

CHAPTER 5

PHOTOSYNTHETIC CARBON ASSIMILATION

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I have discovered what I have long been in quest of, viz, the process in nature by which air rendered noxious by breathing, is restored to its former salubrious condition.

Joseph Priestley, letter to Philus Lindsey, August 1771.

Green plants, and therefore ultimately all other higher forms of life, depend on organic carbon derived from carbon dioxide in processes driven by light energy. Central to every aspect of such photosynthetic carbon assimilation by leaves lies the Calvin cycle. In this cyclic sequence of reactions, carbon dioxide is added to an acceptor molecule. The newly formed addition compound then undergoes a series of changes, including reduction, so that a stable product is formed and set aside. At the same time, the CO₂-acceptor is regenerated and new molecules of acceptor formed so that the process may continue and the green plant may grow.

5.1 Environmental and metabolic role

Remarkably, there is a still a popular belief, reinforced by gardeners who habitually talk about 'feeding' their plants, that green plants obtain most of their nutriment from the soil. In fact, the fallacy of this supposition was demonstrated as long ago as the seventeenth century by van Helmont, who grew a willow in a tub of weighed soil. After watering for five years, the willow weighed 164 lbs. more and the soil only 2 ounces less. This is in accord with contemporary measurements, which show that

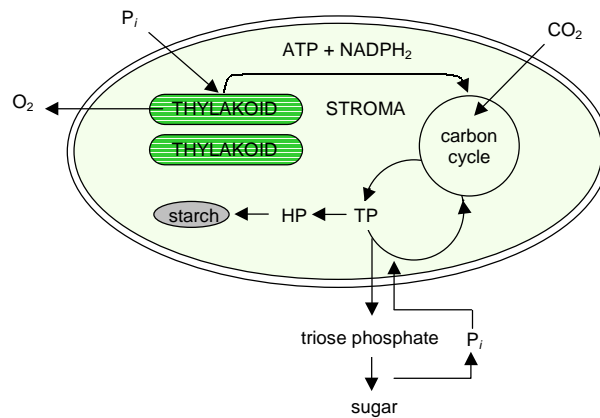


Figure 5.1 A stylised chloroplast bounded by an inner and outer envelope of protein/polar lipid membranes. Because these are devoid of chlorophyll but retain a carotenoid composition similar to that of the thylakoids, they appear yellow when isolated. The inner envelope houses the phosphate translocator that controls the movement of metabolites into and out of the chloroplast. The thylakoid membranes, in which the chlorophylls and other components of the photosynthetic electron transport system are located, are embedded in the stroma, which also contains the soluble enzymes of the Calvin cycle (Section 5.3). The Calvin cycle utilises ATP and NADPH₂, produced by photosynthetic electron transport, to form 3-carbon sugar phosphates (triose phosphates) from CO₂. Such triose phosphates (TP) are either exported through the chloroplast envelopes, via the phosphate translocator, in exchange for external inorganic phosphate (P_i), or utilised in the Calvin cycle or in starch synthesis. Exported triose phosphates are mostly consumed in sucrose synthesis in the cytosol (the aqueous gel, containing many more soluble enzymes, in which the chloroplasts, mitochondria and other cellular organelles are suspended). Sucrose synthesis, like starch synthesis and some of the reactions of the Calvin cycle, liberates P_i needed for the continuation of ATP formation by photochemistry in the thylakoids.

about 90% of the dry weight of green plants is comprised of carbon and oxygen derived from the air by photosynthesis and usually less than 5% (nitrogen and minerals) from the soil.

Photosynthesis, more properly called 'oxygenic photosynthesis' to distinguish it from forms of bacterial photosynthesis that do not liberate oxygen, is not only by far the major source of organic carbon in the biosphere but also the principal mechanism of biological energy transduction. It is the means by which light energy is converted into electrical energy and then into chemical energy. In essence this is achieved by breaking H–O bonds in water (H₂O), liberating the oxygen (O₂) and passing the hydrogen (H₂) through a series of intermediates to carbon dioxide (CO₂), in the process forming carbohydrates such as starch and sucrose, as shown in Fig. 5.1. In plant and animal respiration, this process is largely reversed: oxygen is taken up, carbohydrates are consumed, carbon dioxide is liberated, H–O bonds are re-

established and energy is made available for other metabolic processes. It was oxygenic photosynthesis that created our present atmosphere and continues to maintain it. Oxygenic photosynthesis was also the source of our fossil fuels (Edwards and Walker, 1983; Walker, 1993).

