A Study of the Spontaneous Membrane Insertion of Chloride Intracellular Ion Channel Protein CLIC1 into Model Lipid Membranes

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Certificate of Original Authorship

I, Khondker Rufaka Hossain, certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

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Publications

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Publications arising from collaborations during my PhD degree

[1] Charles C, Thomas B, Holt SA,; Hossain KR, Anton LB, Sonia C, Al Khamici H, Hans C, Valenzuela SM and Bruce C. (2016) Evidence for the Key Role of H_3O in Phospholipid Membrane Morphology, Langmuir, Manuscript ID: la-2016-01988p (Submitted for review).

[2] Al Khamici, H., Brown, L., Hossain, K., Hudson, A., Sinclair-Burton, A., Ng, J., Daniel, E., Hare, J., Cornell, B., Curmi, P., Davey, M., and Valenzuela, S. (2015) Members of the Chloride Intracellular Ion Channel Protein Family Demonstrate Glutaredoxin-Like Enzymatic Activity, PLoS One 10, e115699.

[3] Yepuri, N., Holt, S., Moraes, G., Holden, P., Hossain, K., Valenzuela, S., James, M., and Darwish, T. (2014) Stereoselective synthesis of perdeuterated phytanic acid, its phospholipid derivatives and their formation into lipid model membranes for neutron reflectivity studies., Chem Phys Lipids. 183, 22-33.

Conference Presentations

Oral presentations

- Hossain KR, Al Khamici H, Holt SA and Valenzuela SM. (2016) ELUCIDATING THE MECHANISM FOR STEROL REGULATION OF CHLORIDE INTRACELLULAR ION CHANNEL PROTEIN INTERACTIONS WITH LIPID MEMBRANES. 60th Annual Meeting, Biophysical Society, California, USA.

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- Hossain KR, Al Khamici H, Holt SA and Valenzuela SM. (2014) Effects of Cholesterol on the spontaneous membrane insertion of Chloride Intracellular Ion Channel Protein CLIC1. 12th AINSE-ANBUG Neutron Scattering Symposium (AANSS), Sydney, Australia.

- Hossain KR, Al Khamici H, Holt SA and Valenzuela SM. (2013) A Study of the Spontaneous Membrane Insertion of Chloride Intracellular Ion Channels (CLICs) into Model Lipid Membranes. 11th AINSE-ANBUG Neutron Scattering Symposium (AANSS), Sydney, Australia.

Poster presentations

- Hossain KR, Al Khamici H, Holt SA and Valenzuela SM. (2015) Investigating the mechanism for Sterol Regulation of CLIC1 with Lipid Membranes. New Horizons, Sydney, Australia.

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Abbreviations

2D	Two-dimensional
A	Area per molecule
Å	Angström (10^{-10} m)
A9C	Anthracene-9-carboxylic acid
ACMW	Air Contrast Matched Water
AEBSF	4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride
AKAP	A Kinase anchor protein
AMP	Adenosine monophosphate
Ano	Anoctamin
ANX	Annexin
APP	Amyloid precursor protein
AQP	Aquaporin
Arg	Arginine amino acid
Asn	Asparagine amino acid
ATP	Adenosine triphosphate
Bcl	B-cell lymphoma
BSA	Bovine serum albumin
C-domain	Carboxyl terminal domain
Ca^{2+}	Calcium ion
CaCC	Ca^{2+} - activated Cl- channel
$CaCl_2$	Calcium chloride
CD	Circular Dichroism
CDC	Cholesterol-dependent cytolysins
CFTR	Cystic fibrosis transmembrane conductance regulator
Ch-ane	Cholestane
Ch-one	5-cholesten-3-one
CHO-K1	Chinese hamster ovary cells
Chol	Cholesterol
CIC	Chloride ion channel
Cl^-	Chloride ion
CLIC	Chloride intracellular ion channel
CV	Column Volume
Cys	Cysteine amino acid

d_{31}-POPC	Deuterated 1-palmitoyl-(d31)-2-oleoyl-sn-glycero-3-phosphatidylcholine
*Dm*CLIC	*Drosophila melanogaster* CLIC protein
DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine
DSC	Differential Scanning Calorimetry
DTT	Dithiothreitol
E-64	Epoxide protease inhibitor
E.coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EPR	Electron Paramagnetic Resonance
ErbB	Epidermal growth factor receptor
Erg	Ergosterol
ERK	Extracellular signal-regulated kinase
ERM	Ezrin, Radixin and Moesin proteins
EXC	Excretory canal abnormality
EXL	EXC-like
FRET	Fluorescence Resonance Energy Transfer
GABA	gamma-Aminobutyric acid
Gln	Glutamine amino acid
Glu	Glutamic acid amino acid
Gly	Glycine amino acide
GPHR	Golgi pH Regulator
Grx	Glutaredoxin
GSH	Reduced glutathione
GST	Glutathione S-transferase
H^+	Hydrogen ion
HEDES	2-hydroxyethyl disulphide
Hepes	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
His	Histidine amino acid
Hyd	20-hydroxyecdysone
IAA-94	Indanyloxyacetic acid 94
IPTG	Isopropyl-thio-β-D-galactopyranoside
IQGAP	IQ motif containing GTPase activating protein
IR	Infra-Red
IRS	Interfacial Stress Rheometry
Kan	Kanamycin
KCl	Potassium chloride

