Photosynthesis 2

Calvin Cycle
C4 and CAM Pathways

Lecture 32

Key Concepts

• Three stages of the Calvin Cycle
  – Stage 1: Fixation of CO₂ to form 3-phosphoglycerate
  – Stage 2: Reduction of 3-phosphoglycerate to form hexose sugars
  – Stage 3: Regeneration of ribulose-1,5-bisphosphate

• Regulation of the Calvin Cycle

• The C₄ and CAM pathways reduce photorespiration in hot climates
Three stages of the Calvin Cycle:

**Fixation, Reduction and Regeneration**

Plants store light energy in the form of carbohydrate, primarily *starch* and *sucrose*. The carbon and oxygen for this process comes from CO$_2$, and the energy for carbon fixation is derived from the ATP and NADPH made during photosynthesis.

The conversion of CO$_2$ to carbohydrate is called the Calvin Cycle and is named after Melvin Calvin who discovered it. The Calvin Cycle requires the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase commonly called *rubisco*. The Calvin cycle generates the triose phosphates 3-phosphoglycerate (3-PGA), glyceraldehyde-3P (GAP) and dihydroxyacetone phosphate (DHAP), all of which are used to synthesize the hexose phosphates fructose-1,6-bisphosphate and fructose 6-phosphate.

Hexose phosphates produced by the Calvin Cycle are converted to:

1) *sucrose* for transport to other plant tissues
2) *starch* for energy stores within the cell
3) *cellulose* for cell wall synthesis
4) *pentose phosphates* for metabolic intermediates
Three turns of the cycle results in the fixation of three molecules of CO₂.

Stage 1 is catalyzed by rubisco enzyme which combines three molecules of ribulose-1,5-bisphosphate (RuBP), a C₅ compound, with three molecules of CO₂ to form six molecules of 3-PGA, a C₃ compound. Five 3-phosphoglycerate molecules are used to reform RuBP for another round and one is used for sugar.

The Calvin Cycle is sometimes called the Dark Reactions, but do not be fooled by this name - the Calvin Cycle is the most active during the daylight hours when ATP and NADPH are plentiful.

The net reaction of three turns of the Calvin cycle can be written as:

\[
3 \text{ CO}_2 + 3 \text{ RuBP} + 6 \text{ NADPH} + 9 \text{ ATP} + 6 \text{ H}_2\text{O} \rightarrow 1 \text{ GAP} + 3 \text{ RuBP} + 6 \text{ NADP}^+ + 9 \text{ ADP} + 9 \text{ Pi}
\]

If we just look at the fate of the carbons coming from CO₂ (C₁) in this reaction, we see that one net C₃ compound (GAP) is formed and three C₅ molecules (RuBP) are regenerated:

\[
3 \text{ C}_1 + 3 \text{ C}_5 \rightarrow 1 \text{ C}_3 + 3 \text{ C}_5
\]

Why was the Calvin Cycle originally called the Dark Reactions?
Stage 1: Fixation of CO₂ to form 3-phosphoglycerate

To identify the metabolic intermediates in this process, Calvin and his colleagues used radioactive labeling with $^{14}$CO₂ to follow carbon fixation in photosynthetic algae cells grown in culture.

They found that within a few seconds of adding $^{14}$CO₂ to the culture, the cells accumulated $^{14}$C-labeled 3-PGA, suggesting that this was the first product of the carboxylation reaction. 3-PGA is converted to GAP by two enzymes in the Calvin Cycle.

Within a minute of adding $^{14}$CO₂ to the culture, they found numerous compounds were labeled with $^{14}$C, many of which were later identified as Calvin Cycle intermediates.

Rubisco reaction can be broken down into four basic steps

1) formation of an enediolate intermediate of ribulose-1,5-bisphosphate
2) carboxylation by nucleophilic attack on the CO₂
3) hydration of 2-carboxy-3-keto-D-arabinitol-1,5-bisphosphate
4) aldol cleavage to form two molecules of 3-PGA

The rubisco reaction is very exergonic ($\Delta G^\circ = -35.1$ kJ/mol), with the aldol cleavage step being a major contributor to the favorable change in free energy.
Rubisco is a multisubunit enzyme consisting of eight identical catalytic subunits at the core surrounded by eight smaller subunits that function to stabilize the complex and presumably enhance enzyme activity.

Considering that rubisco plays a central role in all photosynthetic autotrophic organisms on earth, of which ~85% are photosynthetic plants and microorganisms that inhabit the oceans, rubisco is the most abundant protein on planet earth.

**Stage 2: Reduction of 3-phosphoglycerate to form hexose sugars**

3-phosphoglycerate (3-PGA) is converted to 1,3-bispshophoglycerate which is reduced to glyceraldehyde-3-phosphate (GAP). These two reactions require the ATP and NADPH made during the light reactions. Some of the GAP is isomerized to dihydroxyacetone phosphate (DHAP).

Remember that for every 3 CO₂ that are fixed by carboxylation of 3 RuBP molecules, six moles of 3PGA are generated by aldol cleavage.

