**Is photorespiration an effective mechanism for protecting against photoinhibition?**

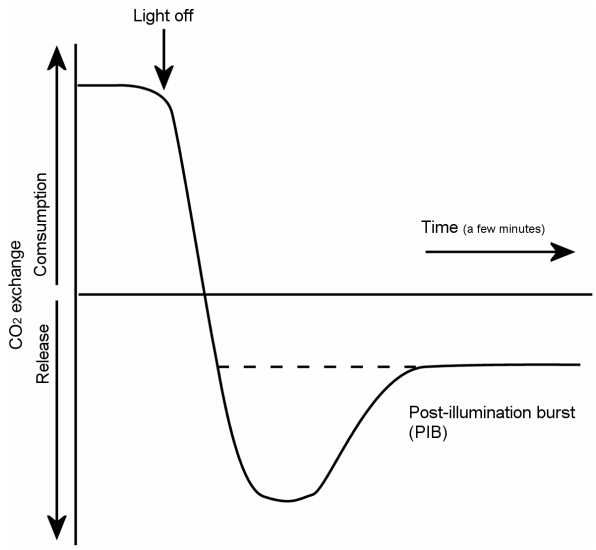
The sessile nature of plants means that they must encounter everything the environment has to throw at them. Most of their life is spent on the resource poverty line, having to make do with what little they have available to them. It would therefore be expected that plants would relish an opportunity to saturate themselves in resources. This is not always the case, the quotation "you can have too much of a good thing" is particularly relevant when talking about a plants response to sunlight. Too little photonic energy will cause photosynthesis to cease and result in starvation as it cannot fix atmospheric carbon, too much will create high energy molecules in the plant capable of doing permanent damage to the photosystems. However plants have not survived for 400 million years without evolving a couple of tricks up their sleeve. In this essay I shall describe how an apparently wasteful process known as photorespiration might play an important role in protecting plants from photoinhibition, setting all of this in the context of the history of their discovery and supplying evidence both for and against photorespirations photoprotective role.

## Background

*Photorespiration*

Photorespiration is the light dependent consumption of $O_2$ and the release of $CO_2$ due to the oxygenase reaction of Rubisco. The first indications that there was a light dependent $O_2$ consumption process came from observations made by Warburg. He noted that altering the levels of $[O_2]$ affected the rate of photosynthesis. It was possible to bring levels down to almost 1-2% $[O_2]$ without affecting mitchondrial respiration and yet still increasing the rate of photosynthesis. Higher concentrations of $O_2$ (>40%) appeared to decrease the rate of photosynthesis. These observations suggested that $[O_2]$ has an inhibitory effect on the rate of photosynthesis.

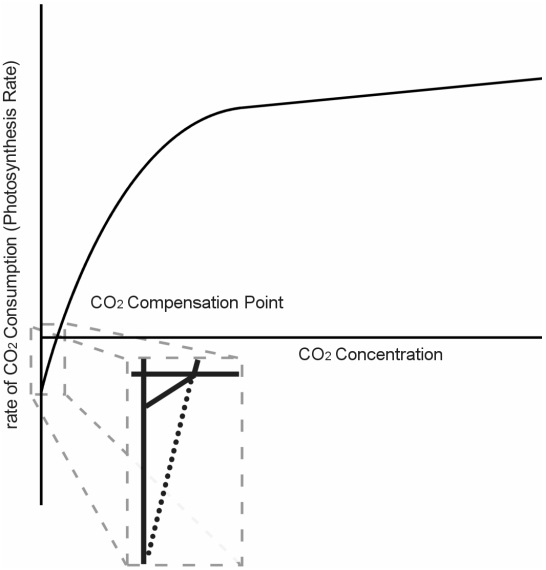
More evidence came from work done by Decker (1955) on the rate of $CO_2$ consumption *figure 1*.




He noted that there was a transient burst of $CO_2$ release soon after the lights were turned off, then return to a steady rate of $CO_2$ release. This was called the Post Illumination Burst (PIB).

Decker deduced that there must be some sort of respiratory process ($CO_2$ release) taking place that was faster than the steady state of respiration in the darkness. It was also able to persist a few minutes after the leaves were exposed to the dark.

Looking at the photosynthesis rate with respect to the [$CO_2$] gave more evidence for the existence of a light dependent respiration process *figure 2*.




