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

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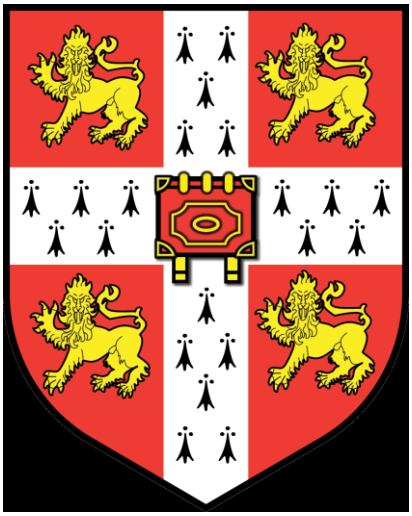

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Declaration

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.

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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 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-atractyloside and bongkrekic 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.

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
$\Delta\Psi$	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	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-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-piperidinyl) 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-Δ3-pyrroline-3-methyl) methanethiosulphonate
mW	microwave
m/z	mass-to-charge ratio
n	sample size
NEM	*N*-ethyl maleimide
nm	nanometre
OD	optical density
OSCP	oligomycin sensitivity conferring protein
P	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
r^2	coefficient of determination
RNA	ribonucleic acid
sarkosyl	*N*-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’-tetramethulethylene-diamine
TIM	translocase of the inner membrane
TOF	time of flight
TOM	tranlocase of the outer membrane
VDAC	voltage-dependent anion channel

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