Investigating the Dual Function of the Chloride Intracellular Ion Channel Proteins

Heba Al Khamici

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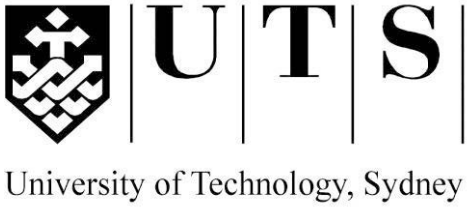

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Dedicated to My Family and all IRAQ with love

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Publications

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List of Abbreviation

λ	Wavelength
A	Absorbance
AA	Ascorbic acid
A9C	Anthracene-9-carboxylic acid
AβP	Amyloid β-protein
ABS	Ammonium persulfate
ac	Alternating current
Ala	Alanine amino acid
AM199	Zwitterioninc lipids
Arg	Arginine amino acid
Asp	Asparagine amino acid
BCA	Bicinchoninic acid assay
AFM	Atomic Force Microscopy
BLM	Black lipid membrane
BSA	Bovine serum albumin
ºC	Degree celsius
$CaCl_2$	Calcium chloride
CDC	Cholesterol-dependent cytolysin
CFTR	Cystic fibrosis transmembrane conductance regulator
CHO-K1	Chinese hamster ovary cells
CIC	Chloride ion channel
Cl^-	Chloride ion
CLIC1 (WT)	Chloride intracellular ion channel protein (wild- type)
Cm	Capacitance
CRAC	Identification of cholesterol recognition amino acid consensus
Cs	Counter electrode capacitance
Cu^{+1}	Cuprous cation
Cu^{+2}	Cupric ion
Cys	Cysteine

DDT	Dithiothreitol
DHA	Dehydroascorbate
DHAR	Dehydroascorbic acid reductase
DIDS	4,4`-diisothiocyano-2,2`stilbene-disulfonic acid
*Dm*CLIC	Drosophila- *melanogaster* CLIC protein
DNA	Deoxyribonucleotides acid
E	Glutamic amino acid
E-coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EIS	Electrochemical impedance spectroscopy
ER	Endoplasmic reticulum
ERK7	Extracellular signal-regulated kinase 7
EXC	Excretory canal abnormality
EXL	EXC4-like
f	Frequency
G	Glycine amino acid
G-site	Glutathione binding site
GABA	Gamma-aminobutyric acid
GS-Se-SG	Selenodiglutathione
Grx	Glutaredoxin
Grx-1, 2 to 5	Glutaredoxin-1, 2 to 5
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidised glutathione
GST	Glutathione-S-transferase
GST-β	Glutathione-S-transferase beta class
GST-Ω	Glutathione-S-transferase omega class
GST-Ω1	Glutathione-S-transferase omega group 1
GST-π	Glutathione-S-transferase pi class
H-site	Hydrophobic region
HSe^- or RSe^-	Selenide
H_2O_2	Hydrogen peroxide

HCSK	High conductance channels with slow kinetics
HcTrx-5	Thioredoxin-related protein in *Haemonchus contortus*
HEDS	2-hydroxyethyl disulfide
HEPES	N-2-hydroxyethylpiperazine-n'-2-ethanesulfonic acid
His	Histidine
ld	Liquid disordered phase
LLO	Listeriolysin-O
lo	Liquid ordered phase
IAA	Indanyloxyacetic acid
ILY	Intermedilysin
IPTG	Isopropyl-β-thiogalactopyranoside
K^+	Potassium ion
KCl	Potassium cholride
kDa	KiloDalton(s)
Km	Dissociation constant of the enzyme-substrate complex
LB	Luria-Bertani medium
M	Molar
MAPK	Mitogen-activated protein kinase
mg	Milligram
min	Minute
MLP	Mobile lipid phase
mM	Millimolar
mV	Millivolt
N	Amino
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide (NAD) + hydrogen (H)
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NaN_3	Sodium Azide
Na_2SeO_3	Sodium selenite
NCC27	Nuclear chloride channel protein-27kDa
N-domain	Amino terminal domain
NEM	N-Ethylmaleimide
nF	Nano-faraday