5.2 Chloroplast and cell

In the final stages of photosynthesis, atmospheric carbon dioxide is assimilated and carbohydrates are formed (for details on mechanisms of acquiring and assimilating carbon dioxide, see Edwards and Walker, 1983; Winter and Smith, 1996; Sage and Monson, 1999 and Leegood *et al.*, 2000). As shown in Fig. 5.1, these events are separated in time and location from the photochemical events that occur in the thylakoid membranes of the chloroplast. Carbon dioxide fixation is sometime called the ‘dark biochemistry’ of photosynthesis, as shown in Fig. 5.2, in order to distinguish it from the ‘photochemistry’, or light reactions but, while it is true that many of the component reactions of CO₂ assimilation can be made to proceed in the dark, they are

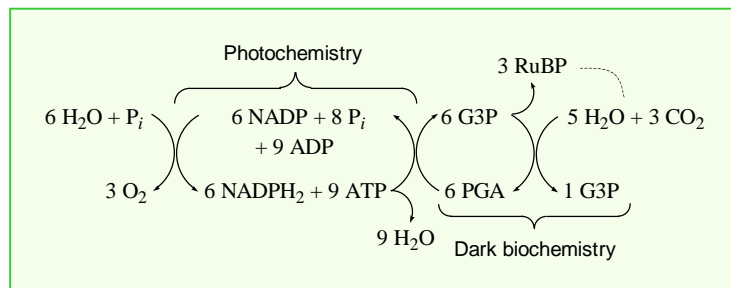


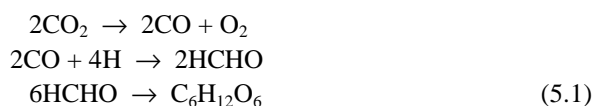
Figure 5.2 Light and dark events in photosynthesis. The first product of photosynthetic carbon assimilation (CO₂ fixation) in the Calvin cycle is 3-phosphoglycerate (PGA), a three-carbon acid. This is converted to a corresponding three-carbon sugar phosphate called glyceraldehyde 3-phosphate (G3P) in a reductive process that consumes two-thirds of the ATP and all of the NADPH₂ generated by the photochemistry. The rest of the ATP is utilised in the regeneration of the CO₂-acceptor, ribulose-1,5-bisphosphate (RuBP) in a process by which five 3-C compounds (triose phosphates) are rearranged to give three 5-C carbon compounds (this is discussed in more detail in Section 5.4.4). The ATP and NADPH₂ generated by photochemical events in the light are consumed in the ‘dark biochemistry’—so called because none of the biochemical reactions involved have an absolute requirement for light even though, in a photosynthetic context, they normally occur only in the light and are often made more active in the light by direct or indirect means (Woodrow and Berry, 1988). Triose phosphates not used in regenerating the CO₂-acceptor undergo subsequent transformations to starch, sucrose *etc.*

all an integral part of the photosynthetic apparatus within the living organism. This chapter describes the manner in which carbon dioxide assimilation is linked to the photochemistry on which it depends and which, in turn, it influences.

Many of the component reactions of CO₂ assimilation and their associated enzymes are physically located in the stroma in which the thylakoid membranes are embedded (Fig. 5.1). All are separated from the surrounding cytosol by the chloroplast envelopes. Nevertheless, events such as sucrose synthesis that occur in the cytosol beyond the chloroplast may be properly regarded as aspects of photosynthesis. Similarly, all aspects of photosynthetic carbon assimilation and much of the photochemistry depend on, or are influenced to a greater or smaller extent by, events that occur outside the chloroplasts.

5.3 C₃ photosynthesis in its relation to the photochemistry

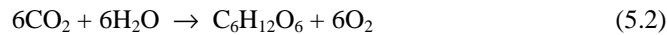
In 'C₃ photosynthesis', the first product of carbon assimilation contains 3 atoms of carbon, as illustrated in Fig. 5.2. In 'C₄ photosynthesis' (C₄ and CAM plants, discussed in Sections 5.7.1 and 5.7.2), we shall see that the first product of CO₂ fixation contains 4 atoms of carbon. Central to both C₃ and C₄ photosynthesis lies the Calvin or Benson–Calvin cycle, the complex metabolic pathway by which plants incorporate CO₂ as carbohydrates.¹ Why, we might ask, did evolution favour the complexity of the Calvin cycle when paper chemistry offers simpler alternatives such as the direct formation of formaldehyde (HCHO), as first proposed by Baeyer in 1870 (eq. 5.1; see Rabinowitch, 1945 for a fuller discussion).



