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Structural characterization of enzyme-ligand complexes of GDP-Mannose dehydrogenase from *P. aeruginosa*

The enzyme GDP-mannose dehydrogenase (GMD) from *Pseudomonas aeruginosa* catalyzes the committed step in the synthesis of the exopolysaccharide alginate. Alginate is a major component of *P. aeruginosa* biofilms that enable the organism to survive under harsh environmental conditions, and also protect it from antibiotic therapy. GMD catalyzes the NAD⁺ dependent, 4-electron oxidation of GDP-mannose to GDP-mannuronic acid, which acts as the donor to the homopolymeric alginate precursor. Previously our laboratory has determined the crystal structure of GMD at 1.55 Å in ternary complex with its cofactor NAD(H) and product GDP-mannuronic acid. The structure provided valuable three-dimensional information on the enzyme's active site and contacts between the protein and its cofactor and product. However, important questions regarding GMD remain, including the structural basis for the enzyme's ability to catalyze two different oxidations in a single active site, and the identification of residues involved in the formation of the proposed enzyme-intermediate complexes. To address these questions, we are characterizing several GMD-ligand complexes by X-ray crystallography. Site-directed mutants based on the crystal structure have been produced and characterized kinetically. These mutant GMD proteins have been used in co-crystallization experiments to capture "snapshots" of the individual steps of the reaction. Structural characterization of these complexes is underway and will illuminate the multi-step reaction mechanism of GMD. They will also serve as templates for efforts at structure-based inhibitor design that could help fight *P. aeruginosa* infections.