Intrinsic Disorder and Coiled Coil formation in Prostate Apoptosis Response factor-4 (Par-4)

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Abstract

Prostate apoptosis response factor-4 (Par-4) is a ubiquitously expressed pro-apoptotic and tumour suppressive protein. Par-4 contains a highly conserved coiled coil (CC) region at the C-terminus, particularly the distal 40 residues fulfil the criteria for a leucine zipper (LZ). This C-terminal domain serves as the primary recognition domain for a large number of binding partners. Par-4 is tightly regulated by the aforementioned binding partners and also by post-translational modifications. Biophysical data presented here describe Par-4 as primarily an intrinsically disordered protein (IDP). Bioinformatic analysis of the highly conserved Par-4 reveals low sequence complexity and enrichment in polar and charged amino acids. High proteolytic susceptibility and increased hydrodynamic radii are consistent with largely extended structures in solution. Spectroscopic measurements using circular dichroism (CD) and nuclear magnetic resonance (NMR) also reveal characteristic features of intrinsic disorder. Under physiological conditions, data show that Par-4 self-associates via the C-terminal domain possibly through coiled coil formation. Analysis of various constructs comprising the Par-4 LZ domain by NMR, CD, light scattering and other techniques reveals an environment-dependent conformational equilibrium between primarily disordered monomers and predominantly coiled coil dimers. Whereas the disordered monomers are easily observed by NMR, the coiled coil fraction is not amenable to NMR studies possibly due to intermediate exchange processes. Mutational approaches that stabilise the coiled coil fraction result in NMR spectra of lower quality compared to the wild-type form. The high degree of sequence conservation suggest that coiled coil formation and intrinsic disorder are essential for Par-4 to function as an effective regulator of apoptosis.

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Glossary of Abbreviations

A_{XXX}	**A**bsorption at **XXX** nm
AATF	**A**poptosis-**a**ntagonising **t**ranscription **f**actor
Apaf-1	**A**poptotic **p**rotease **a**ctivating **f**actor-1
Amp	**Amp**icillin
Aβ	**A**myloid **β**-peptide
aPKC	**a**typical **p**rotein **k**inase **C**
APP	**A**myloid **p**recursor **p**rotein
AR	**A**ndrogen **r**eceptor
AU	**A**nalytical **U**ltracentrifugation
BACE-1	**β**-site **A**PP **c**leaving **e**nzyme-1
BCA	**B**icin**c**honin**a**cid
CAD	**C**aspase **a**ctivated **D**Nase
Cam	**C**hlor**am**phenicol
CARD	**C**aspase **a**ctivation and **r**ecruitment **d**omain
CC	**C**oiled **c**oil
CD	**C**ircular **d**ichroism
cFLIP	**c**ellular **F**LICE-**l**ike **i**nhibitory **p**rotein
CHT1	**Ch**oline **t**ransporter 1
CM	**C**arboxy**m**ethyl
D2DR	**D**opamine **D2 r**eceptor
DAP	**D**eath-**a**ctivated **p**rotein kinase
DD	**D**eath **d**omain
DED	**D**eath **e**ffector **d**omains
DIABLO	**D**irect **I**AP-**b**inding protein with **lo**w pI
DISC	**D**eath-**i**nducing **s**ignalling **c**omplex
Dlk	**D**AP **l**ike **k**inase
DLS	**D**ynamic **l**ight **s**cattering
DOM	**D**is**o**rdered **m**onomer
DTT	**Di**thio**t**hreitol
EDC	1-**e**thyl-3-(3-**d**imethylaminopropyl)**c**arbodiimide
EDTA	**E**thylene**d**iamine**t**etraacetic **a**cid
ER	**E**ndoplasmatic **r**eticulum
ERK	**E**xtracellular signal **r**egulated **k**inase
FADD	**F**as **a**ssociated **d**eath **d**omain
FLICE	**F**ADD-**l**ike **i**nterleukin 1β **c**onverting **e**nzyme (= caspase 8)
GRP78	**G**lucose-**r**egulated **p**rotein-78
GST	**G**lutathione **S**-**T**ransferase

