Exploration of Known Disease-Causing VRK1 Mutants

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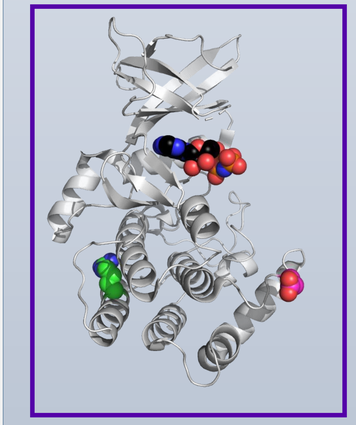
Abstract

VRK1 is a vaccinia-related serine/threonine kinase that contributes to the regulation of cell proliferation. Point mutations within the VRK1 sequence are associated with a variety of complex neurodegenerative disorders which previous research suggests are due to changes in kinase activity and/or structure and stability. Point mutations seen with VRK1 may affect how a tail on the C-terminus of the protein interacts with the protein's active site, possibly altering the ability of the protein to bind to specific substrates.

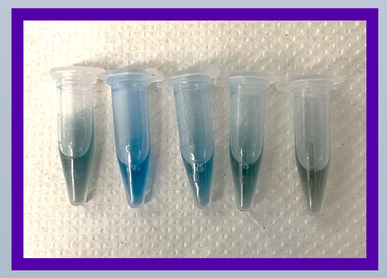
This study looked specifically at the point mutation D263G and its effect on protein stability and ability to bind substrates at its active site. The mutation was produced in a His-tagged VRK1 construct plasmid which was then transformed into *E. coli* bacteria cells, allowing for the purification of the specific mutated VRK1 protein. Experiments were then carried out to determine both the stability of the protein and its substrate binding properties. The stability of the protein was analyzed using circular dichroism and the protein's binding ability was evaluated using differential scanning fluorimetry with ADP and ADP-competitive inhibitors. Results were compared to the wild type VRK1 protein. These experiments are also being extended to other mutations including R321C, which is seen in some patients diagnosed with neuromuscular diseases.

Background



Model of the structure of VRK1 created in PyMOL using PDB ID 6ac9 (Ruff). Gray ribbons: VRK1 backbone. Black spheres: ANP-PNP. Pink spheres: residue Asp263, mutated to Gly in this study. Green spheres: residue Arg321, mutated to Cys in this study.

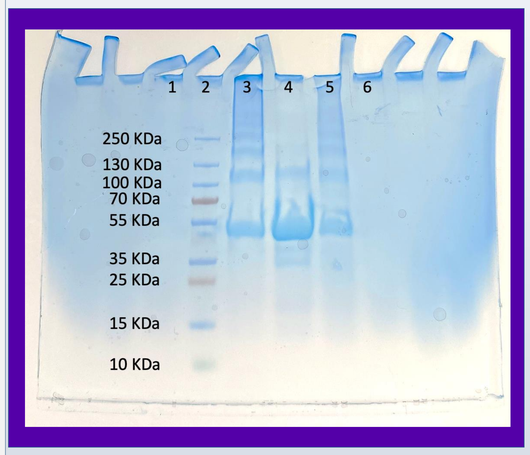
His-tag purification was used to separate VRK1 protein from other cellular components. Charged Ni-NTA resin was used to bind VRK1 by histidine tag. An elution buffer containing high [imidazole] was utilized to remove protein from resin and dialysis was used to remove high levels of imidazole from purified protein.



Quick Bradford assay of five protein elution volumes from the nickel column.

