The Clavin 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 **photsynthesis** 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**.

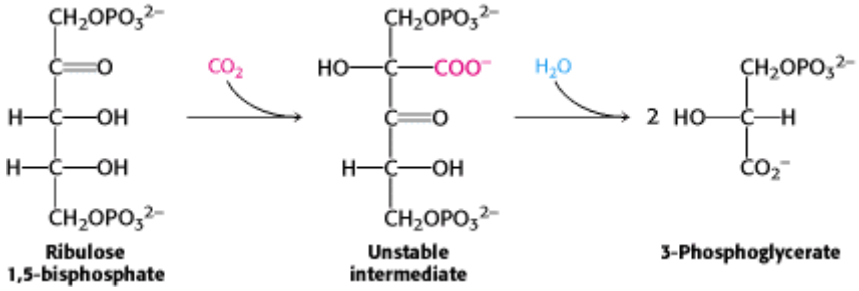
The Calvin Cycle

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**.

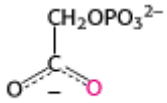
Rubisco is the **most abundant** enzyme, and probably protein, in the **biosphere**, probably because it's **so slow**. It is a **hexadecamer** of **eight large** (containing the active site) and **eight small** subunits.

Initial measurements of $\boldsymbol{K_m}$ 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 $\mathbf{Mg^{2+}}$ ions and an **alkaline pH**, and has to be **reduced**. The steps in the **preparation** of the enzyme are:

1) A $\mathbf{CO_2}$ binds to the **uncharged ε-amino group** of **lysine 201** to form a **carbamate**. This is catalysed by **rubisco activase** [though this also occurs *slowly* when uncatalysed].
2) The $\mathbf{Mg^{2+}}$ can then bind to this **negatively charged carbamate**.
3) The $\mathbf{Mg^{2+}}$ 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 $\mathbf{CO_2}$ 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 $\mathbf{O_2}$. In such a case, as well as **3-phosphoglycerate**, a molecule of **phosphoglycolate** is produced:



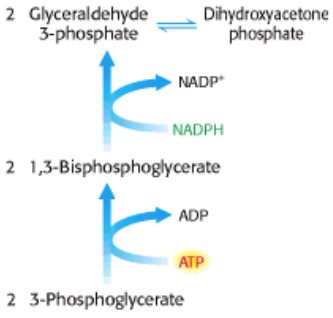
This is problematic, because **phosphoglycolate** is *not* a very versatile metabolite, and this it results in the **loss of CO_2** from the cycle. The cell is partially successful in recovering the carbon by turning **two** such molecules into **one** molecule of 3-phosphoglycerate – a 75% recovery.

Note, however, that since **rubisco** requires **binding of CO_2** to be bound, it will *not* simply mass-produce **phosphoglycolate** if **CO_2** is lacking. The process will simply **pause**.

This process is called **photorespiration**, because **O_2** is consumed and **CO_2** 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**.

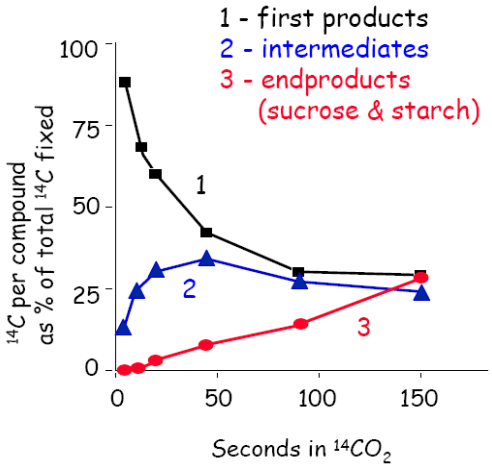
The **stoichometry** of these reactions is:

- **3 CO_2** 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 **CO_2** to **hexose level**.

Experimental Evidence

Experiments were carried out with **radiolabelled carbon compounds**, to establish how **sugar monomers** are made from **CO_2**. The following results were obtained:

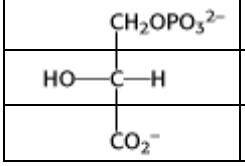


Where:

- was found to be **3-phosphoglycerate**.
- 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.
- were **sucrose** and starch**.**

Further progress came from the **determination** of the **distribution of** ^{14}**C within the *individual molecules***:

- Within **3-phosphoglycerate** (**3-phosphoglyceric acid**)

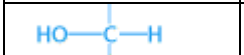
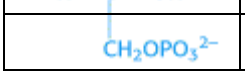
	% of label after	
	5 seconds	**30 seconds**
	2.5	25
	2.5	25
	95	50

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**

	% of label after 5 seconds
	3
	3
	43
	42
	3
	3

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 **C_4 pathway** in **tropical plants** accelerates photosynthesis by **concentrating CO_2**. Four carbon compounds such as **oxaloacetate** and **malate** carry CO_2 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 **CO_2** and its **utilization** in plants growing in **arid environments**, whose **stomata** have to **close** during the day and therefore cannot absorb CO_2. In such plants, C_4 sugars are made from **absorbed CO_2** at **night**, and this **CO_2** is **released** during the day.