Structural Characterization of *The SAC Domain of the Par-4 Protein* by NMR spectroscopy

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ABSTRACT

Prostate Apoptosis Response-4 protein (Par-4): Par-4 gene, first identified in prostate cancer cells undergoing apoptosis, encodes a pro-apoptotic protein. Par-4 selectively induces apoptosis in cancer cells causing regression of tumors in animal models.
Par-4 induces apoptosis in androgen independent prostate cancer cells and Ras-transformed cells but not in androgen-dependent normal cells. Apoptosis induction by the Par-4 involves a complex mechanism that requires activation of the Fas death receptor signalling pathway and coparallel inhibition of NF-kB transcription activity. Par-4 expression is elevated in various neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's diseases and stroke.

Rat Par-4 MW 36kDa, full length aa332 protein that shows high homology to human Par-4. Par-4 has two putative nuclear localization sequences NLS1 (aa20-25) and NLS2 (aa 137-153) localized to the N-terminal half of the molecule. SAC domain: Selectivity for apoptosis induction in cancer cells 59-aa (aa137-195) is necessary for apoptosis. Leucine zipper(LZ) domain of 41-aa (aa 292-332) at C terminus which binds to zinc finger domain of aPKC isoforms, Wilms tumor(WT1) protein, p62 and Dlk. Par-4 Δleucine zip aa1-265, zipperless domain.

Different types of expression vectors have been tried for expression of the Par-4 SAC domain in E. coli cells. The first expression vector was H-MBP-3C was tried with *E.coli* rosetta cells. The fusion protein expressed with this vector was not stable. The Par-4 SAC domain protein gene sequence was cloned in different trial vectors. In the beginning we decided to use a vector without any fusion tag, the pPROEX-HTb which shows low level of expression in *E.coli* BL21 (DE3) CP cells. The pETTEV expression vector with a thioredoxin tag shows good expression level and fusion protein stability. The protein sample after purification was characterized by proton NMR spectroscopy, CD and DLS which does not show any presence of protein folding. pH titration by using NMR spectroscopy to simultaneously observe the protonation state of different ionizing functional groups in peptides and proteins with both acidic and basic condition was also done. There was no evidence of any protein folding in the acidic as well as basic pH range. This suggests that protein is natively unfolded.

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ABBREVIATIONS

aa	Amino acid
CD	Circular Dichroism
DNA	Deoxyribo nucleic acid
Da	Dalton
dNTP	Deoxyribonucleotide triphosphate
DTT	Dithiothretol
DLS	Dynamic Light Scattering
EDTA	Ethylenediaminetetraacetic acid
FPLC	Fast Protein Liquid Chromatography
HSQC	Hetero-nuclear single quantum correlation
HPLC	High Performance Liquid Chromatography
LB broth	Luria Bertani media
IDP's	Intrinsically disordered proteins
IPTG	Isopropyl-β-D-thiogalactopyranoside
IMAC	Immobilzied ion metal affinity chromatography
LZ	Leucine zipper domain
MBP	Maltose binding protein
NMR	Nuclear Magnetic Resonance spectroscopy
OD	Optical density
PCR	Polymerase chain reaction
Par-4	Prostate apoptosis response-4
PBS	Phosphate buffered saline
PKCζ	atypical protein kinase C zeta
Psi	Pounds per square inch
pI	Isoelectric point
SAC	Selective for apoptosis induction in cancer cells
SEC	Size exclusion chromatography
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TCEP	Tris (2-carboxyethyl) phosphine
TEMED	N, N, N', N'-tetramethylethylenediamine
Tris-HCl	2 amino-2-hydroxymethyl propane-1,3 diol

Tricine	N-(-2-hydroxy-1, 1-bis (hydroxymethyl) ethyl) glycine
Thesit	Polyethyleneglycol dodecyl ether
UV	Ultra violet
WT1	Wilms' tumor protein 1
ZF	Zinc finger domain