Enhancement of Photoassimilate Utilization by Manipulation of the ADPglucose Pyrophosphorylase Genes

DoE Grant No. DE-FG06-87ER13699

Thomas W. Okita

PROGRESS REPORT

Overview - The long term goal of this project is to assess the feasibility of increasing the conversion of photosynthate into starch via manipulation of ADPglucose pyrophosphorylase, a key regulatory enzyme in starch biosynthesis. In developing storage tissues such as cereal seeds and tubers, starch biosynthesis is primarily regulated by the gene activation, expression, and allosteric regulation of ADPglucose pyrophosphorylase, as well as starch synthase, and branching enzyme. During the last year we have elucidated the structure of both subunits which compose this tetrameric enzyme and determined the temporal and spatial expression of the genes encoding each subunit as well as their correlation to starch biosynthesis. Genomic clones to both subunits have also been isolated and the gene structure of the small subunit determined. Transgenic potato plants have been produced containing deletions of the small subunit promoter. Currently, cis acting elements and their involvement in spatial and temporal expression are under investigation.

Analysis of the Potato Tuber ADPglucose Pyrophosphorylase Enzyme Structure - To gain insight into the structure/function relationship of the plant ADPglucose pyrophosphorylase we have elucidated the heterotetrameric structure of the potato tuber enzyme (1) and have isolated near-full-length cDNA clones to the small subunit as previously reported (2). Recently, we have isolated and characterized a near-full-length cDNA clone to the large subunit of the tuber enzyme and have derived the missing N-terminal coding sequence of the small subunit from its corresponding genomic clone. Analysis of the primary sequences revealed some interesting findings on possible structure/function relationships of the plant enzyme as summarized below. A more complete description can be found in the appended *Plant Molecular Biology* manuscript (appendix 1).

1. A comparison of the N-terminal sequence of the potato tuber small subunit to the published primary sequence of the

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

This putative transit peptide contains all the general features associated with a plastid targeting sequence. It has a net positive charge, is rich in small hydrophobic and hydroxylated residues, and is deficient in acidic amino acids.

2. Alignment of the small and large potato tuber subunit primary sequences revealed a 52% identity overall. The potato small subunit showed a 91% identity to the rice endosperm small subunit and a 93% identity to the spinach leaf small subunit. The potato tuber large subunit exhibited a 58% and 61% sequence identity to the wheat and maize endosperm large subunits respectively. There appears to be less identity between the two subunits from the same species than between similar subunits from the different plants. The identity studies also suggest that the small subunit is more conserved among the different plants and the larger subunit more divergent. These two observations correlate with the immunological data accumulated thus far (1,3,4). Our results along with another recent homology study (14) suggest that the large and small subunit genes probably arose from a duplication of a common ancestor gene with subsequent divergence.

3. A comparison of the deduced amino acid sequences of the small and large potato tuber subunits to the *E. coli* subunit displayed an overall identity of 37% and 35% respectively. Despite this low overall homology, the domains which are important for allosteric and catalytic activity of the bacterial enzyme (5) are conserved. Since the bacterial and plant enzymes catalyze the same reaction their structural dissimilarities most likely reflect their differences in allosteric specificity. Morell et al. (6) have shown that pyridoxal phosphate (PLP) which mimics the effector molecule 3PGA, equally labels both subunits of the spinach enzyme. For the spinach small subunit, this PLP binding region has been determined and the reactive lysine residue identified. This lysine and surrounding region is identically conserved in the small subunits of potato, rice, and spinach. This region is also highly conserved in the potato tuber large subunit. These observations suggest that the allosteric region of the plant enzymes contains an essential peptide sequence located in its C-terminus rather than in its N-terminus as in the bacterial enzyme (5). The differences in allosteric specificity could also be a result of amino acid changes in other regions of the plant enzyme. Lee et al. (7) and Kumar et al. (8) have shown when Gly336 of the bacterial enzyme is changed to an acidic residue, the bacterial enzyme becomes more refractive towards allosteric activation by fructose 1,6-P₂. It is interesting to note that the amino acid at the equivalent position in the potato, rice, and spinach small subunits are also negatively charged.

Isolation and Structural Elucidation of the Potato Pyrophosphorylase Genes - As mentioned in the previous section cDNA clones to both potato tuber subunits have been isolated and characterized. Using various fragments of each cDNA allowed the isolation of genomic clones to both the small and large subunits from a Lambda EMBL-3 genomic library. The structure of the gene encoding the small subunit has been recently determined by nucleotide sequencing. A comparison of the small subunit genomic and cDNA sequences revealed a complex gene structure, spanning well over 5 kilobases and consisting of 9 exons and 8 introns. Exons ranged in size from 100-400 base pairs and introns from 90-900 base pairs. Such a complex structure is also evident in the gene encoding the rice endosperm ADPglucose pyrophosphorylase small subunit which consists of 10 exons and 9 introns (9). In addition to the high degree of identity between the potato and rice small subunits, further evidence for an evolutionary relationship can be seen in an alignment of the rice and potato genomic sequences which revealed some differences in intron length but showed conserved splice site junctions within their respective coding region. The only exception being intron 9 of the rice gene which is inserted 3 amino acids from the stop codon in a region not contained in the potato gene. The complexity of the pyrophosphorylase genes is also shared by two other genes that encode enzymes involved in starch biosynthesis. These genes, one for sucrose synthase and the other for granule bound starch synthase, display complex gene structures possessing 16 and 14 exons respectively. Whether the presence of a large first intron and the multiple introns contained within the pyrophosphorylase genes are involved in gene expression as observed for the sucrose synthase and granule bound starch synthase genes is unknown and presently being explored.