Therefore, 6 ATP and 6 NADPH are required for every 3 CO₂ that are converted to one net 3PGA. An additional 3 ATP are used in stage 3 to regenerate these 3 RuBP molecules.
NADPH and ATP are produced by the Light Reactions of Photosynthesis

Stage 3: Regeneration of ribulose-1,5-bisphosphate

In this final stage of the Calvin cycle, a series of enzyme reactions convert five C₃ molecules (GAP or DHAP) into three C₅ molecules (RuBP) to replenish supplies of this CO₂ acceptor molecule which is required in the rubisco reaction.

This requires an additional 3 ATP.

Two of the primary enzymes in this carbon shuffle are transketolase and transaldolase which are involved in interconverting C₃, C₄, C₆ and C₇ molecules:
“Carbon shuffle” reactions to replenish RUBP

5 C\textsubscript{3} \rightarrow 3 C\textsubscript{5}

Summary of the Calvin Cycle reactions

Fix 6 CO\textsubscript{2} into one glucose molecule using 12 ATP and 12 NADPH and then regenerate 6 RuBP from using 6 ATP.

Glucose synthesis

6 CO\textsubscript{2} + 6 RuBP + 12 NADPH + 12 ATP + 10 H\textsubscript{2}O \rightarrow 6 GAP + 4 DHAP + Glucose + 12 NADP\textsuperscript{+} + 12 ADP + 16 Pi

Regeneration of RuBP

6 GAP + 4 DHAP + 6 ATP + 2 H\textsubscript{2}O \rightarrow 6 RuBP + 6 ADP + 2 Pi

Net reaction from six turns of the Calvin cycle

6 CO\textsubscript{2} + 12 NADPH + 18 ATP + 12 H\textsubscript{2}O \rightarrow Glucose + 12 NADP\textsuperscript{+} + 18 ADP + 18 Pi
Regulation of the Calvin Cycle

Why must the Calvin Cycle be regulated?

At night, plant cells rely on glycolysis and mitochondrial aerobic respiration to generate ATP for cellular processes.

Since photophosphorylation and NADPH production by the photosynthetic electron transport system is shut down in the dark, it is crucial that the Calvin cycle only be active in the light.

Otherwise, if glycolysis, the pentose phosphate pathway and the Calvin cycle were all active at night, then simultaneous starch degradation and carbohydrate biosynthesis would quickly deplete the ATP and NADPH pools in the stroma.

This is why “Dark Reactions” is a misnomer for the Calvin Cycle; if it were only active at night, then the plant would die from energy loss!

Light stimulation of Calvin Cycle enzymes by two mechanisms:

1. Several enzymes including Rubisco are activated by elevated pH and by increased Mg\(^{2+}\) concentrations in the stroma.

2. Other calvin cycle enzymes are activated thioredoxin-mediated reduction of disulfide bridges.

Why is it important that light stimulate the Calvin Cycle?
Activation by elevated pH and by increased Mg\(^{2+}\) concentrations in stroma

Light activation of the photosynthetic electron transport system causes stromal pH to increase from pH 7 to pH 8 as a result of proton pumping into the thylakoid lumen.

This influx of H\(^+\) into the lumen causes an efflux of Mg\(^{2+}\) to the stroma to balance the charge.

Rubisco activity is maximal under conditions of pH 8 and high Mg\(^{2+}\).

Activation by thioredoxin-mediated reduction of disulfide bridges

Thioredoxin is a small protein of 12 kDa that is found throughout nature and functions as a redox protein that can interconvert disulfide bridges and sulfhydryls in cysteine residues of target proteins.

As long as reduced thioredoxin is present in the stroma, these Calvin Cycle enzymes are maintained in the active state.

However, when the sun goes down, spontaneous oxidation leads to their inactivation.
The C4 and CAM pathways reduce **photorespiration** in hot climates

**What is photorespiration?**
A wasteful oxygenase reaction catalyzed by the enzyme Rubisco that results in the loss of 2 carbons as CO$_2$.

**How do plants limit photorespiration?**
By separating CO$_2$ uptake from CO$_2$ fixation, thereby limiting exposure of Rubisco to O$_2$. This can be done spatially (C4 pathway) or temporally (CAM pathway).

Photorespiration and Rubisco

Rubisco also catalyzes an **oxygenase reaction** that combines RuBP with O$_2$ to generate one molecule of 3-PGA (C$_3$) and one molecule of 2-phosphoglycolate (C$_2$).

It is thought that this **wasteful** reaction (2-phosphoglycolate cannot be used for sugar synthesis) belies the ancient history of the rubisco enzyme which has been around since before O$_2$ levels in the atmosphere were as high as they are today.

In order to salvage the carbon in 2-phosphoglycolate, it must first be converted to **glycolate**, which is exported to peroxisomes to make glyoxylate and glycine which is then exported to mitochondria where two molecules of glycine are converted to one molecule of serine.

