Metabolic Engineering of Starch for Enhanced Plant Growth and Productivity

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ADP-glucose pyrophosphorylase catalyzes a dominant control step in starch biosynthesis in both photosynthetic tissues and reserve organs. In my laboratory we are attempting to understand the structure-function of this enzyme at both the biochemical and physiological levels and to apply this knowledge to engineer plants as a renewable energy source. Previous biochemical-genetic studies have identified residues in both the large and small subunits that affect the binding affinities of substrates and allosteric effectors. Although only the small subunit possesses catalytic capacity, co-expression studies of various large and small subunit mutant combinations showed that the enzyme's regulatory and kinetic properties are not simply additive but, instead, a product of synergy between the two subunit types. Mutagenesis studies identified a specific peptide region of the N-terminal region of the large subunit that modulated allosteric regulatory and catalytic properties. Specifically, mutagenesis of three proline residues located between residues 44 to 66 had a pronounced effect on the allosteric regulatory or catalytic properties of the enzyme. These studies are aided by the development of new vector-host expression system which allowed the co-expression of stably produced subunits using a single inducer, IPTG. These improvements in bacterial expression have aided biochemical studies on structure-function aspects of this enzyme as well as ongoing attempts to obtain highly diffractible crystals for structural elucidation. Several large subunit mutant genes have been introduced into Arabidopsis thaliana TL-46, which contains a missense mutation in the large subunit gene. After backcrossing to TL-46 to eliminate somaclonal variation, stable lines were obtained and evaluated using several physiological parameters. In nearly all instances, these lines showed higher photosynthetic capacity (higher $CO_2$ assimilation rates and/or higher $O_2$ sensitivity), and higher growth rates. Similar studies have been conducted in rice. Field-grown rice plants, expressing a regulatory large subunit in leaf tissue, showed higher productivity and seed yields in two growing seasons. Overall, these results support the view that leaf starch is an important sink to accommodate excess photosynthate and, thereby, lessen photosynthetic feedback.