The intron splice sites of the small subunit were examined for conformity to the GT/AG rule as well as splice site consensus sequences. From this examination, consensus sequences were derived from the 5' and 3' splice sites and were very similar to those described by Brown (10). The region upstream of the first intron was examined for the conserved CAAT and TATA consensus sequences. A putative CAAT box is positioned at -56 and the putative TATA box at -26 bp from the transcriptional start site which was determined by primer extension.

ADPglucose Pyrophosphorylase Gene Expression - The developmental expression of the genes encoding the rice (9), wheat (11), and now potato ADPglucose pyrophosphorylases have been examined. Results from preliminary experiments indicate

that the pattern of mRNA accumulation closely follows the level of pyrophosphorylase activity and starch accumulation. These observations suggest that these genes are controlled transcriptionally. Northern blot and Western blot data (2) indicate that the same potato small subunit gene is expressed in both leaf and tuber. In contrast, Krishnan et al. (12) and Olive et al. (13) have shown in rice and wheat that there are different genes encoding the same type subunit which are expressed either in leaf or endosperm tissue. Recently, we reinvestigated our earlier results using potato plants transformed with constructs containing the potato tuber small subunit promoter transcriptionally fused to B-glucuronidase (GUS). High activity was seen via histochemical and fluorometric assays in tuber tissue, but very little or no activity was evident in leaf tissue. This preliminary result suggests that there maybe tissue specific forms of the small subunit, as seen with the rice and wheat enzymes, that share a high degree of homology at both the nucleotide and amino acid levels. Further experiments will have to be conducted before any firm conclusion can be drawn.

A number of 5' deletions constructs of the potato tuber small subunit promoter have been made, using exonuclease III, and transcriptionally fused to a GUS reporter gene. A stable transformation system has been established which has allowed the production of a number of transgenic potato plants as indicated by both GUS assays and Southern blots. Currently, cis elements and their involvement in spatial and temporal expression are under investigation.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

1. Okita, TW, Nakata, PA, Anderson, JM, Sowokinos, J, Morell, M, Preiss, J: The subunit structure of potato tuber ADPglucose pyrophosphorylase. *Plant Physiol.* 93:785-790 (1990).
2. Anderson, JM, Okita, TW, Preiss, J: Enhancing carbon flow into starch: The role of ADPglucose pyrophosphorylase. In: Vayda, ME, Park, WD (eds) "The Molecular Biology of the potato" pp 159-180. C.A.B. International, Wallingford (1990).
3. Preiss, J, Ball, K, Hutney, J, Smith-White, B, Li, L, Okita, TW: Regulatory mechanisms involved in the biosynthesis of starch. *Pure and Appl. Chem.* 63:535-544 (1991).
4. Preiss, J, Danner, S, Summers, PS, Morell, M, Barton, CR, Yang, L, Nieder, M: Molecular characterization of the *Brittle-2* gene effect on maize endosperm ADPglucose pyrophosphorylase subunits. *Plant Physiol.* 92:881-885 (1990).
5. Baecker, PA, Furlong, CE, Preiss, J: Biosynthesis of bacterial glycogen: Primary structure of *Escherichia coli* ADPglucose synthetase as deduced from the nucleotide sequence of the *glg C* gene. *J. Biol. Chem.* 258:5084-5088 (1983).
6. Morell, M, Bloom, M, Preiss, J: Affinity labeling of the allosteric activator site(s) of spinach leaf ADPglucose pyrophosphorylase. *J. Biol. Chem.* 263:633-637 (1988).
7. Lee, YM, Kumar, A, Preiss, J: Amino acid sequence of an *Escherichia coli* ADPglucose synthetase allosteric mutant as deduced from the DNA sequence of the *glg C* gene. *Nucl. Acids Res.* 15:10603 (1987).
8. Kumar, A, Ghosh, P, Hill, MA, Preiss, J: Biosynthesis of bacterial glycogen: Determination of the amino acid changes that alter the regulatory properties of a mutant *Escherichia coli* ADPglucose synthetase. *J. Biol. Chem.* 264:10464-10471 (1989).
9. Anderson, JM, Larson, R, Landecia, D, Kim, WT, Morrow, D, Okita, TW, Priess, J: Molecular characterization of the gene encoding a rice endosperm specific ADPglucose

pyrophosphorylase subunit and its developmental pattern of transcription. *Gene* 97:199-205 (1991).

10. Brown, JWS: A catalogue of splice site junctions and putative branch-point sequences from plant introns. *Nucl. Acid Res.* 14:9549-9559 (1986).
11. Reeves, CD, Krishnan, HB, Okita, TW: Gene expression in developing wheat endosperm. Accumulation of gliadin and ADPglucose pyrophosphorylase in RNAs and polypeptides. *Plant Physiol.* 82:34-40 (1986).
12. Krishnan, HB, Reeves, CD, Okita, TW: ADPglucose pyrophosphorylase is encoded by different mRNA transcripts in leaf and endosperm of cereals. *Plant Physiol.* 81:642-645 (1986).
13. Olive, MR, Ellis, RJ, Schuch, WW: Isolation and nucleotide sequences of cDNA clones encoding ADPglucose pyrophosphorylase polypeptides from wheat leaf and endosperm. *Plant Mol. Biol.* 12:525-538 (1989).
14. Smith-White, BJ, Preiss, J, Comparison of proteins of ADPglucose pyrophosphorylase from diverse sources. *J. Mol. Evol.* in press.

DATE

FILMED

4 / 29 / 94

END