The graph clearly shows a positive relationship between the amount of $CO_2$ and the rate of photosynthesis. However evidence stems from a section of the graph known as the $CO_2$ compensation point, where the rate of photosynthesis equals the rate of mitochondrial respiration.

When [$CO_2$]s were studied below the compensation point Canvivn found that the levels of $CO_2$ consumption were not as low as expected (the dotted line). This implied that there was a process other than mitochondrial respiration that released $CO_2$ which the plant was using for respiration rather than the controlled [$CO_2$] supplied. Canvivn (1979)

Evidence for a metabolic pathway behind this light dependent respiration came from some of the first work done on Carbon assimilation using $^{14}CO_2$. Plants that were labelled with radioactive carbon showed its inclusion into all the intermediates of the predicted Calvin cycle but also into two compounds, glycollate 2-phosphate (is this the same as phosphoglycollate, I think it is) and glycollate that could not be placed in the cycle.

Work done on glycollate metabolism revealed a relationship between changes in the PIB shape and duration and the levels of endogenous glycollate under different environmental conditions (the higher the temperature the larger and longer the PIB) (Sharkey 1988). The conclusion was that glycollate metabolism gave rise to photorespiratory $CO_2$.

Questions still surrounded the elements contained within the pathway, particularly "where doe the glycollate come from" clearly it must be involved in a carbon assimilatory pathway otherwise it would not have shown up in the $^{14}CO_2$ pathway. Diligent work revealed that the source of glycollate was non other than Rubisco. Rubisco is primarily responsible for the reaction between $CO_2$ and ribulose-1,5-bisphosphate (5C) in the Calvin cycle (Carboxylase reaction) but it also catalyses an Oxygenase reaction, creating a triose phosphate (3C) and glycollate-2-phosphate (2C) from $O_2$ and ribulose-1,5-bisphosphate. Sharkey correctly hypothesised that the oxygenase reaction of rubisco would increase with increasing temperature because of the increase in solubility of $O_2$ relative to $CO_2$ and the specificity of rubisco for $O_2$ over $CO_2$.

Plants are unable to use glycollate as a respiratory substrate and so must convert it glycerate-3-phosphate. However in so doing it costs 25% of the Carbon entering the pathway (as $CO_2$ loss) as well as the loss of $NH_3$ and the consumption of ATP and reducing equivalents. The conversion is achieved by the photorespiratory pathway *figure 3*.

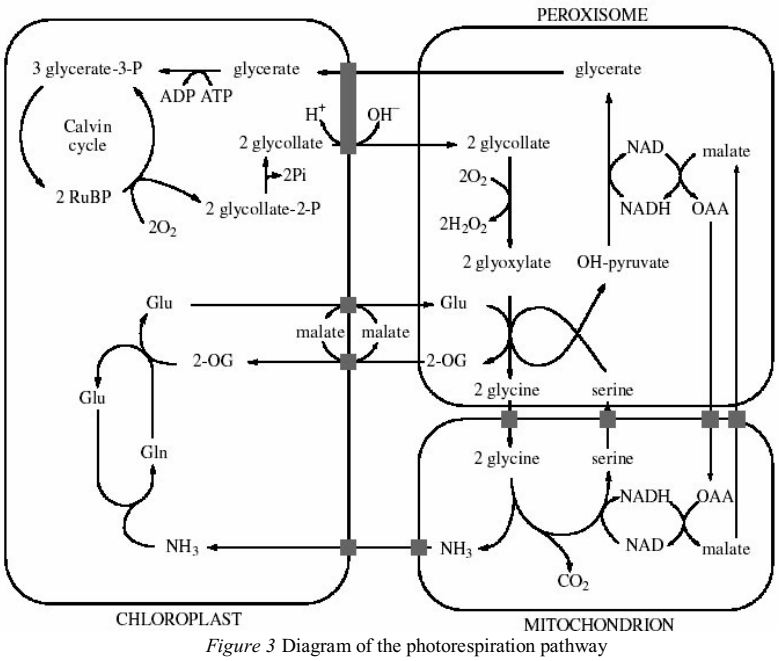



*Figure 3* Diagram of the photorespiration pathway

Glycollate-2-phosphate is hydrolysed to glycollate by chloroplastic phosphoglycollate phosphatase. After transport to the peroxisome, glycollate is oxidised to glyoxylate by glycollate oxidase. Glyoxylate can be transaminated to glycine by serine:glyoxylate aminotranferase (SGAT) or by glutamate:glyoxylate aminotransferase.

