Carbohydrate synthesis in plants

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C3, C4, and CAM plants all have the same goal, to make carbohydrates.

What happens to the triose-phosphates made in the Calvin cycle?

1. Used to synthesize starch for storage in chloroplast.

2. Exported from chloroplast for sucrose synthesis in the cytosol.

How is starch vs. sucrose synthesis regulated?
Why is it regulated?
Triose phosphates produced in the Calvin cycle can be used for starch or sucrose synthesis.
Starch is synthesized in the chloroplast.
What is starch?

• Complex carbohydrate made up of two components

• Components:
  – Amylose
  – Amylopectin

• Properties depend on amounts of the components
glucose + glucose + glucose... = polysaccharide

- starch
- (plant)
Where is it found?

• Roots/Tubers
  – Potato
  – Tapioca

• Cereal
  – Corn
  – Waxy corn
  – Wheat
  – Rice
  – Waxy rice
Amylose

- Linear component of starch
- Contains alpha-1,4 glucosidic bonds
- Molecular weight: less than 0.5 million
- Can form coils which will trap iodine and turn blue
Amylopectin

- Branched component of starch
- Contains alpha-1,4 glucosidic as well as alpha-1,6 glucosidic bonds at the branch points
- Molecular weight: 50-500 million
- Limited coiling causes purplish-red color when iodine added
Amylose vs. Amylopectin

• Starches usually contain more amylopectin than amylose
• Generally roots/tubers contain more amylopectin than cereals
• Roots/Tubers: 80% amylopectin
• Cereals: 75% amylopectin
• Waxy corn contains virtually all amylopectin
Starch Composition

<table>
<thead>
<tr>
<th>Starch</th>
<th>% amylose</th>
<th>% amylopectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tapioca</td>
<td>17%</td>
<td>83%</td>
</tr>
<tr>
<td>Potato</td>
<td>~20%</td>
<td>~80%</td>
</tr>
<tr>
<td>Wheat</td>
<td>25-26%</td>
<td>~75%</td>
</tr>
<tr>
<td>Corn</td>
<td>24-28%</td>
<td>~75%</td>
</tr>
<tr>
<td>Waxy corn</td>
<td>~0%</td>
<td>~100%</td>
</tr>
<tr>
<td>Hi amylose</td>
<td>~75%</td>
<td>~25%</td>
</tr>
<tr>
<td>Rice</td>
<td>22%</td>
<td>78%</td>
</tr>
</tbody>
</table>
Starch Granule

• Made in the cytoplasm of plant cells
• Amylopectin forms in concentric circles with amylose dispersed in between
• Held together by hydrogen bonds
• The granule swells when heated in water
Starch Granule
Starch vs. sucrose synthesis is regulated by level of \textit{cytosolic} P_i as it affects triose-P export from chloroplast.

When cytosolic [P_i] is high, triose-P is exported in exchange for P_i & used to synthesize sucrose.

If cytosolic [P_i] is low, then triose-P is retained in chloroplast and used to synthesize starch.
Biosynthesis of Starch and Sucrose;

• During active photosynthesis in bright light, a plant leaf produces more carbohydrate (as triose phosphates) than it needs for generating energy or synthesizing precursors.

• The excess is converted to sucrose and transported to other parts of the plant, to be used as fuel or stored.

• In most plants, starch is the main storage form, but in a few plants, such as sugar beet and sugarcane, sucrose is the primary storage form.
The synthesis of sucrose and starch occurs in different cellular compartments (cytosol and plastids, respectively), and these processes are coordinated by a variety of regulatory mechanisms that respond to changes in light level and photosynthetic rate.

**ADP-Glucose** is the substrate for starch synthesis in plant plastids and for glycogen synthesis in bacteria.
Starch, like glycogen, is a high molecular weight polymer of D-glucose in (α1, 4) linkage.

Starch is synthesized in chloroplasts for temporary storage as one of the stable end products of photosynthesis, and for long-term storage it is synthesized in the amyloplasts of the nonphotosynthetic parts of plants—seeds, roots, and tubers (underground stems).

The mechanism of glucose activation in starch synthesis is similar to that in glycogen synthesis.

• An activated nucleotide sugar, in this case ADP-glucose, is formed by condensation of glucose 1-phosphate with ATP in a reaction made essentially irreversible by the presence in plastids of inorganic pyrophosphatase.
Starch synthase then transfers glucose residues from ADP-glucose to preexisting starch molecules.