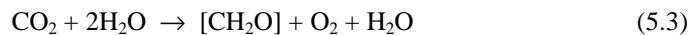
It is tempting to suppose that the more complex solution was favoured because the energy required for carbon assimilation could be provided by ATP, already utilised by anaerobic bacteria as a means of energy conservation at the time that these cyanobacteria, destined to be the precursors of modern chloroplasts, evolved. (For discussion of the unique position of ATP in intermediary metabolism, see Banks and Vernon, 1970.)

¹ The late Melvin Calvin received the Nobel Prize for his work on the elucidation of this metabolic sequence; Andy Benson's name is added by those who rightly wish to acknowledge the contribution of this, his most senior, colleague.

Photosynthetic carbon assimilation (PCA) has often been summarised by the equation



This portrays a reaction sequence, driven by light energy, in which carbon dioxide (CO_2) and water (H_2O) are consumed, oxygen (O_2) is liberated and glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) is the end product. However, although sugars such as glucose and fructose (both of which have the same empirical formula) may eventually appear in plant leaves as a result of photosynthesis, they are not formed directly from carbon dioxide and are not even particularly important compounds in leaf metabolism. A more realistic equation, in which $[\text{CH}_2\text{O}]$ represents a nominal carbohydrate, and molecules of water are included on both sides of the equation, takes account of the fact that the oxygen that is released is derived, not from CO_2 , but from water.



Indeed, with one minor addition, such an equation adequately summarises the combined reactions of the Calvin cycle (Edwards and Walker, 1983; Walker, 1992a) but any such simplification inevitably masks important features, not least the question of precisely where in a leaf photosynthesis occurs and how it is driven and controlled. Here we shall take what we regard as the conventional view: that photosynthetic electron transport is driven by light, that it leads to the formation of ATP and NADPH_2 , and that these are then consumed in the reduction of CO_2 to triose phosphate within the chloroplast.

In terms of energy inputs, we can ascribe a value ($\Delta G'$) of about 29 kJ (7 kcal) to ATP formation (*cf.* Banks and Vernon, 1970) and 218 kJ (52 kcal) to NADPH_2 formation. As represented below, the Calvin cycle consumes 9 molecules of ATP and 6 molecules of NADPH_2 in order to produce one molecule of triose phosphate product (designated G3P in Fig. 5.2) In terms of ATP and NADPH_2 , this would be equivalent to an energy consumption of 1569 kJ (375 kcal). Burning glucose ($\text{C}_6\text{H}_{12}\text{O}_6$) in a calorimeter yields 2822 kJ (672 kcal). This would allow us to put a value of $2822/6 = 470$ kJ (112 kcal) on $[\text{CH}_2\text{O}]$ in eq. 5.3 and a corresponding value of $1569/3 = 523$ kJ (125 kcal) of energy consumed for each molecule of CO_2 incorporated into product in Fig. 5.2. This is consistent with what is known about partial reactions in the entire sequence and implies a high degree of efficiency. However, calculating free energy changes *in vivo* is inevitably something of an approximation depending, as it does, on factors such as the prevailing Mg^{2+} concentration that can rarely be determined with certainty.

5.4 The Calvin cycle

The experiments in which Calvin and his colleagues fed radioactive carbon dioxide to *Chlorella*² implicated the participation of a number of sugar phosphates and related acids in photosynthetic carbon assimilation. Several schemes were considered before everything finally fell into place and the now well-established features of the Calvin cycle became clear. These features are the carboxylation of a 5-carbon sugar phosphate (the CO₂-acceptor), the reduction of the immediate product to yield a 3-carbon sugar phosphate, and the regeneration of the acceptor, as shown in Fig. 5.3.

