Purification of DtpT Using SMALPs as an Alternative to Detergents

Staphylococcus aureus is one of the most widespread bacterial pathogens and antibiotic resistance is common in most infections. The decreasing efficacy of antibiotics has put pressure on medical professionals and researchers to focus efforts on developing alternative treatments for *S. aureus* infections. The Gee lab studies the peptide transporters of *S. aureus* as potential therapeutic targets. Di- and tri-peptide transporter (DtpT) is a membrane protein present in all genomes of *S. aureus.* DtpT is responsible for importing peptides of 2-3 amino acids in length and plays a key role in the nutrient uptake of *S. aureus*. The purpose of this study is to develop an effective procedure to express and purify SaDtpT, so it can be characterized in future experiments. Working with membrane proteins presents unique difficulties, including potential toxicity when overexpressing in *E. coli*, low solubility, and loss of native protein conformation when extracting with detergents. Our goal is to isolate DtpT without compromising the structure of the protein and with minimal modifications to its sequence. To overcome these challenges, we will be using styrene maleic acid lipid particles (SMALPs) as an alternative to detergents when isolating DtpT. SMALPs are a form of lipid nanodisc that are used to extract membrane proteins while mimicking the structure of the native membrane. By using SMALPs as our solubilizing agent, DtpT will go through minimal environmental stresses, allowing for its structure to be conserved when isolating it from its native membrane. The project described was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under Grant # 5P20GM103427. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NIH.