Glucose is added to the *non-reducing end of starch, as in glycogen synthesis*
ADP-glucose pyrophosphorylase

$\text{Glucose-1 P} \rightarrow \text{ADP-glucose}$

$\text{ADP-Glucose pyrophosphorylase}$

$\text{2Pi}$

$\text{Pyrophosphatase}$

$\text{ATP} \rightarrow \text{Pi}$

$\text{ADP} \rightarrow \text{Pi}$

$\text{Starch synthase}$

$\text{Starch (n-residues)} \rightarrow \text{Starch (n+1-residues)}$

$\text{Starch synthase}$

$\text{ADP}$

$\text{Starch (n+1-residues)}$
Glycogen synthase

UDP-glucose

Nonreducing end of a glycogen chain with $n$ residues ($n > 4$)

New nonreducing end

Elongated glycogen with $n + 1$ residues
Although it has generally been assumed that glucose is added to the **nonreducing end of starch, as in glycogen synthesis**, evidence now suggests that starch synthase has **two equivalent active sites** that alternate in inserting a glucosyl residue onto the **reducing end of the growing chain**.

This end remains covalently attached to the enzyme, first at one active site, then at the other.

Attachment to one active site effectively **activates the reducing end of the growing chain** for nucleophilic displacement of the enzyme by the attacking C-4 hydroxyl of a glucosyl moiety bound to the other active site, forming the (α-1, 4) linkage characteristic of starch.
Each of the two reactive groups ($X_a$, $X_b$) at the active site of starch synthase makes a nucleophilic attack on ADP-glucose, displacing ADP and forming a covalent attachment to C-1 of the glucose unit.

The bond holding glucose residue 1 to $X_a$ undergoes nucleophilic attack by the $-OH$ at C-4 of glucose residue 2 on $X_b$, forming an $\alpha(1\rightarrow4)$-disaccharide of residues 2 and 1. This remains attached through glucose 2 to $X_b$, $X_a$, now free, displaces ADP from another ADP-glucose and becomes attached to glucose 3.

The hydroxyl at C-4 of glucose 3 displaces $X_b$ from the disaccharide, forming a trisaccharide attached to $X_a$, $X_b$, now free, acquires glucose residue 4 from another ADP-glucose.

The hydroxyl at C-4 of glucose 4 displaces $X_a$ forming a tetrasaccharide, with its reducing end covalently attached to $X_b$.

Many repetitions of this sequence extend the oligosaccharide, adding glucose residues at its reducing end, with $X_a$ and $X_b$ alternately carrying the growing starch chain. When the chain reaches an appropriate length, it is separated from starch synthase.
(a) Amylose

(b) Branch

(c) Nonreducing ends

Amylose

Reducing ends

Amylopectin
• The amylose of starch is unbranched, but amylopectin has numerous (α-1, 6)-linked branches

• Chloroplasts contain a branching enzyme, similar to glycogen-branching enzyme, that introduces the (α-1, 6) branches of amylopectin.

• Taking into account the hydrolysis by inorganic pyrophosphatase of the PPi produced during ADP-glucose

• Starch synthesis, the overall reaction for starch formation from glucose 1-phosphate.

• Starch synthesis is regulated at the level of ADP-glucose formation (ADP-glucose pyrophosphorylase).

\[
\text{Starch}_n + \text{glucose 1-phosphate} + \text{ATP} \rightarrow \text{starch}_{n+1} + \text{ADP} + 2\text{P}_i
\]

\[
\Delta G^\circ = -50 \text{ kJ/mol}
\]
These branches have an average length of ~20 glucan units
Digesting starch vs. cellulose

**Starch**
- Easy to digest by alpha-amylase

**Cellulose**
- Hard to digest
Cellulose

• Cell walls in plants
  – herbivores can digest cellulose well
  – most carnivores cannot digest cellulose
    • that’s why they **eat meat** to get their energy & nutrients
  • cellulose = roughage
    – stays undigested
    – keeps material moving in your intestines
Sucrose Synthesis
UDP-Glucose Is the Substrate for Sucrose Synthesis in the Cytosol of Leaf Cells

• Most of the triose phosphate generated by CO2 fixation in plants is converted to sucrose or starch.

• Sucrose may have been selected as the transport form of carbon because of its unusual linkage between the anomeric C-1 of glucose and the anomeric C-2 of fructose.

• This bond is not hydrolyzed by amylases or other common carbohydrate-cleaving enzymes, and the unavailability of the anomeric carbons prevents sucrose from reacting non-enzymatically (as does glucose) with amino acids and proteins.
• Sucrose is synthesized in the cytosol, beginning with dihydroxyacetone phosphate and glyceraldehyde 3-phosphate exported from the chloroplast.