NIH	National Institutes of Health
nm	Nanometer
nM	Nanomolar
OD	Optical density
P64	Bovine chloride channel protein -64kDa
PBS	Potassium buffered saline
PC	Phosphotidylcholine
PE	Phosphotidylethanolamine
PFO	Perfringolysin-O
PFT	Pore forming toxin
Phe	Phenylalanine amino acid
POPC	1-palmitoyl-2-oleoylphosphatidylcholine
POPE	1-palmitoyl-2-oleoylphosphatidylethanolamine
POPS	1-palmitoyl-2-oleoylphosphatidylserine
Pro	Proline amino acid
PTMD	Putative transmembrane domain
PtoDHAR2	Dehydroascorbic acid reductase-2 from *Populus tomentosa*
QCM	Quartz Crystal Microbalance
Rm	Resistance
RNR	Ribonucleotide reductase
ROS	Reactive oxygen species
RyR	Ryanodine receptor
s	Second
SAM	Self- assembled monolayer
SAXS	Small-angle X-ray scattering
SCSK	Small conductance channels with slow kinetics
S.E	Standard error
Se^0	Metallic selenium
SEC	Size exclusion chromatography
SeCys	Seleno amino acids
SeMet	Selenomethionine
$SeO(OH)_2$	Selenite
$SeO_2(OH)_2$	Selenate

Ser	Serine amino acid
SDS	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SLB	Supported lipid bilayer
SLO	Streptolysin-O
so	Solid ordered phase
SOH	Sulfenic acid
SO_2H	Sulfinic acid
SO_3H	Sulfonic acid
SPR	Surface Plasmon Resonance
STOML	Stomatin- Like proteins in mammals
tBLM	Tethered bilayer lipid membrane
TCEP	Tris(2-carboxyethyl)phosphine
TEMED	N,N,N,N',N-tetramethylenediamine
Tris	Tris[hydroxymethyl]aminomethane
Triton-X100	Octylphenyl-nonaoxyethylene
Trp35	Tryptophan residue number 35
Trx-1	Thioredoxins-1
TrxR	Thioredoxin reductase
Trxs	Thioredoxins
Tween-20	Polyoxyethylene-sorbitan monolaurate
Tyr	Tyrosine amino acid
µg	Microgram
µM	Micromolar
µS	Microsiemens
UV	Ultraviolet
Val	Valine amino acid
WT	Wild type
X	Any amino acid
Z	Impedance

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Abstract

The Chloride Intracellular Ion Channel (CLIC) family consists of six conserved proteins in humans, CLIC1-CLIC6. These are a group of enigmatic proteins, which adopt both a soluble and membrane bound form. CLIC1 in particular has challenged the widely held view that most proteins adopt one stable native structure essential for their biological function. In contrast, CLIC1 was found to be a metamorphic protein, where under specific environmental triggers it adopts more than one stable soluble structural conformation.

CLIC1 was also found to spontaneously insert into cell membranes and form chloride ion channels. However, factors that control the structural transition of CLIC1 from being soluble into a membrane bound protein have yet to be adequately described. Thus, the first objective of this thesis was to identify factors that are involved in CLIC1's insertion and assembly into membranes using tethered bilayer lipid membranes and impedance spectroscopy as a novel system for the study of ion channel activity.

Our findings demonstrate that CLIC1 ion channel activity is dependent on the type and concentration of sterols in bilayer membranes. These findings suggest that membrane sterols play an essential role in CLIC1's acrobatic switching from a globular soluble form to an integral membrane form, promoting greater ion channel conductance in membranes. What remains unclear is the precise nature of this regulation involving membrane sterols and ultimately determining CLIC1's membrane structure. Furthermore, our impedance spectroscopy results of CLIC1 mutants, suggest that residue Cys24 is not essential for CLIC1's ion channel function however it is important

for its optimal activity in membranes. Therefore oxidation and reduction may not be the only regulators of the ion channel activity of CLIC1.

Structural studies have revealed that, soluble CLIC proteins adopt a glutathione S-transferase fold with a conserved glutaredoxin–like active site motif, similar to the GST-Ω class. Therefore the second aim of this project was to investigate the function of the soluble CLICs.

Using the 2-hydroxyethyl disulfide enzyme assay, we have demonstrated for the first time that CLIC1, CLIC2 and CLIC4 possess "glutaredoxin-like" oxidoreductase activity. CLIC1 was found to catalyse the metabolism of the typical glutaredoxin substrates, sodium selenite and dehydroascrobic acid. As expected, the active site Cys24 was detected to be essential for the enzymatic activity of CLIC1 *in vitro*. Most importantly, indanyloxyacetic acid-94 and anthracene-9-carboxylic acid were found to also inhibit the enzymatic activity of CLIC1.

Members of the CLIC protein family can now be classified as "moonlighting proteins" as they exhibit two independent functions; one as ion channels when in their membrane bound form and the other as oxidoreductase soluble enzymes.