Solving the Structure of Light-Dependent POR

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There are few known, naturally occurring enzymes that are directly induced by light. Two such enzymes exist, one of which is Light-Dependent Protochlorophyllide Oxio-Reductase or LPOR. Research has been done on the general functionality of LPOR; however its crystal structure remains unsolved. Hence, the purpose of this project is to produce, purify, crystallize and solve the structure of LPOR and later make a time-resolved model of the protein in real time. LPOR is responsible for the conversion of protochlorophyllide (pChlide) to chlorophyllide – an important step in producing chlorophyll. During the conversion, a single photon of light breaks the double bond between the 17th and 18th carbons of pChlide allowing NADPH and two amino acid residues to bind to the structure. LPOR contributes the two amino acid residues -Tyrosine and Lysine—to the pChlide and holds the pChlide in place with the NADPH. Thus far in this project, the protein's synthesis has been optimized using nonpathogenic E. Coli, and the substrate (pChlide) has been produced using Rhodobacter bacteria. A protein-substrate activity assay was performed, but the reaction did not occur. Further experimentation is being done to optimize the assay. Crystallization and analysis of the structure will take place once the assay has been carried out and the protein can be combined with its substrate.