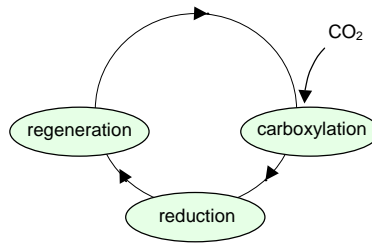


Figure 5.3 Principal features of the Calvin cycle

5.4.1 Carboxylation

A 'carboxylation' is the addition of CO₂ to an acceptor molecule in such a way that a new carboxyl (–COOH) group is formed. In the Calvin cycle, the CO₂-acceptor is a 5-carbon sugar phosphate called ribulose-1,5-bisphosphate (RuBP). The name 'ribulose' indicates that it is a ketose sugar containing a C=O group rather than the aldehyde (CHO) group that characterises the corresponding aldose sugar, ribose. RuBP has two phosphate groups, one at either end (hence 'bis phosphate'). As shown in Fig. 5.4, its carboxylation involves the incorporation of a molecule of water and separation of the addition product into two molecules of 3-phosphoglycerate (PGA). This reaction, in common with others, involves breaking and re-forming bonds. Energy is always used when chemical bonds are broken and released when they are formed. In this instance

² Like Warburg, Emerson, and many others before him, Calvin chose to work with *Chlorella*, a unicellular green alga of great experimental repute. Its advantage was that highly reproducible and uniform cultures could be grown with great ease. Such an algal suspension is, to all effects and purposes, like a green liquid that can be evenly illuminated in a glass 'lollipop' and then run into a killing medium such as boiling ethanol. However, it should be noted that, although the pathway of photosynthesis is the same in algae and higher plants, its exact mode of operation and regulation may not be the same in the two tissues.

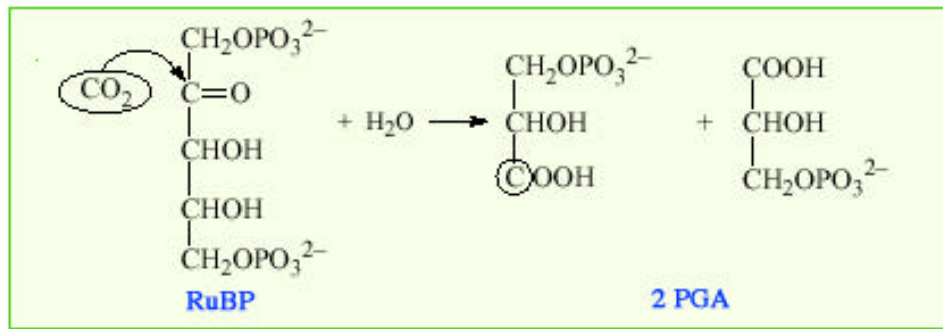


Figure 5.4 The carboxylation of ribulose-1,5-bisphosphate (RuBP). The addition of one molecule of water yields two molecules of 3-phosphoglycerate (PGA).

to the free energy (G^\ominus) of hydrolysis of ATP. Energy is therefore inevitably expended in metabolic carboxylation reactions and this energy bill has to be met (Edwards and Walker, 1983; Walker *et al.*, 1986). In this instance, the energy required at the moment of carboxylation derives from the fact that RuBP is not a 'comfortable' molecule, 'at ease with itself' like carbon dioxide. On the contrary, the molecular structure of RuBP is such that a great deal of internal stress is present. This is relieved by the carboxylation and associated addition of water. As we shall see in Section 5.4.4, this 'uncomfortable' twisting within RuBP, which enables the carboxylation reaction to proceed so effectively in the biological context, is largely imposed by the consumption of ATP in the final stages of regeneration.

Given that the CO₂ concentration in the atmosphere is low (around 0.035%), in addition to having a favourable equilibrium position a biological carboxylation needs to be catalysed by an enzyme with a high affinity for CO₂ (but see sections on C₄ and CAM). When fully activated (see below), the ubiquitous enzyme RuBP carboxylase (rubisco) meets that requirement but it is also subject to competitive inhibition by O₂. The reaction that follows, when O₂ wins this competition, yields one molecule of PGA and one of 2-phosphoglycolate (Keys, 1986). This oxygenation is the biochemical initiator of a form of photorespiration (Section 5.6) and it is for this reason that rubisco is more properly referred to as ribulose-1,5-bisphosphate carboxylase-oxygenase. It occurs in large amounts in leaves and is believed to be the most abundant protein on earth (Ellis, 1979; Ellis and Gray, 1986).

5.4.2 Mechanism

During catalysis by rubisco, the ketose form of RuBP binds to the enzyme and is converted to the enol form (see Fig. 5.5). CO_2 and O_2 then compete to react with the enzyme-bound enol form of RuBP. Carboxylation of enol-RuBP generates 3-keto-carboxyarbinol biphosphate, which is hydrated, and then two molecules of PGA are formed by C–C cleavage between C-2 and C-3. Oxygenation at the C-2 position of RuBP produces a hydroperoxide, which is then converted to P-glycolate and 3-PGA by C–C cleavage between C-2 and C-3, as shown in Fig. 5.6 (for details of other proposed intermediates, see Taylor and Andersson, 1997).

