Synthetic Snake Venom
The Novel Creation of *Crotalid* Phospholipase A2 Using Genetic Engineering

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Introduction

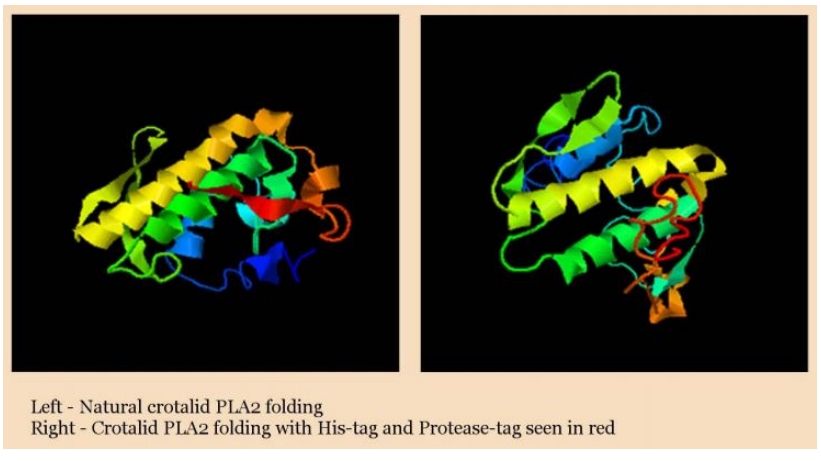
Antivenin is listed as one of the World Health Organization's Essential Medicines, and as such, it is integral to a modern health care system. Antivenin is currently developed through a process of milking venomous animals, in this case, snakes, concentrating the venom, inoculating animals, and isolating antibodies found in their plasma. The aim of this project is to genetically engineer an organism to overexpress common proteins found in snake venom, thereby lowering the cost of antivenin.

Antivenin Manufacturing and Facts

1. Snake Milking - Proteins needed to invoke an immune response are gathered by milking snakes
2. Venom Concentration - Proteins and enzymes are concentrated through freeze-drying
3. Animal Inoculation - Animals are inoculated with small sustained amounts of venom to produce antibodies
4. Antibody Isolation - Antibodies are gathered from the animals plasma and then isolated

One of the most prevalent proteins found in venom is the enzyme Phospholipase A2 (PLA_2). Phospholipase A2's primary function is the breakdown of the cell's phospholipid bi-layer, which causes tissue inflammation and necrosis. Therefore, the production of PLA_2 was the first step in the creation of a synthetic snake venom.

Phospholipase A2 Structure



Design Criteria and Objectives

To genetically engineer an organism, several steps are performed, including: PCR, obtaining the desired DNA, DNA transformation, cell culturing, and DNA extraction. The outlined criteria down below guided our design.

Design Objectives

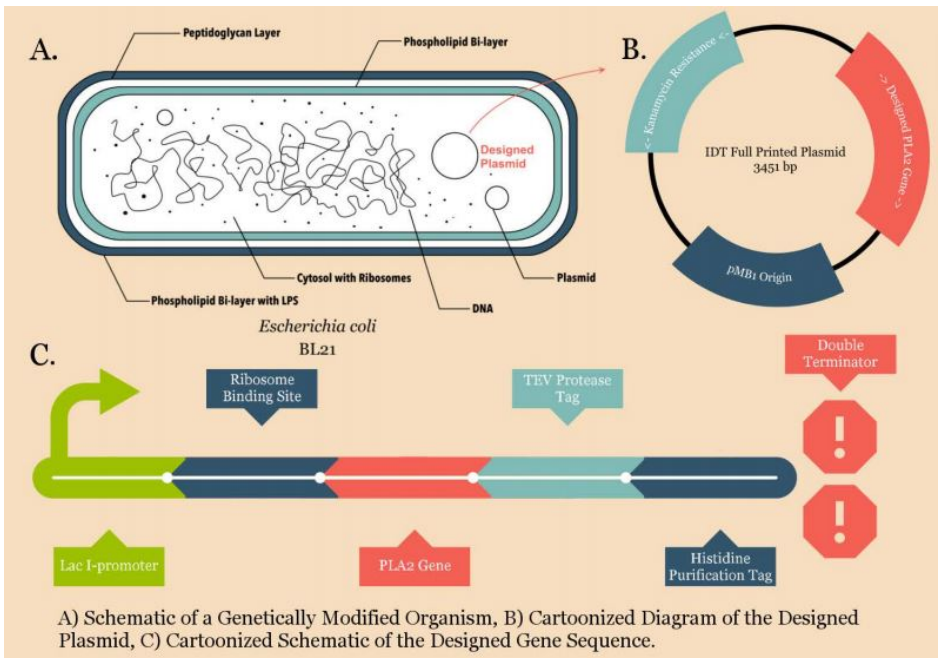
1. Introduce the DNA for PLA_2 into an organism
2. Express PLA_2 production and secretion in an organism
3. Ensure the functionality of PLA_2
4. Evaluate and improve the economic viability of the process

