Influence of Monovalent and Divalent Ions on the Conformational State of the Caspase-cleaved Par-4 (cl-Par-4) Tumor Suppressor

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Abstract

Caspase-cleaved Par-4 (cl-Par-4) is the functionally active part of Par-4 tumor suppressor protein which inhibits nuclear factor NF-κB-mediated cell survival pathways and causes selective apoptosis in various tumor cells. Here, we have characterized the structure of cl-Par-4 protein *in vitro* in the presence of various concentrations of monovalent and divalent ions using a variety of biophysical techniques. We found that divalent cations induce a highly helical tetramer structure at a concentration five times lower than that of monovalent cations. The anion moiety of the salts exerts negligible influence.

Introduction

Prostate apoptosis response-4 (Par-4) is a pro-apoptotic tumor suppressor protein that selectively induces apoptosis in cancer cells. Apoptosis is initiated by the interaction of extracellular Par-4 with the cell surface GRP78 protein. The interaction initiates the Fas/FasL-FADD caspase pathway. The Par-4 protein is predominantly intrinsically disordered. Intracellular cleavage of this 38-kDa full-length protein at Asp-131 generates an approximately 24 kDa functionally active fragment called caspase-cleaved prostate apoptosis response-4 (cl-Par-4). This study aims to characterize the structure of cl-Par-4 *in vitro* in the presence of various ions. The exquisite sensitivity of Par-4 to environmental conditions may be related to the means by which this protein tips the balance between cell survival and cell death in tumor cells.

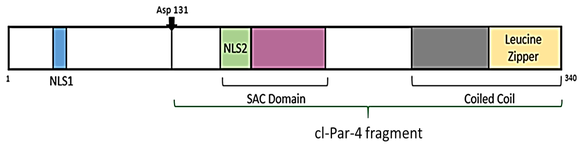

Fig. 1 Full-length Par-4 domain structure and caspase cleavage site. (NLS1: nuclear localization sequence 1; NLS2: nuclear localization sequence 2; and SAC: selective for apoptosis induction in cancer cells)

Objective

To characterize the structure and self-association state of the cl-Par-4 protein *in vitro* in the presence of various ions.

Methods

- recombinant cl-Par-4 was expressed in BL21 *E. coli* cells using the codon-optimized construct of cl-Par-4 in the modified expression vector H-MBP-3C.
- Immobilized metal affinity chromatography (IMAC) was used for the protein purification. Multiple dialyses were used to achieve >99% purity and the desired ionic environment.
- CD spectroscopy, dynamic light scattering (DLS), UV-vis spectroscopy and SDS-PAGE were used to characterize the structure and self-association state of cl-Par-4 *in vitro* in the presence of various concentrations of ions.

Results

cl-Par-4 domain structure and disorder prediction

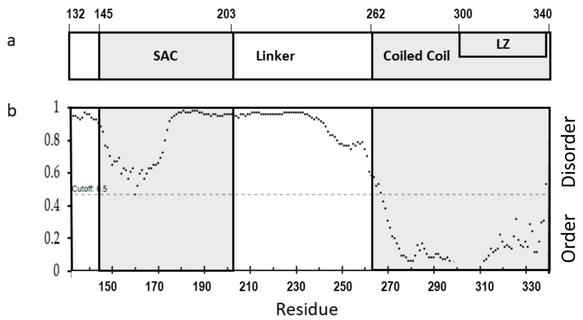

Fig. 2 (a) Domains of cl-Par-4; **(b)** Intrinsic disorder prediction using DISOPRED3.

Cations induce helical CD spectra in cl-Par-4

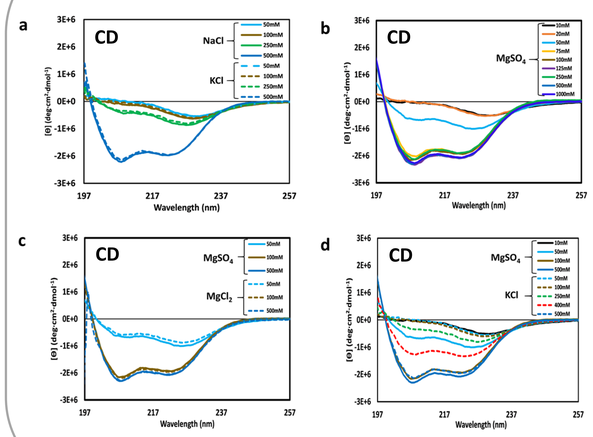

Fig. 3 CD spectra of cl-Par-4 at various concentrations of salts.

