The Calvin Cycle

Introduction

- When sunflower plants are illuminated in the presence of labelled CO$_2$, it is found that of 7.87mg of absorbed CO$_2$, 68% ends up in sucrose and 23% ends up in starch.
- The dark reactions are the second part of photosynthesis which use the ATP and NADPH produced by the light-reactions to reduce carbon atoms from their fully oxidised state as CO$_2$ to a more reduced state such as hexose.
- In contrast to gluconeogenesis, where the energy ultimately comes from the catabolism of other fuels, the Calvin cycle can use energy from sunlight.
- The Calvin cycle takes place in the stroma of chloroplasts.

The Calvin Cycle

The Calvin Cycle consists of three stages:

1) The fixation of CO$_2$ by ribulose 1,5-bisphosphate to form two molecules of 3-phosphoglycerate.
2) The reduction of 3-phosphoglycerate to form hexose sugars.
3) The regeneration of ribulose 1,5-bisphosphate so that more CO$_2$ can be fixed.

Stage (1)

CO$_2$ condenses with ribulose 1,5-bisphosphate and forms an unstable intermediate, which is rapidly hydrolysed to two molecules of 3-phosphoglycerate:
This highly exergonic reaction is catalysed by ribulose 1,5-bisphosphate carboxylase/oxygenase (usually called rubisco), an enzyme located on the stromal side of the thylakoid membrane. This reaction is the rate-limiting step in hexose synthesis.

Rubisco is the most abundant enzyme, and probably protein, in the biosphere, probably because it’s so slow. It is a hexadecamer of eight large (containing the active site) and eight small subunits.

Initial measurements of $K_m$ were so large that the atmospheric concentration of CO$_2$ would have had to be incredibly high for the enzyme to operate at half its maximal velocity. We now know that the enzyme has to be activated by Mg$^{2+}$ ions and an alkaline pH, and has to be reduced. The steps in the preparation of the enzyme are:

1) A CO$_2$ binds to the uncharged $\varepsilon$-amino group of lysine 201 to form a carbamate. This is catalysed by rubisco activase [though this also occurs slowly when uncatalysed].

2) The Mg$^{2+}$ can then bind to this negatively charged carbamate.

3) The Mg$^{2+}$ is also bound to the enzyme via a glutamate residue and an aspartate residue.

The ribulose 1,5-bisphosphate then binds to the Mg$^{2+}$, where it is readily deprotonated [hence the need for alkaline conditions] to form an enediolate intermediate. This readily couples to CO$_2$.

However, note that the enzyme has no binding site for the CO$_2$ molecule to be added to ribulose 1,5-bisphosphate. Therefore, it is unable to discriminate between CO$_2$ and O$_2$, and the enediolate intermediate is sometimes attacked by O$_2$. In such a case, as well as 3-phosphoglycerate, a molecule of phosphoglycolate is produced:

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This is problematic, because phosphoglycolate is not a very versatile metabolite, and this it results in the loss of CO₂ from the cycle. The cell is partially successful in recovering the carbon by turning two such molecules into one molecule of 3-phosphoglycerate – a 75% recovery.

Note, however, that since rubisco requires binding of CO₂ to be bound, it will not simply mass-produce phosphoglycolate if CO₂ is lacking. The process will simply pause.

This process is called photorespiration, because O₂ is consumed and CO₂ is released, without the production of any energy-rich metabolite. This imperfection in rubisco exists because it evolved before oxygen became abundant in the atmosphere. Evolutionary processes, however, have enhanced rubisco to a certain extent – the rubisco of higher plants is eightfold as specific for carboxylation than that of photosynthetic bacteria.

Stage (2)

Two reactions then convert these to glyceraldehyde 3-phosphate:

Which can then be fed into the gluconeogenetic pathway. The two enzymes are similar to those in glyconeogenesis, apart from the fact that they use NADPH instead of NADH.

Stage (3)

Finally, ribulose 1,5-bisphosphate must be regenerated:
1) A series of transformation reactions catalysed by transketolase and aldolase generate ribulose 5-phosphate.

2) This is then phosphorylated by phosphoribulose kinase and ATP to re-form ribulose bisphosphate.

The stoichiometry of these reactions is:

- 3 CO₂ are fixed, which results in SIX 3-carbon sugars. This uses 6 ATP and 6 NADPH.
- The condensation of sugar phosphates in different combinations results in THREE 5 carbon sugars and ONE 3 carbon sugar.
- The 5 carbon sugars are phosphorylated with ATP to regenerate RuBP.
- The 3 carbon sugar (a triose phosphate) is exported to the cytosol to make sucrose.
- Some carbon remains in the chloroplast to make starch.

In total, therefore, THREE ATP and TWO NADPH are needed to bring each CO₂ to hexose level.

