Final Report

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Project Title: NADPH: Protochlorophyllide Oxidoreductase – Structure, Catalytic Function, and Role in Prolamellar Body Formation and Morphogenesis

Abstract

The biosynthesis of chlorophyll is a critical biochemical step in the development of photosynthetic vascular plants and green algae. From photosynthetic bacteria (cyanobacteria) to algae, non-vascular plants, gymnosperms and vascular plants, mechanisms have evolved for protochlorophyllide reduction a key step in chlorophyll synthesis. Protochlorophyllide reduction is carried out by both a light-dependent (POR) and light-independent (LIPOR) mechanisms. NADPH: protochlorophyllide oxidoreductase (EC 1.3.1.33, abbreviated POR) catalyzes the light-dependent reduction of protochlorophyllide (PChlide) to chlorophyllide (Chlide). In contrast, a light-independent protochlorophyllide reductase (LIPOR) involves three plastid gene products (chlL, chlN, and chlB) and several nuclear factors. Our work focused on characterization of both the POR and LIPOR catalyzed processes.

Summary of Research Activities

Our work this past year has focused on further characterization of both the POR and LIPOR catalyzed processes. Using bacterially- (*E. coli*) over-expressed of wild-type and mutant POR proteins, we have carried out studies aimed at defining the residues/domains within POR required for PChlide and NADPH binding and formation of the photoactive POR-PChlide-NADPH ternary complexes within the PLB. We have identified several histidine residues important in determining substrate orientation inside POR-NADPH-PChlide ternary complexes with their removal suppressing formation of the enzyme photoactive state. We have also investigated the role of the unique 35 residue loop in POR in modulating POR::POR interactions and POR assembly in the prolamellar body (PLB).

We have also continued to pursue the crystallization of POR. The studies described above, which are aimed at understanding the factors affecting ternary complex stability, were initiated in an attempt to alleviate what we perceived were stability issues of the complex which compromised our attempts at crystallization of the wild type POR protein. To date we have not been able to identify conditions capable of growing all but small crystals too fragile to allow diffraction analysis using the recombinant pea POR. We continue to pursue these studies in earnest although now looking at other sources of the enzyme as potential starting material.

Our work using subfemtomole MS and MS/MS peptide sequence analysis and nano-HPLC Micro-ESI Fourier Transform Ion Cyclotron Resonance mass spectrometry to build a proteome map of the PLB are also ongoing. Our goal is to generate a comprehensive profile of all proteins and small peptides present in PLBs and identifiable by mass spectroscopic analysis. With this information we hope to identify proteins that interact directly with POR in the formation of

the PLB or participate in the regulation of plastid morphogenesis during photoinduced development.

We established conditions for the routine separation of highly purified PLB fractions from corn etioplasts and have done limited analysis of sequences using various available databases, pattern and profile searches. Among the most abundant components are peptides associated with POR (highly expected) the ATP synthase complex (also expected) several photosynthetic reaction center and electron transport chain components and a number of proteases and lipid-transfer molecules. Among the proteins that we are pursuing is a homolog of the maize non-specific lipid transfer protein precursor (nsLTP). Non-specific lipid-transfer proteins (nsLTPs) are involved in the movement of phospholipids, glycolipids, fatty acids, and steroids between membranes. Several structures of plant nsLTPs have been determined both by X-ray crystallography and nuclear magnetic resonance. However, the detailed structural basis of the non-specific binding of hydrophobic ligands by nsLTPs is still poorly understood. We are exploring whether this protein is essential for PLB dispersal and membrane rearrangement during early stages of etioplast to plastid conversion.

Recently a paper describing the proteome analysis of the rice etioplast was published by Zychlinski et al. (Molecular & Cellular Proteomics 4.8, 2005). This provides a very nice comparison to out work. We are also exploring the use of MALDI-TOF mass spectrometry and microsequencing to identify protein modification sites and sites involved in the *in vivo* and *in vitro* degradation of POR.

In addition to the studies outlined above on structure-function relationships in POR, a portion of the funds provided by the DOE contributed significantly to supporting the PhD work of Shengping Zhang. Shengping is examining the role of nuclear genes in the assembly and function of the light-independent protochlorphyllide reductase (LIPOR) in *Chlamydomonas*. Her studies have employed insertional mutagenesis tagging and complementation cloning as a means of identifying additional *yellow-in-the-dark* or *y*-mutants whose gene products serve to regulate the assembly or activation state of the multisubunit comples formed by the chlL, chlN, and chlB enoded proteins. In her screen, Shengping recovered approximately 28 *yellow-in-the-dark* mutants 12 of which have been fully or partially characterized to date. Among these insertion mutants, several have been shown to be associated with previously known *y*-genes (i.e., y-1 on llinkage group (LG) XVII, y-5, y-10, and y-6 on LG I, y-7 and y-8 on LG III and y-9 on LG II), whereas others map to novel locations in the Chlamydomonas genome. Among the novel *y*-mutants discovered is y-11 on LG III that encodes a protein with both VWA and ATP hydrolysis domains. Homologs of the y-11 encoded protein are found in cyanobacteria (*Anabaena* and *Synechocystis*) and photosynthetic bacteria.

Insertional mutant 51 maps to the region containing y-5 and y-10. Within the region encompassed by scaffold 1 (BACs 8h3 and 1N21) are 5 genes, several of which appear to be likely candidates for involvement in LIPOR activity based upon their proposed function. We are currently attempting to confirm the role of these gene products. In this same region we also identified the gene affected in the *Chlamydomonas reinhardtii* Ac-14 mutant. Ac-14 encodes a novel cyclic nucleotide phosphodiesterase which upon disruption cause the cell to be non-photoautotrophic, acetate requiring.

Students trained (Full or partial support from DOE award)

Shengping Zhang (PhD, University of Virginia, 2007) *Identification of Novel "yellow-in-the-dark" mutants in Chlamydomonas reinhardtii.*

Ian J. Carleo (UVA 2009) *"Use of insertional mutagenesis tagging of y-loci and their confirmation by BAC complementation"*.

Papers in Preparation from these studies:

Title: Identification of Novel "yellow-in-the-dark" Mutants in *Chlamydomonas reinhardtii*
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