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Final Technical Report

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Grant Number: DE-FG02-93ER20121

Title: Structure-Function Relationships of ADP-Glucose Pyrophosphorylase and Branching Enzyme: ;Manipulation of their Genes for Alteration of Startch Quality and Quantity.

Project Period: 08/01/1993 – 07/31/2005

Conversion of the Potato tuber ADP-glucose Pyrophopshorylase Regulatory Subunit into a Catalytic Subunit. ADP-glucose synthesis, a rate-limiting reaction in starch synthesis, is catalyzed by ADP-glucose pyrophosphorylase (ADPGlc PPase). The enzyme in plants is allosterically activated by 3-phosphoglycerate (3PGA) and inhibited by inorganic phosphate (Pi) and is composed of two subunits as a heterotetramer□2□2. Subunit□ is the catalytic subunit and subunit□ is designated as the regulatory subunit.The□ subunit increases the affinty of the activator for the catalytic subunit. Recent results have shown that the subunits are derived from the same ancestor subunit as the regulatory subunit can be converted to a catalytically subunit via mutation of just two amino acids. Lys44 and Thr54 in the large subunit from potato tuber were converted to the homologous catalytic subunit residues, Arg33 and Lys43. The activity of the large subunit mutants cannot be readily tested with a co-expressed wild-type small (catalytic) subunit because of the intrinsic activity of the latter. We co-expressed the regulatory-subunit mutants with SmallD145N, an inactive S subunit in which the catalytic Asp145 was mutated. The activity of the small (catalytic) subunit was reduced more than three orders of magnitude. Coexpression of the L subunit double mutant LargeK44R/T54K with SmallD145N generated an enzyme with considerable activity, 10% and 18% of the wildtype enzyme, in the ADP-glucose synthetic and pyrophosphorolytic direction, respectively. Replacement of those two residues in the small subunit by the homologous amino acids in the L subunits (mutations R33K and K43T) decreased the activity one and two orders of magnitude. The wild-type enzyme and SmallD145NLargeK44R/T54K had very similar kinetic properties indicating that the substrate site has been conserved. The fact that only two mutations in the L subunit restored enzyme activity is very strong evidence that the large subunit is derived from the catalytic ancestor. Previous results showed that Asp145 in the small subunit of the wild-type is essential for catalysis, whereas the homologous Asp160 in the Large WT subunit is not. However, in this study, mutation D160N or D160E in the LK44R/T54K subunit abolished the activity, which shows the ancestral essential role of this residue and confirms that the catalysis of SmallD145NLarge K44R/T54K occurs in the L(□ subunit. A phylogenetic tree of the ADP-Glc PPases present in photosynthetic eukaryotes also sheds information about the origin of the subunits. The tree showed that plant Small and Large subunits can be divided into two and four distinct groups, respectively. The two main groups of S subunits are from dicot and monocot plants, whereas Large subunit groups correlate better with their documented tissue expression. The first Large-subunit group is generally expressed in photosynthetic tissues and comprises Large subunits from dicots and monocots. Group II displays a broader expression pattern, whereas groups III and IV are expressed in storage organs (roots, stems, tubers, seeds). Subunits from group III are only from dicot plants, whereas group IV are seed-specific subunits from monocots. These last two groups stem from the same branch of the phylogenetic tree and split before monocot and dicot separation. Thus few as two mutations turned the L subunit from *Solanum tuberosum* catalytic, showing that L and S subunits share a common catalytic ancestor, rather than a non-catalytic one. The L subunit evolved to have a regulatory role, lost catalytic residues more than 130 million years ago before monocots and dicots diverged, and preserved, possibly as a byproduct, the active site domain.