STRUCTURAL DYNAMICS AND MEMBRANE INTERACTIONS OF THE CHLORIDE INTRACELLULAR CHANNEL PROTEIN, CLIC1

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DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

Christos Nathaniel

day of 2007.

This thesis is dedicated to my loving family: Omiros, Anna and Andreas Nathaniel

"Who looks outside, dreams; who looks inside, awakes"

Carl Gustav Jung

ABSTRACT

The Chloride Intracellular Channel (CLIC) proteins are a family of amphitropic proteins that can convert from soluble to integral membrane forms. CLIC1 is a member of this family that functions as a chloride channel in the plasma and nuclear membranes of cells. Although high-resolution structural data exists for the soluble form of monomeric CLIC1, not much is known about the integral membrane forms' structure. The exact mechanism and signals involved in the conversion of the soluble form to membrane-inserted form are also not clear.

Studies were undertaken in the absence and presence of membrane models. Analysis of the structure and stability of CLIC1 in the absence of membrane investigated the effect of possible signals or triggers that may play a crucial role in the conversion of the soluble form to integral membrane form. Exposing CLIC1 to oxidizing conditions results in the formation of a dimeric form. The CLIC1 dimer was found to be less stable than the monomeric form based on unfolding kinetic studies. The stability of the dimer was also less influenced by salt concentration, compared with the monomer. The effect of pH on the structure of CLIC1 is of physiological relevance since the movement of soluble CLIC1 in the cytoplasm or nucleoplasm toward the membrane will involve the protein being exposed to a lower pH micro-environment. Hydrogen exchange mass spectrometry was used to study the structural dynamics of CLIC1 at pH 7.0 and pH 5.5. At neutral pH, domain II is more stable than the more flexible thioredoxin domain I. The thioredoxin-fold therefore is more likely to unfold and rearrange to insert into membranes. Because of the high stability of domain II this region is probably where the folding nucleus of the protein is. At pH 5.5 it was found that the α1, α3 and α6 helices, which are spatially adjacent to one another across the domain interface, were destabilized. This destabilization may be the trigger for CLIC1 to unfold and rearrange into a membrane insertion-competent form. The role of the primary sequence and unique three-dimensional structure of CLIC1 in membrane insertion was investigated in a bioinformatics-based study that looked at conserved residue features such as hydropathy and charge. Hidden helical propensities and N-capping motifs in the α1-β2 region were found, which may have important implications for locating putative transmembrane regions.

Analysis of the structure and thermodynamics of CLIC1 interacting with membranes investigated changes in secondary structure, tertiary structure, hydrodynamic volume and thermodynamics when CLIC1 is exposed to membrane-mimicking models. The effect of a variety of conditions such as pH and redox, cysteine-modifiying agents (NEM), ligands (GSH), and inhibitors (IAA) on CLIC1 membrane interaction were studied. It was found that CLIC1 interacted with membranes more favourably at lower pH and that NEM completely inhibited CLIC1 interaction with micelles.

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ABBREVIATIONS

A_{280}	Absorbance at 280nm
ANS	8-analino-1-naphtalene sulphonate
Cα	Alpha carbon
CLC	Chloride channel
CLIC	Chloride intracellular channel
CMC	Critical micellar concentration
DEAE	Diethylaminoethyl
DLS	Dynamic light scattering
DOPG	L-α-phosphatidyl-DL-glycerol dioleoyl
DTNB	5,5'-dithiobis(2-nitrobenzoate)
DTT	Dithiothretol
DXMS	Deuterium exchange mass spectrometry
EDTA	Ethylenediaminetetra-acetic acid
e222	Ellipticity at 222nm
Far-UV CD	Far ultraviolet circular dichroism
ΔG	Gibbs free energy obtained under standard conditions
Grx2	Glutaredoxin 2
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutahtione transferase
GuHCL	Guanadinium hydrochloride
hGSTA1-1	Human class α glutathione transferase
H_20_2	Hydrogen peroxide
ΔH	Enthalpy change
HXMS	Hydrogen exchange mass spectrometry
IAA-94	Indanyloxyacetic acid 94
IPTG	Isopropyl-D-thiogalactoside
kDa	Kilodalton
LUV	Large unilamellar vesicle
MLV	Multilamellar vesicles
NaCl	Sodium chloride

Na_2HPO_4	Sodium Phosphate
NATA	N-acetyl-tryptophanamide
NEM	N-ethyl maleimide
OD_{600}	Optical density at 600 nm
PC	Phosphatidylcholine
PDI	Polydispersity index
PE	Phosphatidylethanolamine
PS	Phosphatidylserine
PDB	Protein data bank
p*I*	Isolelecric point
POPC	L-α-phosphatidylcholine β-oleoyl-γ-palmitoyl
rGSTK1-1	Rat class kappa glutathione transferase
Rmsd	Root mean square of deviation
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEC-HPLC	Size exclusion high performance liquid chromatography
SCOP	Structural classification of proteins
Sj26GST	*Schistosoma japonicum* glutathione transferase
SUV	Small unilamellar vesicle
Δ*S*	Entropy change
TFE	Trifluoroethanol
Ure2p	Yeast prion protein

The IUPAC-IUBMB one and three letter codes for amino acids are used.

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