Characterization of Cl-Par-4: WT vs. Mutant

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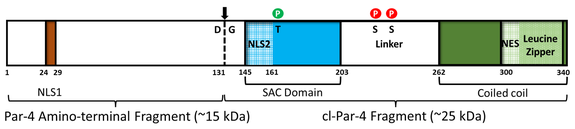
ABSTRACT

Intrinsically disordered proteins (IDPs) play important roles in regulation of cell signaling pathways as well as cellular processes. Dysregulation of these proteins is associated with several human diseases. Prostate apoptosis response-4 (Par-4), a proapoptotic tumor suppressor protein, is categorized as an intrinsically disordered protein and downregulation of this protein has been reported in a myriad of cancers including glioma, breast cancers, and prostate cancers. The caspase-cleaved fragment of Par-4 (cl-Par-4) plays an active role in tumor suppression by inhibiting several cell survival pathways. Here, we employed site-directed mutagenesis to introduce a point mutation in the cl-Par-4 wildtype (WT) to generate the D313K cl-Par-4 mutant. We have characterized both the mutant and the WT using various biophysical techniques such as CD, DLS, and NMR to determine the effect of the D313K mutation. The results show that D313K cl-Par-4 attains a compact and well-folded helical conformation, possibly a tetramer, similar to that of the WT in presence of high salt at physiological pH. However, D313K does so with half the amount of salt required for the WT. This establishes that the substitution of a basic residue for an acidic residue at position 313 alleviates inter-helical charge repulsion between dimer partners and helps to stabilize the structural conformation.

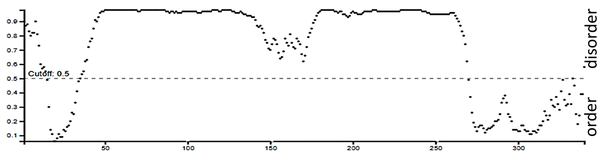
INTRODUCTION

- Prostate apoptosis response-4 (Par-4): tumor suppressor
- cl-Par-4: active fragment of Par-4
- Human diseases: cancers, neurodegenerative, & others
- Cancers: down-regulation of Par-4
- Neurodegeneration: upregulation of Par-4
- Par-4: selective apoptosis in cancer cells
- Target for chemotherapeutic agents: potential
- Structure: unknown

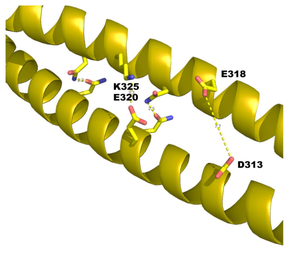
Full-length Par-4



Disorder prediction in Par-4



Crystal structure of rat Par-4 CC domain



Polar interaction showing D313-E318 repulsion

HYPOTHESIS

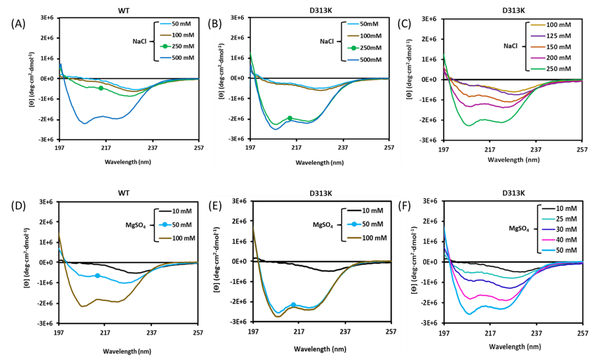
- Requirement of high salt or low pH to stabilize structural conformation could be due to inter-helical electrostatic repulsion between D313 and E318 residues in the leucine zipper dimer
- D313K point mutation in cl-Par-4 helps alleviate inter-helical charge repulsion and stabilizes structural conformation at low salt under physiological pH

METHODS

- cl-Par-4 WT vs. mutant: D313K (site-directed mutagenesis)
- Template: cl-Par-4 (codon-optimized)
- Expression vector: modified H-MBP-3C
- Purification: immobilized metal affinity chromatography
- Characterization: CD, DLS, NMR

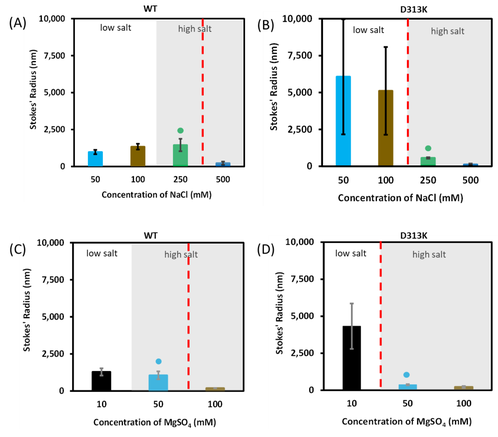
RESULTS

CD: WT vs. D313K (effect of salts)



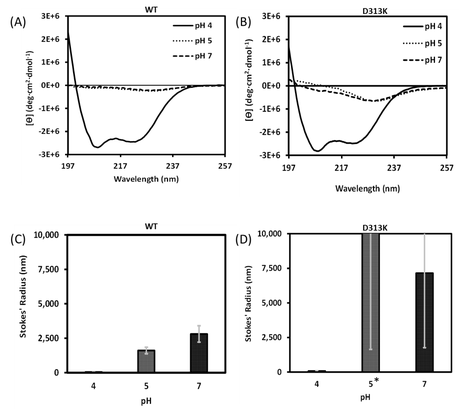
NaCl: (top panel) D313K requires less Na^+
$MgSO_4$: (bottom panel) D313K requires less Mg^{2+}

DLS: WT vs. D313K (effect of salts)



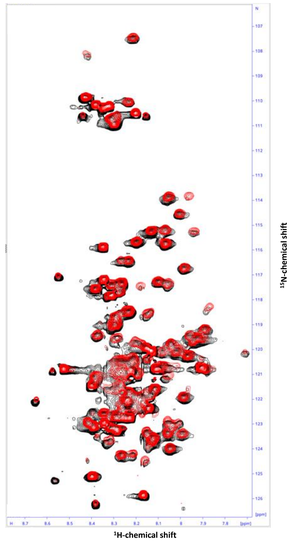
NaCl: (top panel) D313K mutation changes particle size
$MgSO_4$: (bottom panel) D313K mutation changes particle size

WT vs. D313K (effect of pH)



D313K mutation has less effect on conformation vs. pH
*At pH 5 stokes' radius extends off the graph, to 17,800 nm

NMR: WT vs. D313K (^1H-^{15}N HSQC overlay)



D313K mutation retains the structure

CONCLUSIONS

- D313K forms stable, compact, and helical conformation with half the amount of salt required for WT at pH 7
- These compact structures are similar to structures formed by WT cl-Par-4 at higher salt

FUTURE WORKS

- ❑ Generate E318K mutant cl-Par-4, characterize and compare the results to WT
- ❑ Introduction of mutation *in vivo*: test effect on apoptosis, cancer, and neurodegenerative disease

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2. Clark, A. M.; Ponniah, K.; Warden, M. S.; Raitt, E. M.; Yawn, A. C.; Pascal, S. M., pH-Induced Folding of the Caspase-Cleaved Par-4 Tumor Suppressor: Evidence of Structure Outside of the Coiled Coil Domain. *Biomolecules* **2018,** *8*, 162.

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