HCA	**H**ydrophobic **c**luster **a**nalysis
HMBP-3Cpro	**H**exa-histidine-**MBP**-tagged **3C** **pro**tease
HMQC	**H**eteronuclear **m**ultiple-**q**uantum **c**oherence
HSQC	**H**eteronuclear **s**ingle-**q**uantum **c**oherence
IAP	**I**nhibitors of **ap**optosis
ICAD	**I**nhibitor of **c**aspase **a**ctivated **D**Nase
ID	**I**ntrinsic **d**isorder
IDP	**I**ntrinsically **d**isordered **p**rotein
IEC	**I**on-**e**xchange **c**hromatography
IκB	**I**nhibitor of **κB** family
IKK	**Iκ**B **k**inase
IMAC	**I**mmobilised **m**etal ion **a**ffinity **c**hromatography
IPTG	**I**so**p**ropyl-β-D-**t**hio**g**alactopyranoside
Kan	**Kan**amycin
LB	**L**uria **B**ertani medium
LZ	**L**eucine **Z**ipper
MALLS	**M**ulti-**A**ngle **L**aser **L**ight **S**cattering
MAPK	**M**itogen-**a**ctivated **p**rotein **k**inases
MBP	**M**altose **b**inding **p**rotein
Mes	2-**Mo**rpholino**e**thane**s**ulfonic acid
MOPS	3-(N-**Mo**rpholino)**p**ropane**s**ulfonic acid
MS	**M**ass **s**pectrometry
MW	**M**olecular **w**eight
MWCO	**M**olecular **w**eight **c**ut **o**ff
NFκB	**N**uclear **f**actor **κB**
NLS	**N**uclear **l**ocalisation **s**equence
NMR	**N**uclear **m**agnetic **r**esonance
NOESY	**N**uclear **O**verhauser **E**ffect **S**pectroscop**y**
NRMSD	**N**ormalised **r**oot **m**ean **s**quare **d**eviation
NTA	**N**itrilo**tri**a**c**etic acid
OD_{XXX}	**O**ptical **d**ensity at **XXX** nm
Par-4	**P**rostate **a**poptosis **r**esponse factor-**4**
PAGE	**P**oly**a**crylamide **g**el **e**lectrophoresis
PBS	**P**hosphate **b**uffered **s**aline
PCD	**P**rogrammed **c**ell **d**eath
PCR	**P**olymerase **c**hain **r**eaction
PI3K	**P**hospho**i**nositide **3**-**k**inase
PKA, PKB or PKC	**P**rotein **k**inase **A**, **B** or **C**
PML	**P**ro**m**yelocytic **l**eukemia
POD	**P**redominantly **o**rdered **d**imer

PTEN	**P**hosphatase and **ten**sin homolog deleted on chromosome 10
RP-HPLC	**R**eversed **p**hase - **h**igh **p**erformance **l**iquid **c**hromatography
rpm	**R**evolutions **p**er **m**inute
rrPar-4	**r**ecombinant **r**at **P**rostate **a**poptosis **r**esponse factor-**4**
R_S	**S**tokes **r**adius
rTEV	**r**ecombinant **t**obacco **e**tch **v**irus protease
SAC	**S**elective **a**poptosis induction in **c**ancer cells
SAXS	**S**mall-**a**ngle **X**-ray **s**cattering
SEC	**S**ize **e**xclusion **c**hromatography
SDS	**S**odium **d**odecyl **s**ulphate
SMAC	**S**econd **m**itochondria-derived **a**ctivator of **c**aspase
SOC	**S**uper **o**ptimal broth with **c**atabolite repression
SP	**S**ulfo**p**ropyl
SSB1, 2, 4	**S**PRY-domain containing **S**OCS **b**ox proteins 1, 2, 4
TCEP	**T**ris(2-**c**arboxy**e**thyl)**p**hosphine
TFA	**T**ri**f**luoro**a**cetic acid
TFE	**T**ri**f**luoro**e**thanol
T_m	**M**elting **t**emperature
TNFα	**T**umour **n**ecrosis **f**actor **α**
TNFR1	**T**umour **n**ecrosis **f**actor **r**eceptor 1
TOP1	**Top**oisomerase 1
TRAIL	**T**NF-**r**elated **a**poptosis **i**nducing **l**igand
WT	**W**ild **t**ype
WT1	**W**ilm's **t**umour protein **1**
XIAP	**X**-linked **i**nhibitor of **ap**optosis

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