**Experimental Evidence**

Experiments were carried out with radiolabelled carbon compounds, to establish how sugar monomers are made from CO₂. The following results were obtained:

Where:
o was found to be 3-phosphoglycerate.
o was found to consist of various intermediates formed from 1 and generating 3 (C3, C4, C5, C6 and C7 phosphorylated sugars). The rates at which these were labelled was so similar that the sequence of labelling could not be determined directly.
o were sucrose and starch.

Further progress came from the determination of the distribution of 14C within the individual molecules:

- Within 3-phosphoglycerate (3-phosphoglyceric acid)

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<th>5 seconds</th>
<th>30 seconds</th>
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<tbody>
<tr>
<td>CH$_2$OPO$_3^{2-}$</td>
<td>2.5</td>
<td>25</td>
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<tr>
<td>HO−C−H</td>
<td>2.5</td>
<td>25</td>
</tr>
<tr>
<td>CO$_2$−</td>
<td>95</td>
<td>50</td>
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This established that

a. The CO$_2$ is incorporated into the carboxyl group of phosphoglycerate.
b. The fact that a label rapidly appeared in the other carbons established the existence of a cyclic pathway whereby the acceptor of the CO$_2$ was formed from the product of the carboxylation [3-phosphoglycerate itself].

- Within one of the C6 sugar phosphates, fructose 6-phosphate

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<tr>
<td>CH$_2$OH</td>
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<td>HO−C−H</td>
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<tr>
<td>CH$_2$OPO$_3^{2-}$</td>
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This strongly suggested that the two 3-carbon compounds formed from 3-phosphoglycerate were combined to give a 6-carbon sugar phosphate.

**Sucrose & Starch**

**Sucrose** and **starch** are the major carbohydrate stores in plants – the former is useful for **storage** (we use glycogen), the latter for **transport** (we use glucose):

- **Starch** is synthesised and stored in the chloroplast. It is a polymer of glucose residues, but it is less branched than glycogen (its animal counterpart) because it contains a smaller proportion of α-1,6-glycosidic linkages. Another difference is that ADP-glucose rather than UDP-glucose is its precursor.

- **Sucrose**, however, is a disaccharide synthesised in the cytoplasm. Since plants are unable to transport hexose phosphates across the cytoplasm, they transport instead triose phosphates such as glyceraldehyde 3-phosphate into the cytoplasm, in exchange for phosphate through abundant phosphate translocators. These then form fructose 6-phosphate which joins the glucose unit of UDP-glucose to form sucrose 6-phosphate. The hydrolysis yields sucrose, which is a readily transportable and mobilizable sugar.

**Dependence on Environmental Conditions**

In general, **photosynthesis** takes place during the day, whereas **catabolic metabolism** takes place at night. How are they co-ordinately controlled?

- We mentioned before that the **rate limiting step** in the dark reactions was the action of rubisco. Now, it turns out that in the presence of light:
  - The **pH increases from 7 to 8** in the stroma (due to **proton pumping**).
  - Levels of **Mg$^{2+}$** rise (to “balance” the **proton pumping**). Both of these **favour** the formation of the **carbamate** necessary for enzyme activity. In the **dark**, this is **unlikely to happen**.

- **Thioredoxin** plays a **central role** in the regulation of the **calvin cycle**. The presence of **NADPH** and reduced **ferrodoxin** are **good signals** that
conditions are right for biosynthesis. One way this is conveyed to biosynthetic enzymes is through thioredoxin, a 12-kd protein that contains to neighbouring cysteine residues that cycle between a reduced sulfhydryl form and an oxidised disulfide form. The oxidised form can active enzymes by reducing disulfide bridges that control their activity, and inhibit several degradative enzymes in the same way.

In the chloroplast, oxidised thioredoxin is reduced by ferrodoxin by an enzyme called ferrodoxin-thioredoxin reductase. The enzyme contains a 4Fe-4S cluster that couples two one-electron oxidations of reduced ferrodoxin to one two-electron reduction of thioredoxin.

- In the dark phosphoribulose kinase and glyceraldehydes 3-phosphate dehydrogenase are inhibited by association with a small protein called CP12. NADPH disrupts this association, leading to the release of the active enzymes.

- The C₄ pathway in tropical plants accelerates photosynthesis by concentrating CO₂. Four carbon compounds such as oxaloacetate and malate carry CO₂ from mesophyl cells, which are in contact with air, to bundle-sheath cells, which are the major site of photosynthesis. ATP is used in the process. This is important at high temperature, when the oxygenase action of rubisco becomes more and more significant.

The pathway can also be used to temporally rather than spatially separate the absorption of CO₂ and its utilization in plants growing in arid environments, whose stomata have to close during the day and therefore cannot absorb CO₂. In such plants, C₄ sugars are made from absorbed CO₂ at night, and this CO₂ is released during the day.