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Improving Purification Method of the F-plasmid Exclusion Protein TraT

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Introduction

The increased use of antimicrobial agents has given rise to antibiotic resistance among various microbes. As a result, bacteria are becoming harder to kill, thus, creating an environment where disease transmission is more prevalent. Our research focuses on the TraT exclusion protein. Previous research provided evidence of TraT inhibiting conjugation among bacteria via surface exclusion, thus slowing the transfer of antimicrobial resistance genes. Purification of TraT will allow for further examination of its structure and function.

Our group had previously purified soluble domain of TraT using recombinantly added His_6 tag and Ni-Affinity chromatography. When loaded into an SDS PAGE gel this protein migrates at ~25 kDa as expected (Fig.1). However, it also displays bands above and below the main band signifying contamination or degradation.

Project Objective

This project aims to develop steps for further purification of TraT-His_6 via Ion Exchange chromatography.

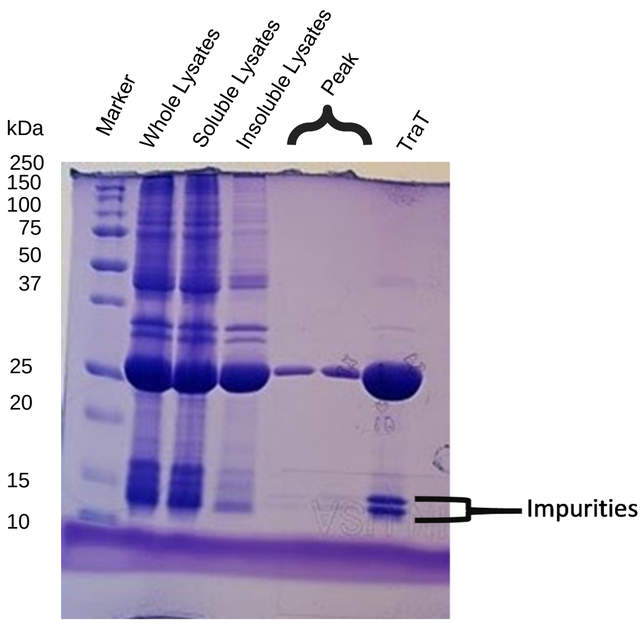

Figure 1: Qualitative analysis of TraT Protein purified via Ni-Affinity Purification. Proteins are separated on SDS-PAGE and stained with Coomassie Blue. Whole lysate is the entire non-purified sample, soluble lysate is the supernatant, insoluble lysate is the resuspended pellet. The peaks are the purified TraT samples and TraT is the entire purified sample.

Approach

FPLC chromatography was used with low and high salt NaCl buffer. %B represents the high salt buffer that was used to elute TraT that is bound to the column. Two ion exchange runs were conducted with anion-exchange (Q) and cation-exchange (S) columns to determine interaction with TraT.

Results

- Cation exchange run results in one TraT peak at 280 nm (orange star) and one TraT peak at 215 nm (blue star). Arrows point to non-binding TraT fraction detected at 215 nm and 280 nm.

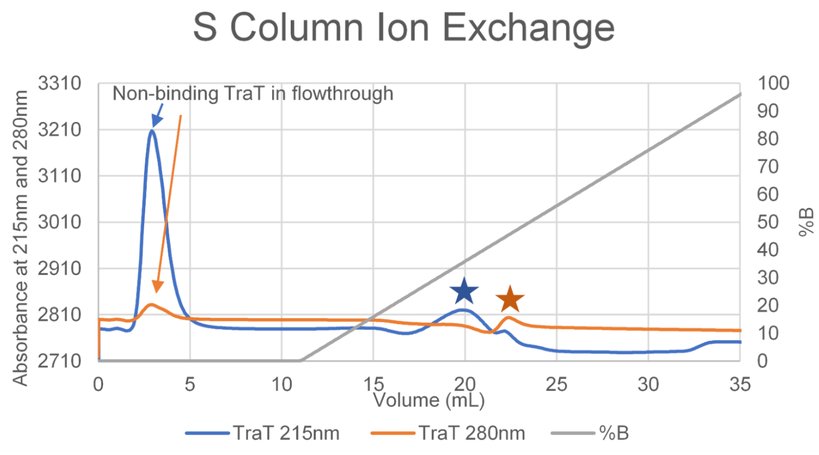

Figure 2: Cation-Exchange Chromatogram of TraT Protein

- Anion exchange run resulting in two TraT peaks at 280 nm (orange stars) and one TraT peak at 215 nm (blue star).

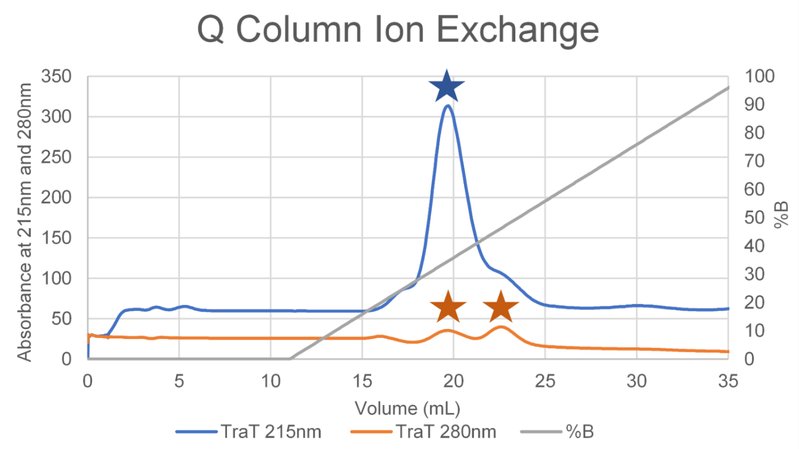

Figure 3: Anion-Exchange Chromatogram of TraT Protein

Summary

- TraT-His_6 protein bound to both S and Q columns. It likely suggests that the protein has portions that exhibit different electrical charges and allow for such binding.
- Separation of the protein into two fractions on both columns also suggests heterogeneity of the initial Ni-affinity purified protein with structural or chemical differences yet to be identified.
- Future research should examine additional purification via size exclusion chromatography that will assess homogeneity and oligomerization state of the TraT protein and allow for further analysis using X-ray crystallography or other structural methods.

Acknowledgements

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