Cations reduce cl-Par-4 particle size

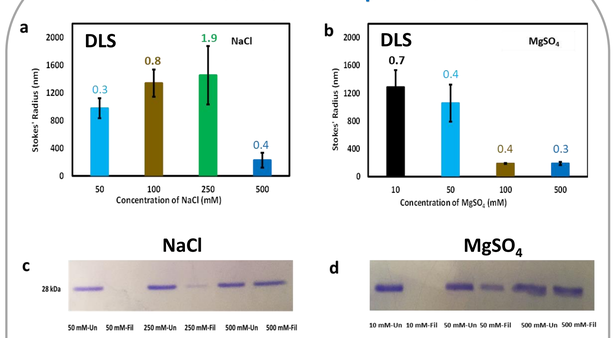

Fig. 4 Stokes' radii of cl-Par-4 particles under the influence of **(a)** sodium and **(b)** magnesium. Polydispersity values are shown above the bars in parts (a) and (b). In panels **(c)** and **(d)**, SDS-PAGE analysis of unfiltered and filtered samples of cl-Par-4 confirm that low salt induces large particle sizes.

Filtration retains small and medium-sized particles, which are highly helical

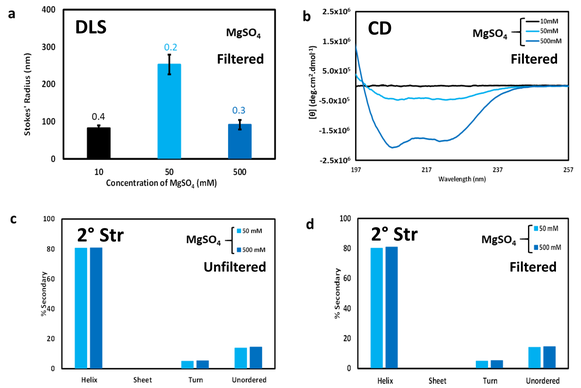

Fig. 5 Effect of filtration on cl-Par-4 samples vs. $MgSO_4$ concentration.

Low salt induces scattering

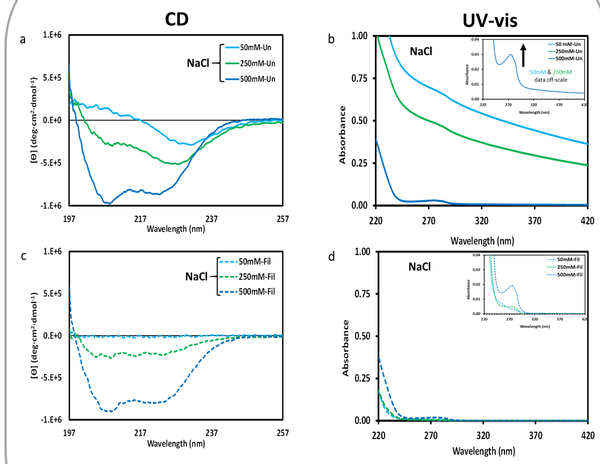

Fig. 6 CD & corresponding UV-vis spectra of cl-Par-4 as a function of NaCl concentration: Unfiltered **(a)** & **(b)**; Filtered **(c)** & **(d)**.

Formation of large soluble aggregates under low salt conditions is reversible

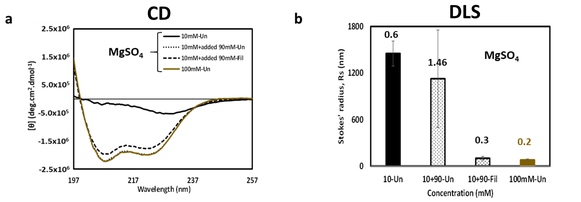

Fig. 7 Reintroduction of salt into a low salt sample of cl-Par-4 produces **(a)** CD and **(b)** DLS results similar to samples that have never been at low salt.

With time, filtered and unfiltered CD spectra converge

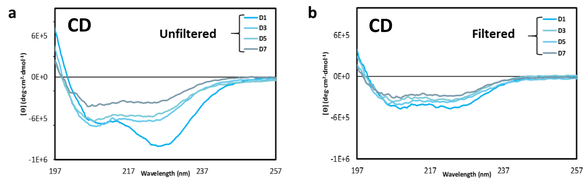

Fig. 8 Time-dependence of CD spectra in the presence of 50 mM $MgSO_4$: Unfiltered **(a)**; Filtered **(b)**.

Conclusions

- Divalent cations exert similar effects on cl-Par-4 structure as monovalent cations, but at approximately five times lower concentration.
- Anion identity shows almost negligible effect.
- At lower concentration of cations, cl-Par-4 forms large soluble aggregates which are not permanent and can be reversed by addition of salt into the solution.

Future works

- Determine the self-association state of cl-Par-4 under combined conditions of altered pH and various salts.
- Determine the three-dimensional structure of cl-Par-4 protein using NMR, SAXS and X-ray crystallography.

References

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Acknowledgement

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