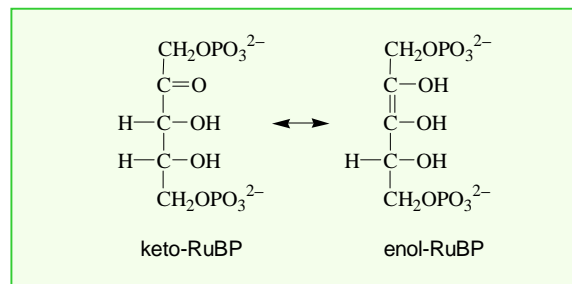


Figure 5.5 Conversion of keto-RuBP to enol-RuBP, the first step in rubisco catalysis.

The capacity of rubisco to function as a catalyst is controlled through a unique mechanism of interconversion of the enzyme between active and inactive forms. The enzyme is converted to an inactive form in the dark and an active form in the light. This, along with control of other enzymatic steps, assures that the substrate-consuming reactions of photosynthesis are shut down at night. Control of the degree of activation during the day can prevent the RuBP pool from being depleted in the face of changing environmental conditions. For example, as the light intensity decreases and the energy-dependent process of regeneration of RuBP slows, decreased activation of rubisco can maintain the RuBP pool. The catalytically active form of rubisco is carbamylated by a molecule of CO_2 at a lysine residue in the active site, and that CO_2 is different from the one used as a substrate in carboxylation. This allows binding of Mg^{2+} ion, which is essential for catalysis and completes the activation process, creating the so-called ECM (enzyme- CO_2 -Mg) complex.

The catalytically active ECM complex uses RuBP and CO_2/O_2 as substrates in the light. In addition to its role in metal binding, the carbamate is thought to catalyse the conversion of keto-RuBP to enol-RuBP, the first step in the catalytic process. X-ray

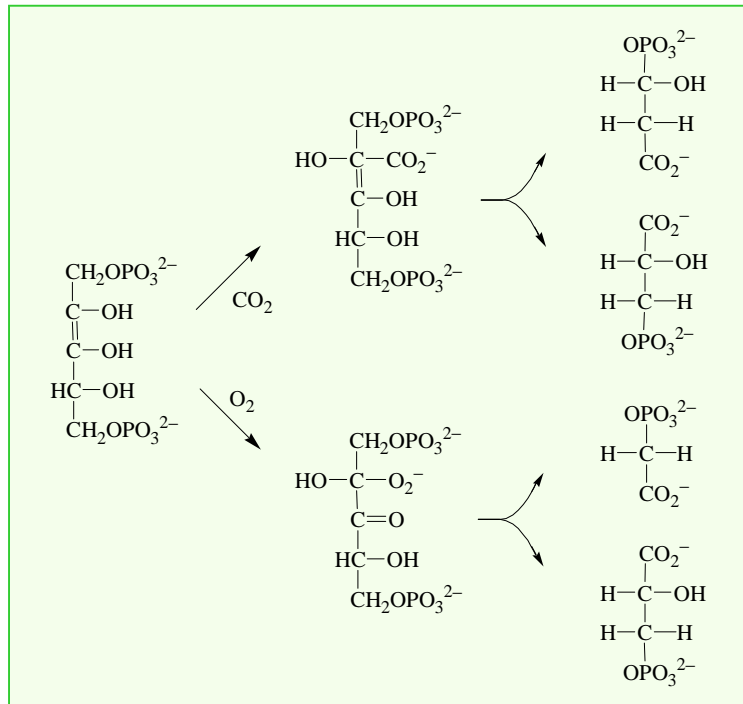


Figure 5.6 Reaction of CO₂ or O₂ with enol-RuBP.

crystallographic analysis of rubisco indicates flexible loop regions close around the active site, sequestering sugar phosphates from the solvent and protecting reaction intermediates; as reaction products are formed the active site reopens. Decarbamylation, and inactivation of the enzyme at night is favoured by the lower Mg²⁺ concentration and pH in the chloroplast stroma. Binding of RuBP to the decarbamylated form of the enzyme in the light has a role in controlling the level of inactive enzyme. RuBP binds to this inactive form of the enzyme, resulting in closure of the active site and formation of a dead-end complex that traps RuBP and prevents carbamylation of the enzyme. ATP and a protein called activase are required to open the traps and dissociate RuBP from this complex. ATP may convert activase to an active conformation so that it can open the closed catalytic site, allow RuBP to dissociate and spontaneous carbamylation to proceed. This indicates that ATP, which is formed in the chloroplast in the light, probably plays an important role in controlling the state of activation of rubisco (Andrews, 1996; Taylor and Andersson, 1997).