Oxygenation of RuBP, and metabolism of 2-phosphoglycolate by the **C$_2$ glycolate pathway**, is collectively called **photorespiration** because O$_2$ is consumed and CO$_2$ is released.
Rubisco is a carboxylase and an oxygenase

The 2 carbons in glycolate are lost as either CO₂ or serine

2 ATP are Consumed in the cycle
Plants in hot climates are especially susceptible to photorespiration due to high O\textsubscript{2}:CO\textsubscript{2} ratios under these environmental conditions. Increased rates of photorespiration in C3 plants (normal carbon fixation) reduces growth.

In the 1960s, Marshall Hatch and Roger Slack, plant biochemists at the Colonial Sugar Refining Company in Brisbane, Australia, used \textsuperscript{14}CO\textsubscript{2} labeling experiments to determine what the initial products were in the carbon fixation reactions of sugarcane plants.

To their surprise, they found that malate was more quickly labeled with \textsuperscript{14}C than was 3-PGA. Follow up work showed that plants such as sugarcane and corn, and weeds like crabgrass, thrive under high temperature conditions by having very low levels of photorespiration.

The mechanism involves the carboxylation of phosphoenolpyruvate (PEP) by the enzyme PEP carboxylase to form oxaloacetate (OAA), a four carbon (C\textsubscript{4}) intermediate that serves as a transient CO\textsubscript{2} carrier molecule. OAA is then converted to malate which functions as a storage form of CO\textsubscript{2}.

Two variations of the "Hatch-Slack" pathway have been described;

1) C\textsubscript{4} pathway in tropical plants utilize two separate cell types to reduce photorespiration during the day. In this case, CO\textsubscript{2} is stored in the C\textsubscript{4} intermediate malate which is made in one cell type and then transported to a second cell type containing Rubisco where it is decarboxylated to release the CO\textsubscript{2}.

2) CAM pathway found in desert succulents captures CO\textsubscript{2} in the form of malate at night when it is cool and O\textsubscript{2}:CO\textsubscript{2} ratios are low. During the day when photosynthesis is active, the stomata are closed and malate is decarboxylated to release the CO\textsubscript{2} in the same cell for use by Rubisco.
C4 Pathway in Sugarcane

Mesophyll cells are responsible for CO$_2$ capture. Interior bundle sheath cells (further away from atmospheric O$_2$), use CO$_2$ released from the C$_4$ intermediate malate to carry out the Calvin cycle reactions. This "separation in space" between the two cell types essentially eliminates the oxygenase reaction in rubisco and thereby blocks photorespiration. Two high energy phosphate bonds are required to convert pyruvate to phosphoenolpyruvate (rxn 4) which has an energy cost to C$_4$ plants.

Key Enzymes in the C$_4$ Pathway in Sugarcane

PEP carboxylase (rxn 1) catalyzes a reaction combining HCO$_3^-$ with PEP to form OAA.

OAA is reduced to malate by the enzyme NADP-malate dehydrogenase (rxn 2). Other C$_4$ plants transaminate OAA to form aspartate which functions as the CO$_2$ intermediate.

Malate is then transported to the bundle sheath cells through special protein-lined channels connecting the cells where it is oxidized and decarboxylated in the bundle sheath cell chloroplast by NADP-malic enzyme (rxn 3) to form CO$_2$ and pyruvate.

The released CO$_2$ is incorporated into RuBP by rubisco and the resulting 3-phosphoglycerate is metabolized by the C$_3$ pathway.

In sugarcane, pyruvate is transported back to the mesophyll cells where it is phosphorylated by the enzyme pyruvate phosphate dikinase in the presence of ATP (rxn 4) to yield PEP + AMP.
CAM Pathway

First discovered in succulent plants of the Crassulaceae family therefore called Crassulacean Acid Metabolism (CAM) pathway.

The CAM pathway functions to concentrate CO$_2$ levels in the chloroplast stroma to limit the oxygenase activity of rubisco. CAM plants like the saguaro cactus use a temporal separation.

During the night when the stomata are open to obtain moisture, CO$_2$ is captured by the mesophyll cells and incorporated into OAA by PEP carboxylase. OAA is then reduced by NAD-malate dehydrogenase to form malate.

During the day, the CO$_2$ is released from malate by NADP-malic enzyme, allowing the Calvin Cycle to fix the CO$_2$ when ATP and NADPH are plentiful from photosynthesis.

Importantly, tropical plants obtain moisture and sunlight during the day, so spatial separation is optimal, whereas, cacti only open stomata at night, so temporal separation allows photosynthesis to take place during the day even with the stomata closed.

CAM Plants Temporally Separate CO$_2$ Uptake from CO$_2$ Fixation

1. PEP Carboxylase
2. Malate Dehydrogenase
3. NADP-Malic Enzyme

Buchanon et al., Fig. 12.51
C3 plants have an advantage in mild temperatures, whereas, C4 plants have an advantage in high temperatures.

Springtime (20º C)
Turf (C3) outgrows crabgrass (C4) because of increased energy efficiency

Summertime (30º C)
Crabgrass (C4) outgrows turf (C3) because of reduced photorespiration.