In the mitochondria half of the glycine molecules are converted to $N^5N^{10}$-methylene THF in the serine hydroxymethyl transferase (SHMT) reaction to form serine, releasing $NH_3$ and $CO_2$. Since photorespiration occurs at very high rates it has been estimated that the production of $NH_3$ by photorespiration is at least an order of magnitude greater than the primary assimilation of nitrogen from nitrate reduction (Keys *et al* 1978). Because of this the $NH_3$ immediately enters the GS-GOGAT cycle in the chloroplast for reassimilation. From a control point of view the system also exhibits interdependence of reactions one part of the cycle relying on the output of another. Thus the conversion of glyoxylate to glycine requires the availability of amino groups resulting from the recycling of nitrogen by the GOGAT cycle.

After transport back into the peroxisome serine is converted by SGAT to hydroxypyruvate which is reduced to glycerate by hydroxypyruvate reductase (HPR). Glycerate is finally transported back to the chloroplast in which it is converted into glycerate-3-phospahate (G-3-P), which enters the Calvin cycle.

In summary, the photorespiration pathway takes the metabolically useless glycollate produced by the oxygenase activity of Rubisco and converts it into G-3-P, which can re-enter the Calvin cycle. This is achieved at the cost of ATP and reducing molecules, $CO_2$ and $NH_3$ (or is it $NH_2$ I have read it differently in different papers ). How could such an energetically unfavourable process remain in plants without being selected against? There are many hypotheses for persistence photorespiration, ranging from an unavoidable evolutionary artefact which hijacked alongside carboxylase Rubisco action in a high [$CO_2$]

environment to providing important metabolic intermediates, although there has been no conclusive evidence for all plant species.

**Photoinhibition**

Although plants require light for photosynthesis there are dire consequences for them if they are exposed to light intensities in excess of those that can be used. Excess light energy can result in photo-oxidative damage to the photosynthetic apparatus and other cell components. Photoinhibition was defined by Kok as "the debilitating effect of high intensities of visible light on the photosynthetic capacity of green organisms.

Problems arise because the excess light energy excites electrons in the photosystem complex without there being the usual terminal electron acceptor to accept them. The high-energy electrons soon find another oxidising agent to reduce and it is these species which can cause the damage. The two most common outcomes are: (I am not sure how singlet oxygen fits into the grand scheme oft things as I have only seen references to damage cause by superoxide radicals )

1. *Cytochrome b/f complex reduction*. This occurs when photosystem II ( PSII) is working harder than photosystem I (PSI), resulting in a backlog of electrons and a reduction of the cytochrome b/f complex. Because the complex is highly reduced it can no longer accept electrons and so free $O_2$, acts as the oxidant creating singlet oxygen.

$$O_2 \xrightarrow{\text{High-energy } e^-} {}^1O_2 \text{ Singlet Oxygen}$$

2. *NADP reduction*. If NADPH is not used as fast as it is being produced then there will be no available NADP left to act as the terminal electron acceptor for PSI. This results in creating of a much more dangerous species than before, a superoxide radical.

$$O_2 \xrightarrow{\text{High-energy } e^-} O_2^{\bullet} \text{ Superoxide Radical}$$

   This is quickly dealt with via superoxide dismutase (SOD), but in so doing it creates $OH^{\bullet}$ which is much more dangerous and can lead to DNA damage, protein modification or even the exponential process of lipid peroxidation.

Plants avoid photoinhibition in two ways; by either lowering their ability to absorb light, reducing the efficiency of their photosystems, or they find ways of dissipating the energy absorbed by the photosystem through non-assimilatory electron transport.

*Reducing photon absorption*

Reducing the efficiency of light capture can be achieved on a developmental, cellular and biochemical level in plants. Developmentally differences in the leaf anatomy of sun and shade leaves goes further than simply altering the thickness and the available leaf area but also resource partitioning is tuned to increasing light capture in shade leaves and increasing Calvin cycle components in sun leaves. There are also examples of negative heliotropism, plants bending away from bright light in order to protect

themselves. Within the photosynthetic cells themselves orientation of the chloroplasts largest side away from the light reduces the efficiency of light capture.

