

“Starch Conversion to Sucrose in Plant Leaves”

(\$110,000) 9/15/2002 through 03/14/2004.

In this project, the pathway of conversion of transitory starch to sucrose in plant leaves was investigated. This basic biological information is important for understanding how starch synthesis during the day can increase the plant's ability to accumulate and store biomass. The results will also allow for manipulation of end products of photosynthesis to direct more of the energy-rich compounds of plants into useful products.

Maltose is the major export form of carbon from chloroplasts at night

(Weise et al., 2004) Chloroplasts of bean or spinach were incubated in a centrifuge tube over silicon oil in the dark. After 2.5 hours the tubes were centrifuged and the medium (minus chloroplasts) were assayed to determine what products were exported from the chloroplasts. It was found that between 50% and 80% of the exported carbon was in the form of maltose. This was in direct conflict with many reports of phosphorylation being important to starch conversion to sucrose. The advance used in these studies was to use starch-loaded isolated chloroplasts rather than relatively-starch deficient chloroplasts labeled with radioactive carbon. We applied a new method for measuring maltose that did not rely on radioactivity.

The Role of Amylomaltase in Maltose Metabolism in the Cytosol of Photosynthetic Cells

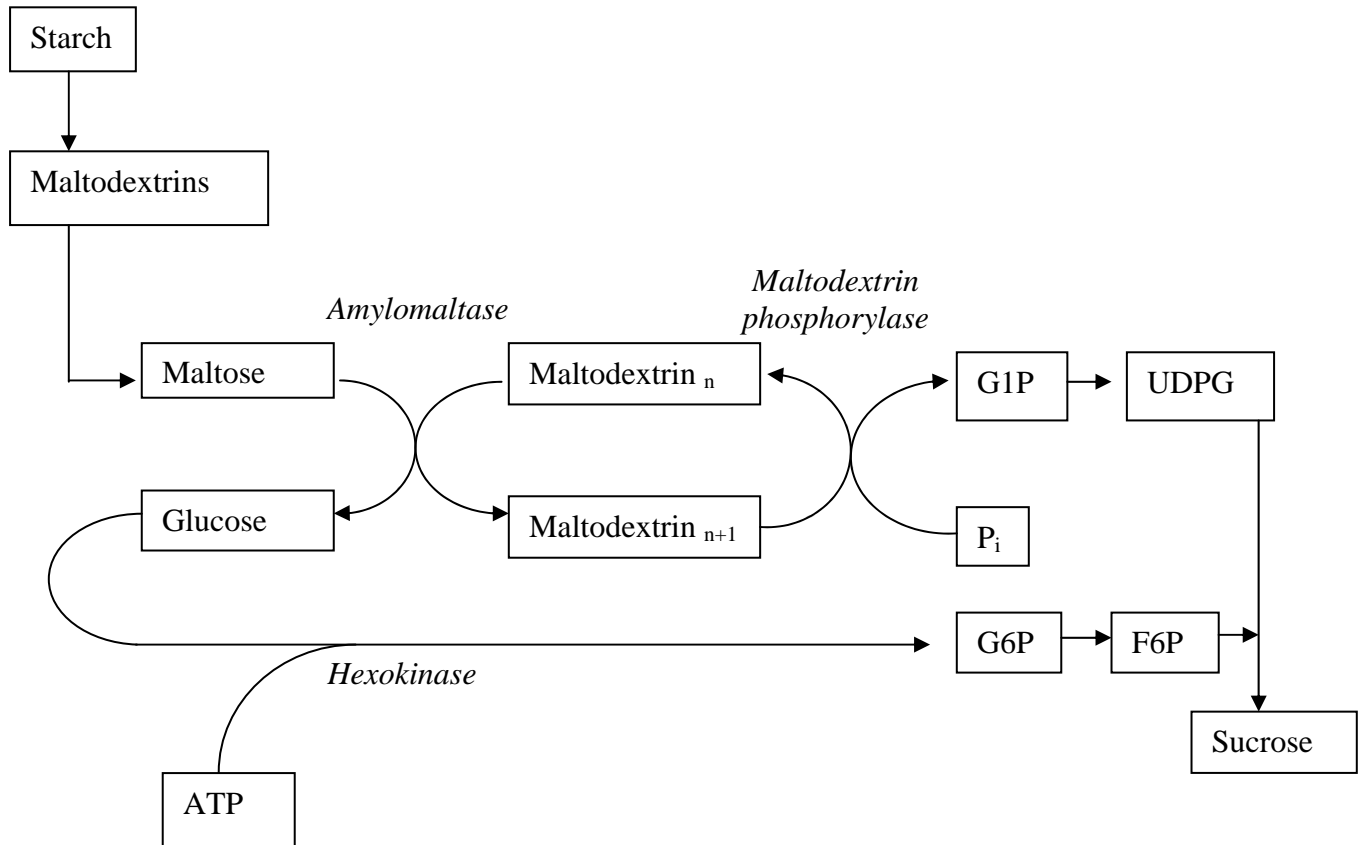
(Lu and Sharkey, 2004) The Wisconsin population of T-DNA plants was screened for lines with inserts in what was annotated as a cytosolic D enzyme. D-enzyme, or disproportionating enzyme, is a type II 4- α -glucanotransferase. The similar enzyme in the plastid does not work with maltose but some similar enzymes in bacteria do. Two lines of amylomaltase knockouts were found. Plants lacking the cytosolic glucanotransferase accumulated large pools of maltose and high molecular weight dextrans. This work indicates that the metabolism of maltose in the cytosol has properties of the malQ/malP system of *E. coli* in which amylomaltase uses maltose to extend maltodextrins while maltodextrin phosphorylase shortens the maltodextrins and makes G1P (see Fig. 1). To show that the amylomaltase knockouts eliminated the mRNA for these enzymes we probed blots made from RNA of these plants (northern blots). As expected, the knockouts had no mRNA but what was unexpected was that the wild type plants had much more mRNA during the day than at night. We do not yet have data on the amount of the protein present and suspect it is not more abundant during the day. However, we speculate that a light-sensitivity of transcription of this gene could help coordinate starch degradation with the previous day's photosynthesis. On bright days, when more photosynthate is likely shuttled into starch, more amylomaltase would be available the following night to convert that starch to sucrose. We consider this a hint that amylomaltase may play a role in matching starch degradation capability with starch synthesis. We are currently trying to express this gene in *E. coli* in a plasmid that also has a His tag to allow quick purification. This will allow an analysis of the specific functions of this gene.