5.4.3 Reduction

Once formed by carboxylation, PGA is rapidly reduced to triose phosphate in the two-stage reaction shown in Fig. 5.7. The first stage consumes all of the NADPH_2 and two-thirds of the ATP used in the cycle. Nevertheless it is energetically unfavourable and gives rise to no significant quantities of free glycerate 1,3-bisphosphate (GBP); the overall sequence is made energetically favourable by the subsequent hydrolysis of this bisphosphate. The resulting triose phosphate, glyceraldehyde 3-phosphate (G3P), can be regarded as the immediate end product of the Calvin cycle. Some of it may be stored within the chloroplast, as starch (thereby releasing P_i *in situ*), though not all plant species make leaf starch. Some triose phosphate is exported from the chloroplast (via the 'phosphate translocator') as G3P or dihydroxyacetonephosphate (DHAP) in exchange for inorganic phosphate (P_i) from the cytosol and, once in this compartment, it is commonly consumed producing sucrose which is transported to other parts of the plant. Sucrose synthesis, from triose phosphate, releases P_i , which re-enters the chloroplast, enabling photosynthetic phosphorylation and the continuation of the Calvin cycle. Isolated chloroplasts are not able to synthesise sucrose because they lack the necessary enzymes and would, if not supplied with the P_i that is made available by sucrose synthesis in the living cell, soon cease to evolve O_2 at normal rates (Edwards and Walker, 1983; Walker, 1992a).

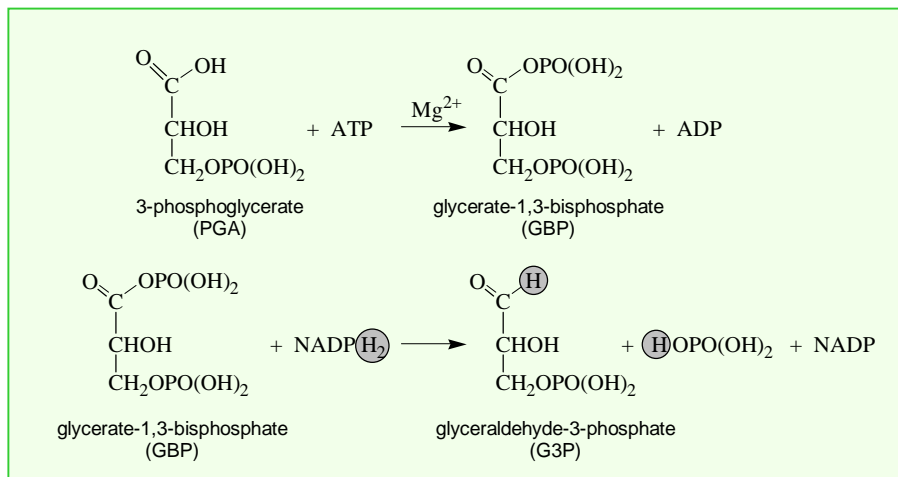


Figure 5.7 The reduction of PGA to triose phosphate (G3P) at the expense of ATP and NADPH_2 produced by photosynthetic electron transport.

5.4.4 Regeneration

Each time CO₂ is added to RuBP, a molecule of that compound is consumed. In order that this process can continue it is necessary to regenerate new RuBP from the newly formed product. Arithmetic demands that, in order to make a 5-carbon CO₂ acceptor from 3-carbon products without loss, it is necessary to convert five threes into three fives, as shown in Fig. 5.8. Accordingly, a large fraction of the triose phosphate formed by reduction of the PGA formed in the initial carboxylation is retained within the chloroplast for this purpose. There follows a sequence of reactions that may at first see very complex. On closer examination a repeated pattern emerges.

