The Importance of the C2 Domain in VSP

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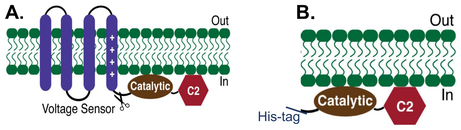
Abstract

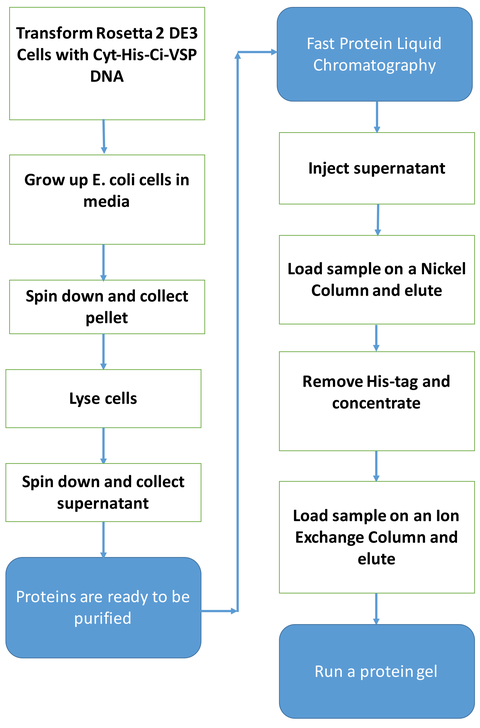
The voltage sensing phosphatase (VSP) is a unique transmembrane protein that uses changes in voltage to dephosphorylate phosphatidylinositol phosphates (PIPs). PIPs regulate many cell processes, including growth, movement, and even death. VSP is made up of three domains, the voltage-sensing domain (VSD), the catalytic domain and the C2 domain. The function of the VSD and catalytic domain is well understood; however, the role of the C2 domain remains a mystery. Recent research in oocyte cells has shown that VSP lacking the C2 domain is not able to dephosphorylate PIPs. C2 domains are known lipid-binding domains and may also have that function in VSP. My research is focused on studying the C2 domain of Ci-VSP. The goal of this work is to test the role of the C2 domain under controlled conditions. The first step is to purify the cytosolic domain of Ci-VSP (wild type) from E. coli, using chromatography techniques. I will then test VSP for its dephosphorylation activity using a standard colorimetic assay. Purified VSP will allow me to properly characterize the catalytic activity of VSP in a detailed way not possible inside a cell. I will use standard molecular biology techniques to delete the C2 domain and this truncated protein will be purified and characterized in the same way as wild type. Using Förster Resonance Energy Transfer (FRET) based methods, VSP and the VSP without the C2 domain will be evaluated for lipid binding properties. This work will assist me to determine the precise function of the C2 domain.

Introduction



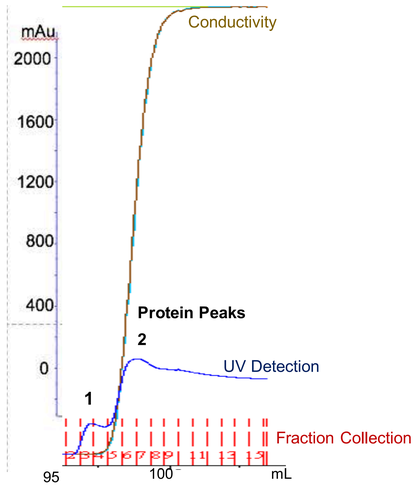
(A) Full Length Ci-VSP. (B) Cytosolic His-tag Ci-VSP. The 6x His-tag is on the N-terminus.

