. DNA methods	60
2.3.1. Electroporation competent <i>Escherichia coli</i> and <i>Lactococcus lactis</i> cells	60
2.3.2. Agarose gel electrophoresis	61
2.3.3. Polymerase chain reaction	62
2.3.4. Restriction endonuclease digestion	65
2.3.5. DNA precipitation	65
2.3.6. Electroporation	65
2.3.7. Plasmid DNA extraction and cell preservation in glycerol	66
2.3.8. DNA sequencing	66
2.4. <i>Lactococcus lactis</i> cultures and harvesting	66
2.5. Labelling methods	67
2.5.1. Labelling of whole <i>Lactococcus lactis</i> cells with eosin-5-maleimide	67
2.5.2. Labelling of whole <i>Lactococcus lactis</i> cells with 1,2-ethanediyI bismethanethiosulphonate	68
2.5.3. Labelling of whole <i>Lactococcus lactis</i> cells with eosin-5-maleimide and (1-Oxyl-2,2,6,6-tetramethyl-4-piperidinyI) maleimide	68
2.6. General protein methods	69
2.6.1. Lysis of whole cells of <i>Lactococcus lactis</i>	69
2.6.2. Isolation of <i>Lactococcus lactis</i> membranes	69
2.6.3. Estimation of protein concentration	70
2.6.4. Gel electrophoresis	70
2.6.5. Fluorescence imaging and quantification	70
2.6.6. Western blotting and quantification	71
2.6.7. Coomassie gel staining	72
2.7. Transport assays	72
2.7.1. Fused <i>Lactococcus lactis</i> membrane preparation	72
2.7.2. Fused <i>Lactococcus lactis</i> membrane transport assays	73
2.7.3. Whole cells of <i>Lactococcus lactis</i> transport assays	73
2.8. Mass spectrometry	74
2.9. Electron paramagnetic resonance spectroscopy	74

Chapter 3 Transport activities of single cysteine mutants of the ADP/ATP carrier	77
3.1. General introduction	77
3.2. Expression of $\Delta 2-19$ cysteine-less Aac2p	78
3.3. Site-directed mutagenesis for the generation of single cysteine mutants of Aac2p	80
3.4. Expression level of single cysteine mutants	87
3.5. Specific initial uptake rates of single cysteine mutants in <i>Lactococcus lactis</i>	93
3.5.1. Effect of single cysteine mutations on transport	93
3.5.2. Effect of carboxy-atractyloside and bongkreikic acid	102
Chapter 4 Orientation of single cysteine mutants of the ADP/ATP carrier in <i>Lactococcus lactis</i> membranes	109
4.1. Introduction	109
4.2. Selection of a thiol-specific, membrane-impermeable probe	109
4.3. Selection of mutants	116
4.4. Expression level of single cysteine mutants	119
4.5. Optimisation of labelling	122
4.6. Specific labelling in the presence of bongkreikic acid and carboxy-atractyloside	127
4.7. Specific initial uptake rates in the presence of carboxy-atractyloside	137
Chapter 5 Accessibility of single cysteine mutants of the ADP/ATP carrier in different transport states	139
5.1. Introduction	139
5.2. Selection of mutants	139
5.3. Expression level of single cysteine mutants	140
5.4. Eosin-5-maleimide labelling of single cysteine mutants in the presence of bongkreikic acid or carboxy-atractyloside	141
5.4.1. Specific labelling	141
5.4.2. Difference in labelling	150
5.5. Effect of eosin-5-maleimide on the specific initial uptake rates of single cysteine mutants	154
5.6. Effect of nucleotides on eosin-5-maleimide labelling of single cysteine mutants	157
Chapter 6 Probing the formation of the cytoplasmic salt bridge network	163
6.1. Introduction	163
6.2. Probing the putative cytoplasmic salt bridge network	163
6.2.1. Selection of a thiol-specific cross-linking probe	163
6.2.2. Selection of single and double cysteine mutants	164

6.2.3. Expression and cross-linking of single and double cysteine mutants in the presence or absence of bongkreikic acid and carboxy-atractyloside	164
6.2.4. Transport activity of cross-linked ADP/ATP carrier	169
6.2.5. Matrix-assisted laser desorption/ionisation time of flight mass spectrometry of cross-linked ADP/ATP carrier	172
6.3. Probing the distance between alpha-helices adjacent to the putative cytoplasmic salt bridge by pulsed double electron resonance	176
6.3.1. Selection of thiol-specific spin labels	176
6.3.2. Selection of single and double cysteine mutants	176
6.3.3. Expression of single and double cysteine mutants	178
6.3.4. Transport activity of single and double cysteine mutants in fused lactococcal membranes	179
6.3.5. Electron paramagnetic resonance of single and double cysteine mutants	180
6.3.5.1. Labelling with MTSL	180
6.3.5.2. Labelling with MAL-6	182
6.3.6. Competition between MAL-6 and eosin-5-maleimide	188
Chapter 7 General discussion	191
7.1. Expression and transport activity of mutant Aac2p	192
7.2. Orientation of single cysteine mutant carriers in the <i>Lactococcus lactis</i> membrane	195
7.3. Accessibility of single cysteine mutants in different transport states	197
7.4. The formation of the cytoplasmic salt bridge network	202
Appendix I Generation of mutants and list of primers	205
Appendix II Transport and labelling statistics	221
Appendix III Specific initial uptake rates of single cysteine mutants	225
Appendix IV Expression level of single cysteine mutants	227
Bibliography	237