kDa	kilo-Dalton
KH_2PO_4	Potassium dihydrogen phosphate
LC	Liquid condensed phase
LM	Langmuir monolayer
LSM	U6 snRNA-associated Sm-like protein
Lys	Lysine amino acid
M	Moles
MAP	Mitogen activated protein
MC	Monte-Carlo
mCLIC	mouse Chloride intracellular ion channel protein
$MgCl_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
ml	milli-Litre
mM	milli-Molar
N-domain	Amino terminal domain
NaCl	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate
NBD	Nucleotide binding domain
NCC27	Nuclear chloride channel protein 27kDa
NEM	N-Ethylmaleimide
Ni-NTA	nickel-nitrilotriacetic acid
NMR	Nuclear Magnetic Resonance
NR	Neutron Reflectivity
p64	Bovine chloride channel protein 64kDa
Panc	Pancreatic cancer cells
PC	Phosphatidylcholine
PCR	Polymerase chain reaction
PE	Phosphatidylethanolamine
PFT	Pore-forming toxin
Phe	Phenylalanine amino acid
PHR	Pam Highwire RPM-1 proteins
pI	Isoelectronic point
POPC	1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine
POPE	1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylethanolamine
POPS	1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylserine
PPI2	Serine/threonine phosphatase (PP1) isoform PP1 gamma 2
Pro	Proline amino acid

PS	Phosphatidylserine
PTMD	Putative Transmembrane Domain
Q	Momentum transfer
RyR	Ryanodine receptor
SANS	Small Angle Neutron Scattering
SAXS	Small Angle X-ray Scattering
SDS-PAGE	Sodium Dodecyl Sulfate-PolyAcrylamide
SEC	Size Exclusion Chromatography
Ser	Serine amino acid
Sito	ß-sitosterol
SLD	Scattering Length Density
SLS	Surface Light Scattering
t	Thickness
T84	Human colon cancer cell
tBLM	Tethered lipid bilayers
TCEP	tris-2-carboxyethyl-phosphine
TEMED	NNNN'-tetramethylethylenediamine
TMD	Transmembrane domain
TMR	Transmembrane region
TNF-α	Tumour necrosis factor- alpha
Trp	Tryptophan amino acid
Val	Valine amino acid
VGAT	Vesicular GABA transporter
VGLUT	Vesicular glutamate transporter
X	Any amino acid
XR	X-ray Reflectivity
XRD	X-ray Diffraction
Γ	Surface excess/ Surface coverage
ΔA	Percentage surface area expansion
λ	Wavelength
µl	micro-Litre
π	Surface pressure
σ	Roughness
Ω-GST	Omega class GST

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Abstract

Sterols have been reported to modulate conformation and hence the function of several membrane proteins. One such group is the Chloride Intracellular Ion Channel (CLIC) family of proteins. These largely soluble proteins possess the intriguing property of spontaneous insertion into phospholipid bilayers to form integral membrane ion channels. To date, the structure of their membrane-bound form and factors influencing their auto-insertion remains largely unknown. In this thesis, we have performed Langmuir-film, X-ray, and neutron reflectivity experiments to study the interaction of wild-type or mutant versions of the protein CLIC1 with monolayers prepared using various mixtures of different phospholipids and sterol molecules, in order to investigate the regulatory role of the membrane lipid combination on the spontaneous membrane insertion of CLIC1 and to elucidate the structural features of the CLIC1 membrane-bound form within the lipid monolayers.

Our findings have demonstrated that the spontaneous membrane insertion of CLIC1 is dependent on the presence of cholesterol in lipid monolayers. In phospholipid monolayers only, CLIC1 was able to insert within the phospholipid head-group region with no penetration into the acyl chain region of the monolayers. However, in the presence of cholesterol, CLIC1 showed significant interaction with the phospholipid acyl chains thereby, suggesting that cholesterol is required for the penetration of CLIC1 into the hydrophobic tails of the lipid monolayer, which is considered necessary for the formation of functional ion channels. From reflectivity experiments, we were able to show that approximately 0.8 mg/m^2 of CLIC1 inserted into phospholipid monolayers containing cholesterol such that the protein occupied an area per molecule between 5 ~ 7 nm^2 with a total CLIC1 thickness ranging from ~ 51 Å to 59 Å throughout the entire monolayer. We have also demonstrated for the first time that the GXXXG motif in CLIC1 acts as the cholesterol-binding site used by the protein for its initial recognition and binding to membrane cholesterol. Furthermore, Langmuir and reflectivity experiments using different sterols have confirmed that the interaction between CLIC1 and sterols is dependent on an intact 3β-OH group in the sterol ring. Modification of the sterol structure by the introduction of additional hydroxyl

groups and methylation of the sterol alkyl chain was shown to facilitate greater spontaneous membrane insertion of the protein within the phospholipid monolayer. Taken together these findings provide clear evidence for the important role of sterols in the regulation of CLIC1 membrane interactions and a putative mechanism for its initial binding and membrane integration.