Design Process

Gene Design -- To begin the genetic engineering process, gene parts and an organism would first need to be selected, after which

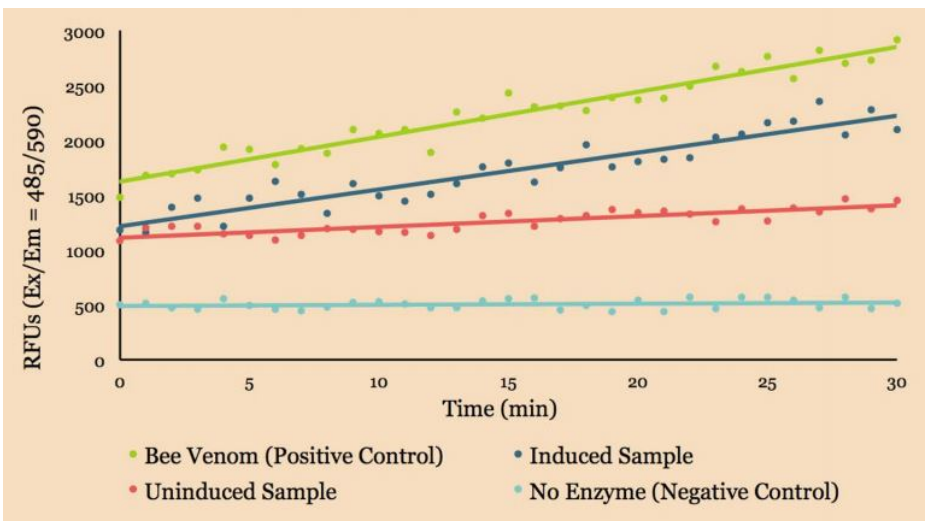
Design and Decision Tree

a genetic construct would need to be made. The design of the recombinant gene began by identifying the components needed for the synthesis and isolation of PLA_2 in addition to the preservation of its activity. Dues to PLA_2's breakdown of phospholipids, it was also necessary to incorporate a promoter so the production of protein could be controlled through induction. After the organism, the BL_{21} strain of *E. coli*, and the genetic parts were selected, a genetic construct was synthesized by IDT. The design of this construct can be seen below.