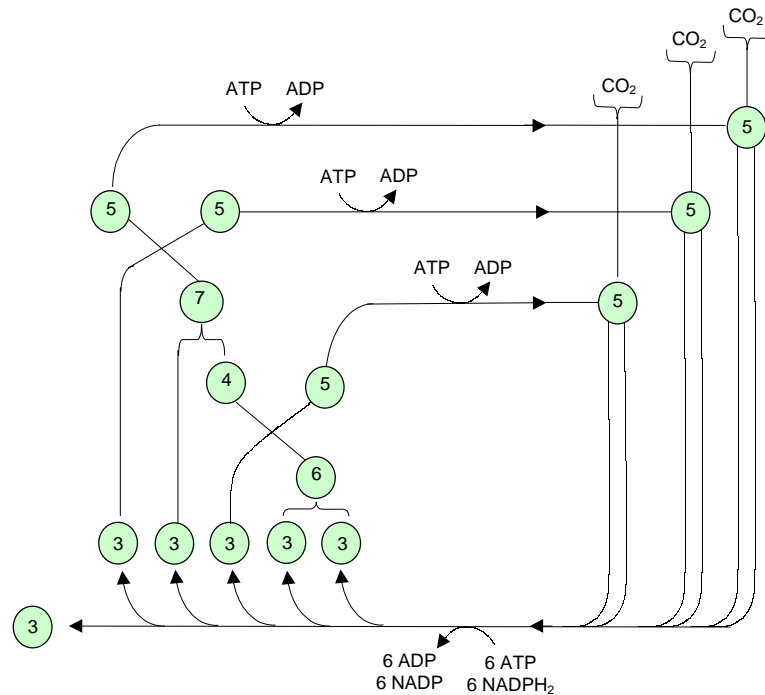


Figure 5.8 The Calvin cycle in outline, showing how three molecules of CO₂ give rise to one molecule of 3-carbon product and how five 3-C molecules are rearranged to give three 5-C acceptors. The 2-C unit transferred (e.g. from a 7-C molecule to a 3-C to give two 5-C molecules) is the ketose glycoaldehyde (CH₂OHCHO), and the enzyme that catalyses its transfer is called transketolase.

Figure 5.9 shows the metabolites involved in these molecular rearrangements. Two types of reaction are involved. The first (eq. 5.4) joins (or condenses) two triose (3-C) phosphates to give one hexose (6-C) biphosphate



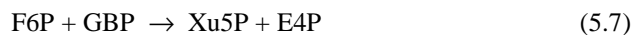
The triose phosphates that are condensed at this stage are G3P and its isomer dihydroxyacetonephosphate (DHAP).



The condensation product is fructose-1,6-bisphosphate (FBP). Reaction 5.5 is classified as an aldol condensation because it involves an aldehyde and an alcohol group. Accordingly the enzyme which catalyses it is called an aldolase. Once formed, FBP is hydrolysed by a specific phosphatase.



A 2-C (ketose) unit is then transferred (as shown in Figs. 5.8 and 5.9) from the top of F6P to G3P, creating new pentose (5-C) and tetrose (4-C) phosphates called xylulose-5-phosphate (Xu5P) and erythrose-4-phosphate (E4P) respectively.



This sequence (eqs. 5.5–5.7) of three reactions (aldol condensation, hydrolysis and 2-C transfer), which is portrayed in the centre of Fig. 5.9, is now repeated, at least in principle. This time, although the condensation involves G3P as before, the 4-C molecule (E4P) also participates (eq. 5.8) so that the product is the 7-C compound sedoheptulose-1,7-bisphosphate (SBP).



This is then hydrolysed by a specific phosphatase to give sedoheptulose-7-phosphate (S7P) and P_i



Finally, a 2-C unit (carbons 1 and 2) is transferred (Fig. 5.9, top left) from the ‘top’ of S7P to the last (G3P) of the five molecules of triose phosphate that participate in this process in which five 3-C sugar phosphates are rearranged to give three 5-C sugar phosphates.



Since G3P is again the recipient of the 2-C ketose unit, one of the products is Xu5P as before (in eq. 5.7), but what is left of a 7-C sugar that loses two of its carbons must be a 5-C compound and, indeed, the second product is the pentose sugar ribose-5-phosphate (R5P).

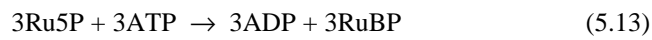
All three pentose phosphates (*i.e.* two molecules of Xu5P and one of R5P) are now converted to ribulose-5-phosphate (Ru5P) in one of two reactions. One of these converts Xu5P to its epimer, and the enzyme concerned is hence called an epimerase.



The other converts R5P to its isomer (eq. 5.12) and the catalyst is called an isomerase



As a consequence of reactions 5.11 and 5.12, three molecules of Ru5P have been created, largely by rearranging 5 molecules of triose phosphate. All that remains is the final phosphorylation, brought about by ATP, which re-creates the CO₂ acceptor, RuBP.