However these methods may not be sufficient and so plants also use biochemical pathways to reduce efficiency, mainly by converting the excess light energy to heat before it can excite an electron. Chlorophyll can be de-excited in order to increase the intensity of light at which photosynthesis begins to plateau off. Certain photoprotective chemicals can be produced through the phenylpropanoid pathway which aid in absorbing harmful light radiation (whether this is only for non visible light i.e. UV protectants I am not sure). The increase in intrathylakoidal space [$H^+$] as a side effect of increased photosynthetic activity results in the interconversion, via epoxidation, of high efficiency pigments (Violaxanthin) to lower efficiency ones (Zeaxanthin). The opposite also occurs and evidence for this process is a correlation between the ratio of pigments and the energy dissipation of the system.

During long-term exposure to high light intensities the most drastic method of reducing photosystem efficiency is the sacrificial protection PSII undergoes in order to protect PSI from photoinhibition. PSII contains a metabolically in expensive polypeptide D1, which, much like the crumple zone of a car, is designed to become photochemically damaged the expense of the safety of the other components of the photosystems (as PSII lies before PSI). D1 is then quickly proteolysed, PSII degraded, a new D1 synthesised and then PSII is rebuilt.

*Non-Assimilatory Electron Transport*

Photorespiration has been thought to act in this mechanism in order to protect the plant from photoinhibition. It provides a perfect cycle for the system to burn off extra energy, in the form of ATP and NADPH. Theoretically the glycollate pathway makes it possible for the Calvin cycle to turn over without net gain of carbon with the normal input of photochemical energy per turn of the cycle (Osmond *et al* 1995). Correlative observations have already shown that an increase in heat (usually along with an increase in light intensity) favours photorespiration but more quantitative evidence is required. Also the intermediates of the cycle (particularly the amino groups) can be siphoned off into other pathways to create pigments (or perhaps the phenylpropanoids?) which would also aid in photoprotection.

Previous studies have involved reducing the [$O_2$] down to below 1-2%, thus inhibiting the oxygenase activity of Rubisco. Whilst this has provided us with good correlative data, what is really needed is an example where different levels of photorespiration compared to wild type are obtained and the overall contribution it has to protection. One such study was performed by Kozaki and Takeba (1996). They constructed transgenic tobacco plants enriched or reduced in plastidic glutamine synthase (GS2). Modifying the amounts of GS2 would alter the rate of photorespiration and thus the supply of intermediates to the Calvin cycle. They hypothesised that an increase of GS2 leading to an increase in photorespiration flux would lead to an increase in the flux of metabolites through the Calvin cycle, burning more energy and protecting the plant from photoinhibition. Insufficient GS2 and high light intensity would lease to an accumulation of ammonia and a depletion off the amino acid donor, which would slow down the Calvin cycle and lead to photoinhibition.

Results showed that plants with twice the wild type level of GS2 had an improved capacity for photorespiration and an increased tolerance to high intensity light. Those with a reduced amount of GS2 had a diminished capacity for photorespiration and were photoinhibited more severely by high intensity light compared with control plants.

However altering a specific enzyme in the middle of the photorespiratory pathway may not be the most accurate way of determining the contribution to protection against photoinhibition. GDC mutants mentioned in Winger (2000) have been found to bypass the normal photorespiratory pathway by oxidative decarboxylation of glyoxylate and the formation of serine from formate, thereby compensating for the lack of GDC activity. The redundancy seen in the pathway may extend to all the downstream enzymes of Rubisco and so in order to ensure an accurate analysis the oxygenase activity of Rubisco must be altered through genetic manipulation. (There must be some examples out there although admittedly I have not looked, could we talk about a couple? )

However there is evidence to suggest that removal of the photorespiratory pathway may lead to reduced rates of photosynthesis.

1. An impairment of the recycling of carbon in the photorespiratory pathway could result in the depletion of Calvin cycle intermediates. The first indications of this were that when mutants were fed with intermediates that they were unable to synthesise in the photorespiratory pathway they had partial activity of the Calvin cycle restored. For example partial activity was restored in GS2 mutants fed with glutamine.
2. An impairment of the photorespiratory nitrogen reassimilation could result in a decline in the nitrogen status of a leaf and a reduction in the amount of photosynthesis proteins. This has already been mentioned in the GS2 mutant where $NH_3$ production increased whilst glutamine content decreased.
3. Accumulation of photorespiratory metabolites could have a feedback on Calvin cycle activity. There is little evidence that accumulation of $NH_3$ in GS2 mutants directly inhibits photosynthesis by uncoupling photosynthetic electron transport. This was shown by feeding the plants glutamine and seeing a partial restoration of photosynthesis despite the elevated levels of $NH_3$. Also there is little evidence for serine playing a part because in protoplast cultures deficient in SGAT serine levels rose up to nine fold before photosynthesis was affected. Of all of the studies performed it appears that glyoxylate is most likely to have an effect as *in vitro* studies have revealed a negative correlation between glyoxylate levels and Rubisco activation (Häusler *et al* 1996).

