Analysis of the Vir-A Histidine Kinase in Agrobacterium tumefaciens

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Abstract

Agrobacterium tumefaciens is a plant pathogen that causes billions of dollars in plant damage yearly. *A. tumefaciens* uses a transmembrane histidine kinase (VirA) to initiate pathogenesis in response to a chemical signal upon plant wounding. VirA is a protein dimer and contains four major regions: Periplasmic (P), Linker (L), Kinase (K), and Receiver (R). The Linker region initiates a signal when it binds phenols, a class of small molecules that are produced by the host plant. Binding of phenols to the linker region leads to conformational changes at the periplasmic and linker regions of VirA, which is transmitted to the kinase region, where VirA auto-phosphorylates at a conserved histidine residue and transfers this phosphate to the response regulator, VirG. The phosphorylated VirG acts as a transcription factor for the expression of a set of virulence genes (*vir* genes), which incorporate into the genome of the plant, triggering the plant cell to divide uncontrollably into a crown gall tumor.

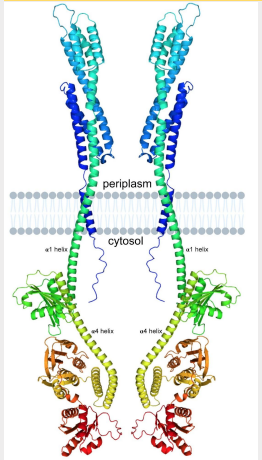
Despite the importance of the phenol-linker region interaction in *A. tumefaciens* pathogenesis, the atomic level insight into how phenols and VirA interact is yet to be elucidated. This study involves protein expression and purification of VirA linker region to obtain soluble proteins for crystallization to elucidate the crystal structure of VirA with and without phenol derivatives.

The VirA linker region 2802447 cloned was expressed in E. coli cells at 20°C, and after reaching an $O.D._{600nm}$ greater than 0.6 were induced with 0.1 mM IPTG. The cells were grown overnight, and were then harvested. The cell pellet suspended in lysis buffer was lysed with the Avestin emulsiflex cell disruptor, followed by centrifugation at 20K rpm, 4°C for 2 hours, after which the supernatant was separated from the pellets and purified manually using a Ni-NTA column. Protein fractions with single band at 18K Da as checked by SDS-PAGE were combined and dialyzed overnight in a buffer containing 50mM Tris, 500mM NaCl, 1mM TCEP, 5% glycerol, pH 7.5. The pure protein (>90%) as judged by SDS-PAGE was saved and used later for crystallization.

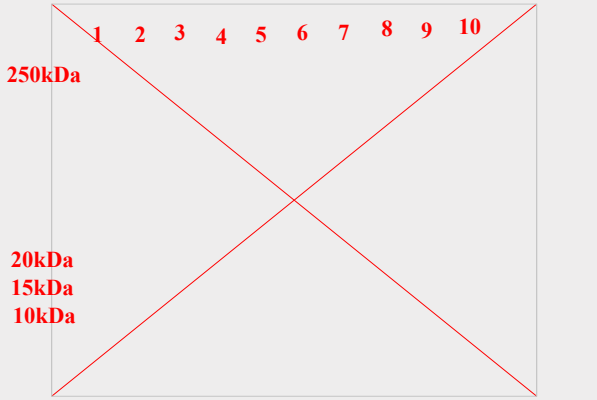
Background

- Agrobacterium tumefaciens is a bacterium which causes crown gall disease on various different plant species by introducing its T-DNA into the host's genome
  - T-DNA transforms the host cell into a tumor cell resulting in large tumor like growths (crown gall) to form on the plants
- Histidine kinases play the role of sensing external environmental changes for organisms such as bacteria, fungi, and plants.
- Vir-A is the histidine kinase in Agrobacterium tumefaciens.
- It serves as the It serves as the initiating transcriptional factor for A. tumefaciens when certain signals such as low pH, sugar, and phenols are received causing tumor like growths to form on the plants.

Results



Model displaying the structure of the Vir-A protein.



1- Marker
2- 150 Mm Imidazole fraction
3- 150 Mm Imidazole fraction
4- 150 Mm Imidazole fraction
5- 150 Mm Imidazole fraction
6- 150 Mm Imidazole fraction
7- 150 Mm Imidazole fraction
8- flowthrough
9- cell lysis
10- pellet

Conclusion/Future Direction

As stated previously the purpose of this experiment was to determine whether the protein Vir-A was being expressed in a pure soluble form. In order for this to be classified as true a single band must be present at 18KDa on the gel. As seen in the image provided on the previous slide of the gel we ran, there is a prominent line at around 18KDa from well 2 which was ran with 150mM Imidazole fraction and from well 10 which was ran with the pellet. This means that in well 2 specifically, there is the expression of the pure, soluble Vir-A protein. Unfortunately wells 3 through 7 which were also ran with 150mM Imidazole fraction showed no results (which could be an error during the run).

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