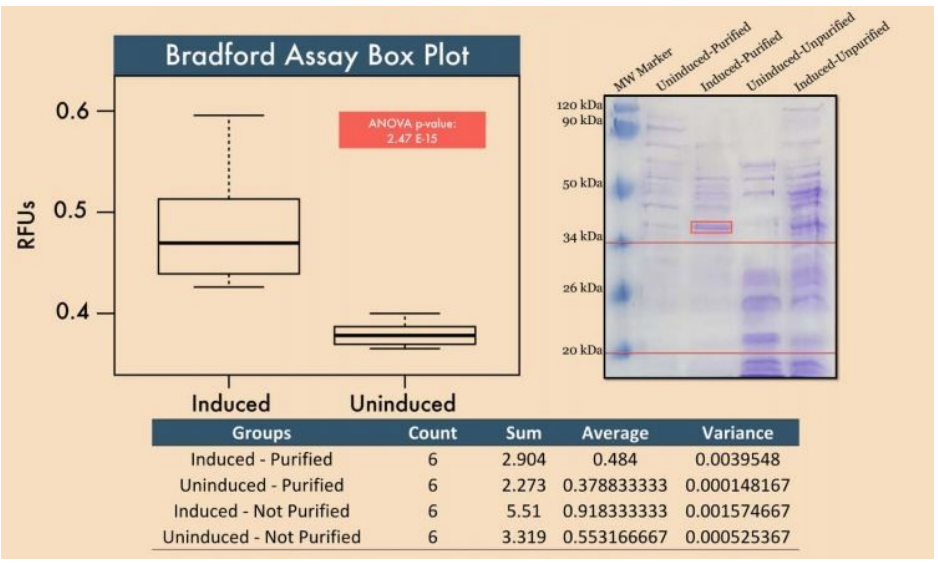


A) Schematic of a Genetically Modified Organism, B) Cartoonized Diagram of the Designed Plasmid, C) Cartoonized Schematic of the Designed Gene Sequence.

Bradford Assay and SDS Page Gel -- A Bradford Assay and a SDS-PAGE Gel were used to measure the overexpression of PLA_2. The results of the Bradford Assay, which measured protein concentration, are shown in the table to the right and graphed in a box plot. An ANOVA analysis of the data returned a p-value of 2.47E-15. PLA_2 weighs approximately 18 kDa which does not have an obvious band, however, upon closer inspection of the SDS-PAGE Gel, a PLA_2 dimer band can be observed at approximately 40 kDa.



Economic Evaluation and Viability -- When produced in a bench-top system, PLA_2 from crotalid venom can be produces for $0.60 per unit. More data is needed in order to scale up this operation, but currently the average purchasing price for PLA_2 is $0.60 per unit. Although crotalid PLA_2 is more expensive, since there is no commercial source for crotalid PLA_2 currently, it is reasonable to expect a much higher retail rate.



Groups	Count	Sum	Average	Variance
Induced - Purified	6	2.904	0.484	0.0039548
Uninduced - Purified	6	2.273	0.378833333	0.000148167
Induced - Not Purified	6	5.51	0.918333333	0.001574667
Uninduced - Not Purified	6	3.319	0.553166667	0.000525367

Activity Assay -- The activity of PLA_2 was measured with EnzCheck Phospholipase A2 Fluorescence Assay. The various samples of expressed protein were compared to a positive control (PLA_2 from bee venom) and a negative control (no PLA_2 enzyme) and observed at different concentrations over time, observed in the graph to the left, illustrates enzymatic activity as PLA_2 breaks down phospholipids into organic acids. The lack of fluorescence change over time for the negative controls shows difference in the enzymatic activity between the samples which were induced and those that were not. this illustrates the success of the design as the induced sample exhibited noticeably more fluorescence than that of the uninduced sample. The fluorescence that remains in the uninduced sample could be attributed to the leaky regulation of the promoter.

Conclusions and Future Work

The final design must be compared to the design criteria determined at the beginning of the design process.

Satisfaction of Design Criteria

1. The final gene was successfully cloned into *E. coli* using molecular cloning standards
2. Overexpression of PLA_2 was achieved in BL_{21} *E. coli*. The concentration was calculated using data from the Bradford Assay and the SDS-PAGE Gel and was found to me 2 mg/L
3. An activity assay showed positive results by exhibiting increasing fluorescence over time, indicating that the synthesized proteins' activity was unaffected by the isolation mechanism
4. The process developed reliably produces one unit of crotalid PLA_2 for $0.60, compared to the industry standard of one unit of PLA_2 (non-crotalid) for $0.60

The overexpression of phospholipase A2 by recombinant assembly and insertion into *E. coli* provides a reliable source for active phospholipase A2. In addition, the recombinant assembly allowed for the creation of an isolation mechanism durable enough to survive sonication, yet small enough not to affect the activity of the product.

The design criteria outlined at the beginning of the design process were satisfied and the product was determined to be economically viable given the small-scale results obtained

Future Work

1. Synthesizing the other prevalent proteins that compose snake venom to create a generic antivenin
2. Create a recombinant assembly that encodes for the enzymes that carry out post translational modifications
3. Purchase antibodies for PLA_2 to check for affinity
4. Move to in-vitro testing of PLA_2 and then to in-vivo testing by large animal immunization