There are doubts regarding the contribution photorespiration makes to protection from photoinhibition. Firstly that photorespiration occurs even in low light levels, what is the point of it working in these conditions, it is truly wasting energy. Secondly studies of the GS mutants by Kozaki and Takeba (1996) offer no exact explanation for the increase in photorespiration and it is thought to be due to some sort of pleitotropic effect (this was taken directly from you lectures and I am a bit confused about this ). Thirdly doubts stem from work on the oxygenase activity of Rubisco from non-oxygenic photosynthetic bacteria, which are obligate anaerobes (????? not mentioned in lectures). The bacteria have modified their light harvesting complexes so that they do not release $O_2$ and so should not be susceptible to photorespiration and yet the oxygenase activity is even higher when

tested (couldn't his be an artefact much like the hypothesis that oxygenase activity is hijacking along with carboxylase activity and the bacteria were able to deactivate it whilst plants made use of it ).

However one of the greatest shadows cast over the photorespiration camp is that there is already another non-assimilatory electron transport chain in place that also deactivates the oxidising agents produced by the photosystems in times of excess light. This is called the Asada (or Water Water) pathway and it occurs so fast that the active oxygen's are unable to damage any of the photosystems.

1 $2H_2O \rightarrow 4e^- + 4H^+ + O_2$ (from photolysis of water)
2 $2O_2 + 2e^- \rightarrow 2O_2^{\bullet}$ (photoreduction of $O_2$ in PSI)
3 $2O_2^{\bullet} + 2H^+ \rightarrow H_2O_2 + O_2$ (SOD-catalysed disproportionation of $O_2^{\bullet}$)
4 $H_2O_2 + 2\text{Ascorbate} \rightarrow 2H_2O + 2\text{MDA}$ (APX-catalysed reduction of $H_2O_2$)
5 $2\text{MDA} + 2e^- + 2H^+ \rightarrow 2\text{Ascorbate}$

*Note 2 and 3 are also known as the Mehler reaction*

Despite its apparently complex steps all this cycle does is convert light energy (in the form of an excited electron) into heat energy, all the elements are returned to their native states, hence the term "Water Water" cycle.

$$\Sigma\ 2H_2O + O_2^{\bullet} \rightarrow O_2 + 2H_2O$$

The Asada cycle also creates a proton gradient which can be used to create ATP which is not consumed in the cycle itself. The creation of the proton gradient also serves to convert high efficiency pigments into lower efficiency ones as previously mentioned.

The major difference between the two non-assimilatory electron transport pathways is that whilst photorespiration uses ATP and NADPH the Asada pathway generates ATP. Both have suspected secondary roles in promoting photoprotection and I believe that a mix of the two is required for effective protection against photoinhibition. The subtle differences described above contributing to this, if there is an excess of ATP and NADPH then photorespiration can be used to lower their levels whereas if there is too much light and active oxygen species are being produced then the Asada pathway will come into play. It is not possible for one to exist without the other and that this mix is dependant on the environment. Heber *et al* (1996) found that in *Chenopdium bonus* photorespiration is essential for maintaining electron flow in high light whilst the Asada pathway was insufficient to prevent photoinhibition. (I do not know if they showed the photorespiration was *sufficient* in this species).

## Discussion

Photorespiration is apparently here to stay. What is the exact origin for the oxygenase activity of Rubisco, are they here today because of their role in photoprotection or usefulness in providing intermediates for the Calvin cycle or is it here merely as an evolutionary hijacker to the selective advantage conferred by the carboxylase activity and plants have merely made the best of a bad situation. I believe it is a mixture of the two although I am sure that in time the answers will reveal themselves. As for photorespirations role in protecting against photoinhibition there is enough evidence to establish in some plants that it is used however undoing the tangled knot of photoprotection control may take a long time.

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