The Maltodextrin Phosphorylase Knockout is Embryo-lethal

Y. Lu and T.D. Sharkey

We searched the Wisconsin collection of tDNA plants for a knockout in what had been called cytosolic starch phosphorylase. One line was found. When the knocked out gene is heterozygous, the plants appear normal but in no homozygous plants can be recovered and it appears that this knockout causes embryos to arrest at the heart stage. Because it is embryo-lethal, we cannot conclude anything about the role of this enzyme in photosynthesis, but we hypothesize it is a polyglucan phosphorylase that cleaves G1P units from polyglucans made by amylomaltase and maltose. This explains why there are what would seem to be starch-degrading enzymes in the cytosol but does not explain why this knockout causes an embryo-lethal phenotype.

Prog. Rpt. Figure 1. Hypothetical scheme for maltose breakdown in the cytosol based on the amylose, amylopectin metabolism of *E. coli*.



Rapid Starch Breakdown in Photorespiratory Conditions

A. Motzko, S.E. Weise, and T.D. Sharkey - In this work leaves were held in CO₂-free air for over five hours to see if starch breakdown could account for CO₂ loss during photorespiration. The decline in starch was ten times more than the total CO₂ lost during these experiments and the maltose concentration doubled. After 5 hrs, the starch content of the leaves fell nearly 60% even though the experiment was carried out entirely during the normal light period and so circadian rhythms would have worked against starch degradation. We postulate that the photorespiratory conditions stimulated starch conversion to maltodextrins in the chloroplast which were then attacked by starch phosphorylase (to provide a carbon source for the Calvin cycle) and by β -amylase (resulting in high levels of maltose). We next tested whether starch degradation showed any circadian regulation. When leaves were put into darkness in the middle of their normal day they did not accumulate maltose for at least 5 hours whereas darkness at the end of the normal day caused maltose to increase 50 to 100% within one hour. We conclude that there are metabolite level changes that can initiate starch degradation (the photorespiration results) but that

circadian regulation can also influence starch breakdown. Experiments are underway to understand the relative importance of these regulation mechanisms.

Transitory Starch in Basal Land Plant Lineages

(Sharkey et al., 2005) While it is generally assumed that transitory starch is widespread among land plants, we were not aware of any systematic study of this. Using the rich collection of plants from basal land plant lineages available in the Birge Hall, UW-Madison greenhouse, it was shown that liverworts, a number of mosses, lycopodium, and ferns all show pronounced accumulation of starch during the day and catabolism of starch at night.

Publications

Lu Y, Sharkey TD (2004) The role of amylomaltase in maltose metabolism in the cytosol of photosynthetic cells. *Planta* 218: 466-473

Sharkey TD, Weise SE, Standish AJ (2005) CO₂ processing: From the chloroplast to the leaf. In WK Smith, TC Vogelmann, C Critchley, eds, *Photosynthetic Adaptation from the Chloroplast to the Landscape*, Academic Press,

Weise SE, Weber A, Sharkey TD (2004) Maltose is the major form of carbon exported from the chloroplast at night. *Planta* 218: 474-482