Charles Smarr

Major: Biochemistry
University: University of Missouri-Columbia
Faculty Mentor: Dr. Peter Tipton
Mentor Department: Biochemistry
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A kinetic study of active site mutations in GDP-mannose dehydrogenase

Charles Smarr, Mairi Lough and Jennifer Kimmel

In the human pathogenic bacterium *Pseudomonas aeruginosa*, GDP-mannose dehydrogenase (GMD) serves as a critical enzyme in the biosynthesis of alginate, a polysaccharide that functions as a protective coating against antibiotics and immune system responses. Infection is particularly problematic in cystic fibrosis and burn victims, as *P. aeruginosa* uses alginate to form dense, protected colonies that can often be fatal to the host. GMD catalyzes the oxidation of GDP-mannose to GDP-mannuronic acid, using two equivalents of NAD+ as cofactors for the reaction. The gene for GMD, AlgD, has been sequenced and the enzyme has been examined via X-ray crystallography, allowing for an analysis of mutations of specific amino acid residues within the active site of the enzyme. Kinetic studies of single and double mutations of cysteine and glutamate residues within the active site, C268 and E157, respectively, have been carried out using ultraviolet spectrophotometry and fluorimetry. These mutations have been characterized and compared with wild-type enzyme, using Michaelis-Menten and Hill analysis to determine the effect of the mutated residue on specific activity, substrate binding and inhibition, and the allosteric effects of GMP, a known inhibitor of wild-type GMD. Through analysis of these mutations, a more thorough understanding of the structure of GMD and the mechanism by which it acts can be reached. In conjunction with further characterization of C268 and E157, a study of other potential active site residues will give insight into the mechanism of GMD and potentially allow for work to begin on a functional inhibitor of this committed step in the biosynthetic pathway of bacterial alginate.