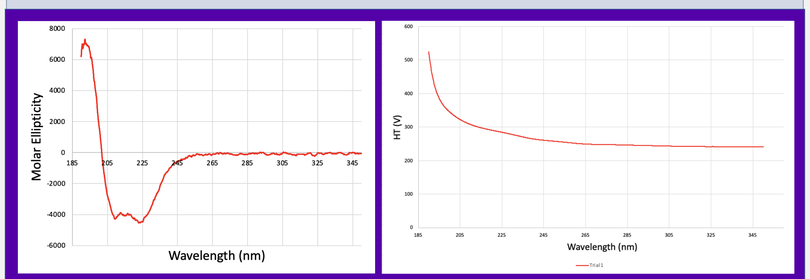
Results

SDS-PAGE

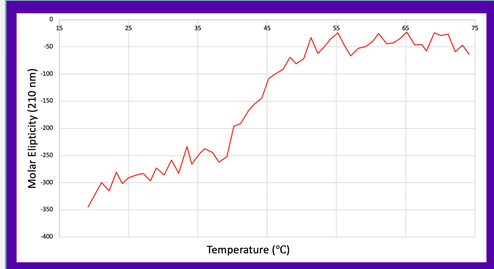


Dark bands indicate the size of protein which can then be compared to the sizes associated with the ladder. Wild Type VRK1 (column 3), D263G (column 4), and R321C (column 5) all appear at about 45.5 kDa, the expected size of VRK1 protein.

Circular Dichroism



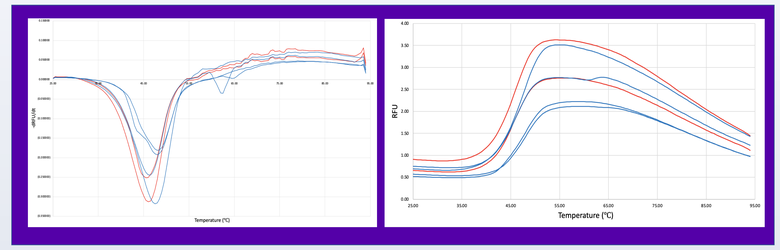
Wavelength scan of the VRK1 D263G protein with corresponding HT voltage of spectrometer from one representative experiment (protein concentration: 0.1 mg/mL). The scan suggests the protein is a mix of alpha helices and beta sheets.



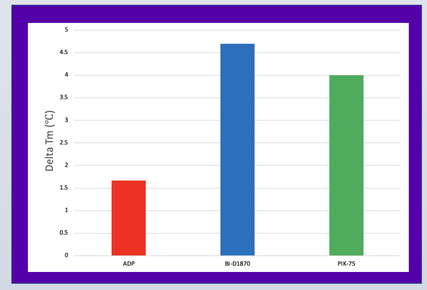
VRK1	Tm Measured by CD (℃)	Tm Measured by DSF (℃)
D263G	41.95	46
Wild Type	48	46

Thermal denaturation scan of D263G from one representative experiment is shown on the left. The table on the right shows the average Tm values for both the D263G variant and the wild type VRK1. 2 independent trials were completed for both the CD and DSF experiments.

Differential Scanning Fluorimetry



DSF experiments with VRK1 D263G with ADP (blue) and without ADP (red). SYPRO Orange dye was used to track unfolding of protein throughout an increase in temperature. Tm values were calculated from the minimum –dRFU/dt values.



Average Delta Tm values for DSF experiments run with ADP or drug inhibitors. (ADP: average of 2 trials, BI-D1870: average of 3 trials, PIK-75: single trial)

Conclusions

- SDS-PAGE indicates that our Ni-NTA purifications of wild-type, D263G and R321C VRK1 were successful and high-yield.
- VRK1 D263G has a lower Tm value than the wild-type enzyme. This suggests the mutant is less stable, even though its structure is similar structure to the wild type VRK1.
- DSF shows D263G binds ADP with a similar affinity to wild-type. It also has relatively high ΔTm values for binding BI-D1870 and PIK-75. This data suggests that the D263G mutant active site may be more pliable for binding either inhibitors or ADP.

Acknowledgements and References

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References

1. Frederick, M., Hurley, D., Mai, E. *et al*. Investigation of Disease-Causing Point Variants of VRK1. (poster) Winona State University, (2022).

2. Martin-Doncel, E., Rojas, A.M., Cantarero, L. *et al.* VRK1 functional insufficiency due to alterations in protein stability or kinase activity of human *VRK1* pathogenic variants implicated in neuromotor syndromes. *Sci Rep* 9, 13381 (2019). https://doi.org/10.1038/s41598-019-49821-7