Optimization of Protocol for the Purification of Type I Signal Peptidase (SPaseI)

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Abstract
Type I signal peptidases (SPases) are essential to protein excretion, and inhibiting this protein can lead to cell death. This makes SPase a potential new target for antibiotics. In this research, the purification of SPase I was optimized by changing the sonication protocol. This first step was to use a different resuspension buffer, and then the amount of lysozyme was changed. It was found that 5 mM sodium phosphate and 2 uL/mL lysosome produced the best results.

Introduction

Antibiotics are important to healthcare as they help fight bacterial infections. Within the last few decades, there has been an increasing number of drug-resistant bacteria, which limits treatment options for those strains. One approach to this problem is finding new antibiotic targets, like proteins essential to protein excretion, such as signal peptidases (SPases). SPase I cleaves signal peptides from proteins that are translocated across biological membranes. The inhibition of SPase I can cause the acclamation of secretory proteins, which can lead to cell death.

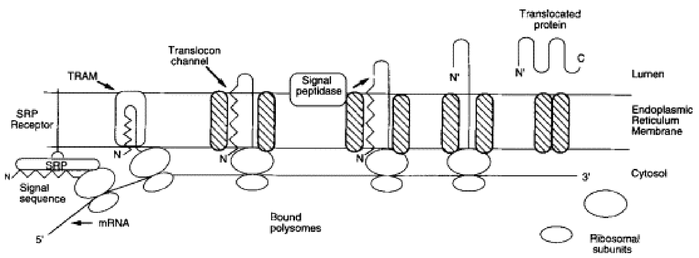

Figure 1: diagram of the release of translocated protein with the aid of signal pepdidase

Methods:

- Overnight growth
- Inoculate bacteria
  - Pre-induction aliquot at OD=0.7
  - 500 uL IPTG added to induce expression
  - Post-induction aliquot taken
  - Centrifuge (20 min, 4°C, 4500 RMP)
  - Resuspend (5mM sodium phosphate buffer)
- Sonication
  - Protocol 1: (1 uL/mL PMSF & lysozyme)
  - Protocol 2: (1 uL/mL PMSF & 2 uL/mL lysozyme)
  - 9:00 min 5 sec on, 10 sec off

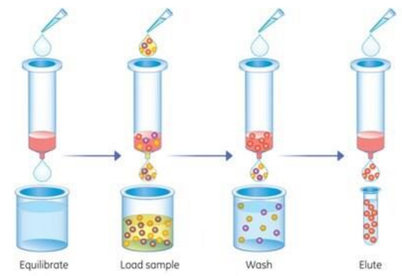

Figure 2: Diagram of a His-Tag purification

- His-Tag Purification
- Gel electrophoresis

Results

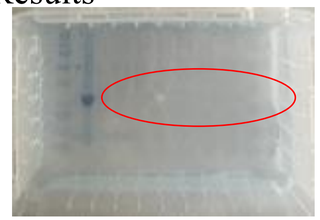

Figure 3: Semester 1 SDS-Page gel of SPase purification. Lanes as follows: Ladder, pre-induction, post-induction, pre-load with imidazole, load without imidazole, then with, wash without, then with imidazole, elutes 1-3 without imidazole then elutes 1-4 with imidazole.

The red circles indicate where the purified protein should/is located

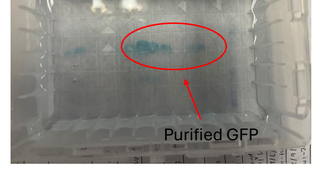

Figure 4: SDS-Page gel of GFP purification with two protocols. Lanes as follows: Ladder, Pre-induction 2/6/24, Post-induction 2/7/24, pre-load, load, wash, elute 1, 2, and 3, pre-load, load, wash, elute 1, and 2.

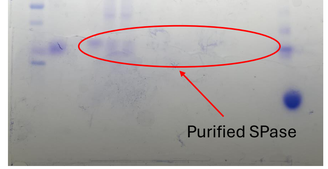

Figure 5: SDS-Page gel of SPase Purification. Lanes as follows: Ladder, GFP standard, Pre induction, Post induction, Preload, Load, Wash, Elute 1-6, lysozyme

Conclusions

- Semester 1 purification: no visible bands showing unsuccessful purification
- GFP purification to optimize protocol
  - 5 mM sodium phosphate is better than 1X Native Purification Buffer
  - 2X lysozyme better than 1X
- Final purification had visible bands after modifications to protocols

Future Directions

- Activity assays to assess catalytic activity

References
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