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## Purification and site mutagenesis in GDP-mannose dehydrogenase

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A current research focus is the biosynthetic pathway used in alginate production by the bacterium *Pseudomonas aeruginosa*. This polysaccharide provides the bacteria with resistance to immune and antibiotic response leading to serious health complications and death in patients, specifically cystic fibrosis patients. This summer's research was centralized on the step in the pathway controlled by the AlgD gene which oxidizes GDP-mannose to GDP-mannuronic acid by the enzyme GDP-mannose dehydrogenase (GMD). In order to characterize this reaction, it is necessary to create mutants of GMD to compare with the wild type. Mutations are created by introducing amino acid substitutions into the wild-type sequence for residues thought to be important for enzyme catalysis. The single site mutations purified thus far include E157Q, C268A, C268S, C268D, and K210A while the double site mutants include E157Q/C268A and E157Q/C268S. The genes for both wild type and mutant GMD have been cloned from *Pseudomonas aeruginosa* and are transformed into *E. coli* which allows for an abundance of protein to be over-expressed. Once the mutants are confirmed, the desired protein is isolated by use of chemical precipitation, heat denaturation, and column chromatography. The purification procedure is confirmed using gel electrophoresis. The purified and concentrated protein can then be used to determine kinetic parameters are then compared to those predetermined for wild type.