Protein Purification Flowchart

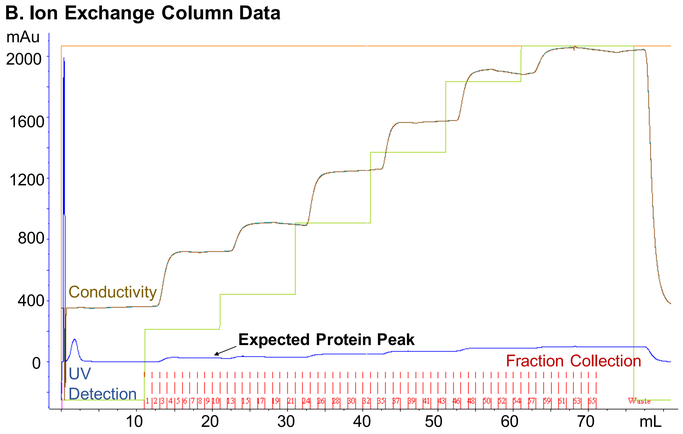


Cyt-His-Ci-VSP Purification Results

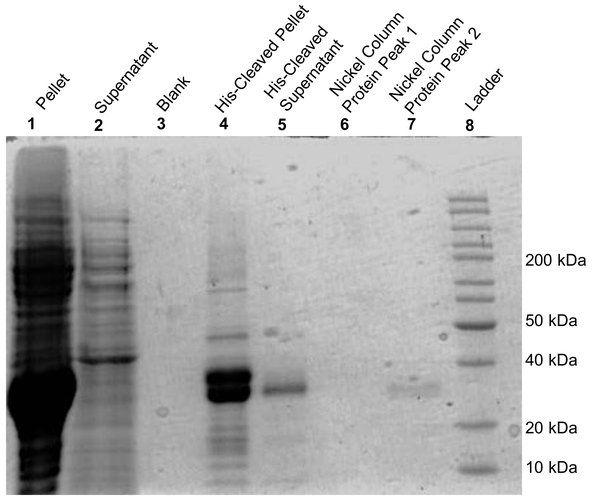
A. Nickel Column Data



B. Ion Exchange Column Data



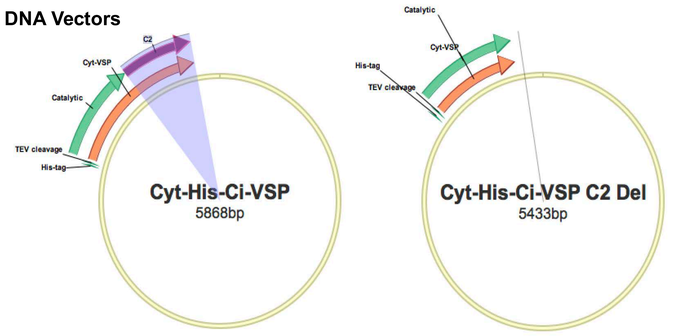
C. SDS PAGE Protein Gel



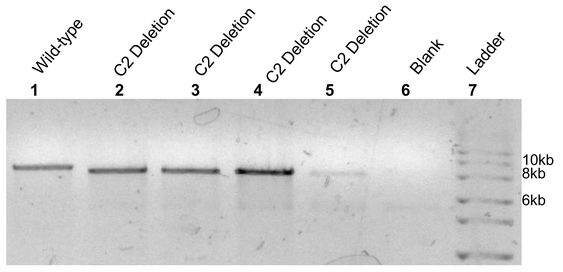
(A) Nickel Column Data. UV Detection was set at 280 nm to detect protein in injected sample. Protein peak 1 is indicative of nonspecific binding, while peak 2 is Cyt-His-Ci-VSP being eluted out of the nickel column. 1 mL fractions of protein peak 2 are collected and concentrated. (B) Ion Exchange Column Data. No protein peak was observed. Possible explanations include protein not eluting out of the column. (C) SDS Page Protein Gel. Protein from lane 2 was used on the nickel column. Protein from lane 5 was run over the ion exchange column. Protein is seen in lane 7 at 38.5 kDa, as expected.

Molecular Biology to Delete the C2 Domain

A. DNA Vectors



B. Agarose DNA Gel



C. DNA Sequences

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Wildtype      aagaattacggaggacaacttcccccgatg
Expected      aagaattacggaggatagtaggcggccgcc
Expermental   aagaattacggagga---taggcggccgcc
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(A) Representation of the C2 domain being deleted out of VSP. The C2 domain is 435 base pairs. (B) Agarose DNA Gel of potential C2 deleted Cyt-His-Ci-VPS. Lane 2, 3, 4, and 5 contain samples of DNA run at different PCR temperatures. These samples can be compared to wildtype DNA (Lane 1). The C2 deleted DNA should be 435 base pairs smaller than wildtype, which is arguably seen in the gel. (C) The DNA of the catalytic domain is green in each sequence. The DNA of the C2 domain is in purple. The stop codon is red. The experimental C2 deletion DNA lacks the stop codon.

Conclusion

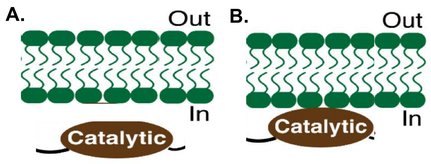
- ✓ The protein purification process is nearly optimized. This process will be used to study the C2 domain of VSP in the future.
- ✓ While the C2 deletion within VSP was unsuccessful, small changes to the primers should result in a successful deletion.

Future Work

This work will benefit future research in identifying the role of the C2 domain in VSP.

Once Cyt-His-Ci-VSP is successfully purified, its ability to dephosphorylate PIPS will be tested using a Biomol Green Malachite Assay. This technique is able to measure free phosphate concentration. VSP will serve as a control when testing the activity of VSP lacking the C2 domain. Purification of C2 deleted Cyt-His-Ci-VSP will be performed similarly and tested for activity. Due to previous oocyte work, it is hypothesized that VSP lacking the C2 domain will not be active.

The lipid-binding properties of purified VSP will be studied and compared to that of VSP without the C2 domain. It is expected that the catalytic domain will be slightly detached from the lipid membrane once the C2 domain is deleted.



(A) Cartoon depicts the hypothesis that the C2 domain has lipid binding properties. (B) Cartoon shows an alternative hypothesis that the catalytic domain has lipid binding abilities, independent from the C2 domain.

If VSP, without the C2 domain, is inactive and shown to have lipid-binding properties, it can be concluded that the C2 domain has a role in catalysis, potentially through orientating the catalytic domain.

References

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