• After condensation of two triose phosphates to form fructose 1,6-bisphosphate (catalyzed by aldolase), hydrolysis by fructose 1,6-bisphosphatase yields fructose 6-phosphate.
Net reaction: Sugar phosphate + NTP $\rightarrow$ NDP-sugar + 2P$_i$
d-Glucosyl group

UDP-glucose
(a sugar nucleotide)
• **Sucrose 6-phosphate synthase** then catalyzes the reaction of fructose 6-phosphate with UDP-glucose to form sucrose 6-phosphate.

• Finally, **sucrose 6-phosphate phosphatase** removes the phosphate group, making sucrose available for export to other tissues.

• The reaction catalyzed by sucrose 6-phosphate synthase is a low-energy process ($\Delta G = -5.7 \text{ kJ/mol}$), but the hydrolysis of sucrose 6-phosphate to sucrose is sufficiently exergonic ($\Delta G = -16.5 \text{ kJ/mol}$) to make the overall synthesis of sucrose essentially **irreversible**. Sucrose synthesis is regulated and closely coordinated with starch synthesis.
Conversion of Triose Phosphates to Sucrose and Starch Is Tightly Regulated

- Triose phosphates produced by the Calvin cycle in bright sunlight, as we have noted, may be stored temporarily in the chloroplast as starch, or converted to sucrose and exported to nonphotosynthetic parts of the plant, or both.

- The balance between the two processes is tightly regulated, and both must be coordinated with the rate of carbon fixation.

- Five-sixths of the triose phosphate formed in the Calvin cycle must be recycled to ribulose 1,5-bisphosphate.

- If more than one-sixth of the triose phosphate is drawn out of the cycle to make sucrose and starch, the cycle will slow or stop.

- However, insufficient conversion of triose phosphate to starch or sucrose would tie up phosphate, leaving a chloroplast deficient in Pi, which is also essential for operation of the Calvin cycle.
• The flow of triose phosphates into sucrose is regulated by the activity of fructose 1,6-bisphosphatase (FBPase-1) and the enzyme that effectively reverses its action, PPI-dependent phosphofructokinase (PP-PFK-1).

• These enzymes are therefore critical points for determining the fate of triose phosphates produced by photosynthesis.

• Both enzymes are regulated by fructose 2,6-bisphosphate (F2,6BP), which inhibits FBPase-1 and stimulates PP-PFK-1.

• In vascular plants, the concentration of F2,6BP varies inversely with the rate of photosynthesis.
• **Phosphofructokinase-2**, favors greater flux of triose phosphate into fructose 6-phosphate formation and **sucrose synthesis**.

• With this regulatory system, sucrose synthesis occurs when the level of triose phosphate produced by the Calvin cycle exceeds that needed to maintain the operation of the cycle.
Sucrose synthesis is also regulated at the level of sucrose 6-phosphate synthase, which is allosterically activated by glucose 6-phosphate and inhibited by Pi.

This enzyme is further regulated by phosphorylation and dephosphorylation; a protein kinase phosphorylates the enzyme on a specific Ser residue, making it less active, and a phosphatase reverses this inactivation by removing the phosphate.
• The key regulatory enzyme in starch synthesis is **ADP-glucose pyrophosphorylase**

• It is activated by 3-phosphoglycerate (which accumulates during active photosynthesis) and inhibited by Pi (which accumulates when light-driven condensation of ADP and Pi slows).

• When sucrose synthesis slows, 3-phosphoglycerate formed by CO2 fixation accumulates, activating this enzyme and stimulating the synthesis of starch.
Fructans

- Fructans are probably the most abundant storage carbohydrate in plants next to starch and sucrose.
- Fructans are linear or branched polymers of mostly \( \beta \)-fructosyl-fructose linkages.
- Unlike sucrose they are synthesized and stored in vacuoles and can accumulate in the stems, bulbs and tubers of a number of plants.
- The basic structure of a fructan is a trisaccharide, known as a kestose, which has a sucrose molecule linked to one additional fructose:
• They are found as oligosaccharides of 5-10 fructose molecules, ~50 residues in the inulin (β_{2,1}) and ~200 for the levan (β_{2,6}).

• Branched fructans found in grasses, known as graminanes.
Inulin

inulin (β 2,1)
Levan

Levan (β 2,6)
• Fructan synthesis occurs in vacuoles and sucrose is the precursor.

• In the first step of fructan synthesis, the fructose moiety of a sucrose molecule is transferred to a second sucrose molecule by sucrose-sucrose fructosyl transferase forming a kestose + glucose
Fructose groups are preferentially transferred from a trisaccharide kestose to longer kestoses rather than from sucrose by *fructan-fructan fructosyl transferases*.
• **Fructan exohydrolases** degrade fructan polymers by cleaving terminal fructose residues.