As we have seen, RuBP is an intrinsically unstable molecule. In effect, the energy required to drive the carboxylation in an energetically favourable reaction (*i.e.* one with an equilibrium position which overwhelmingly favours carboxylation, despite the very low concentrations of CO₂ in the atmosphere) is provided by ATP at the time of phosphorylation of Ru5P (eq. 5.13). The remaining one-third of the ATP consumed in the Calvin cycle is used at this point.

5.4.4 The phosphate translocator

It is important not to lose sight of the role played by the phosphatases in regeneration of RuBP (eqs. 5.6 and 5.9) because, in addition to their direct role in this rearrangement, they also contribute to another key aspect of photosynthetic carbon assimilation, *i.e.* the maintenance of P_i availability for ATP formation. The Calvin cycle does not itself produce free sugars. Starch is a sugar polymer and, to the extent that it is formed within the chloroplast stroma in many species, it constitutes a means of releasing P_i for further photophosphorylation of ADP to ATP. Nevertheless, such starch formation is largely a mechanism by which a plant maintains a supply of metabolites in darkness

and in any other circumstances in which the rate of photosynthesis is insufficient to meet consumption of photosynthetic products in other cellular processes. That part of the newly created triose phosphate pool that is not consumed in re-creating or increasing the amount of the CO₂ acceptor (RuBP) is directly exported to the cytosol through the P_i translocator located in the innermost chloroplast envelope. Such export occurs by exchange and this is the means by which P_i released from triose phosphate in cytosolic sucrose synthesis re-enters the chloroplast. Photosynthesis, depending as it does on P_i for ATP synthesis, can only meet this requirement by internal release of P_i (e.g. in starch synthesis and in the phosphatase-catalysed reactions) and by importing P_i in exchange for triose phosphate. The Calvin cycle, as portrayed in Fig. 5.9, requires 9 molecules of ATP and therefore 9 molecules of P_i must be made available if it is to continue. Two are released by the hydrolysis of FBP and SBP (via eqs. 5.6 and 5.9) and a further six in the reduction of GBP (as shown in Figs. 5.8 and 5.9). Only one is made re-available by import of P_i from the cytosol (in exchange for every molecule of triose phosphate exported for sucrose synthesis *etc.*) or for triose phosphate consumed in internal starch synthesis. Even so, it is these molecules of P_i that play a pivotal role in regulation (Woodrow and Berry, 1988) and poisoning of the entire system. There is no doubt that, in some circumstances, experimental manipulation of cytosolic P_i availability can have profound effects on photosynthesis, which reach right back to the early events of the photochemistry (Walker and Sivak, 1985).

5.5 Autocatalysis: adding to the triose phosphate pool

Had carbon assimilation evolved in such a way that it involved direct reduction of CO₂ to formaldehyde, there would have been no need to regenerate a CO₂ acceptor. Since in fact it involves addition of CO₂ to a pre-existing acceptor (RuBP), there is not only a requirement for regeneration of this acceptor but a need to create more. The Calvin cycle is a mechanism by which one molecule of triose phosphate product is produced from three molecules of CO₂. The original amount of acceptor is then regenerated as five molecules of triose phosphate (G3P) are rearranged and transformed to give three molecules of pentose bisphosphate (RuBP). However, it will be immediately apparent from Fig. 5.9 that, if more than one out of every six molecules of triose phosphate were withdrawn as product, the entire sequence would rapidly spiral to a halt. Conversely, if a proportion of the potential triose phosphate product is retained within the cycle, the amount of acceptor formed will automatically increase. It is this 'autocatalytic' increase that allows both for growth and the rapid changes in RuBP quantities that are necessary if the plant is to optimise CO₂

assimilation in response to changes in light, temperature, availability of water *etc.* When leaves are abruptly illuminated after a period of darkness, PCA does not start immediately. Instead there is a period of induction, a lag before the full rate of assimilation is achieved. Induction lags are longer at lower than at high temperatures (Edwards and Walker, 1983). They are due in part to delays imposed while light activation of enzymes and the building up of Calvin cycle intermediates occurs. In all of this there is a need to regulate the operation of the cycle in such a way that an appropriate balance between export of triose phosphate from the cycle and regeneration and utilisation of RuBP within the cycle is maintained. In certain circumstances, abrupt illumination initiates pronounced (but dampening) oscillations in the rate of carbon assimilation, as regulatory responses appear to struggle to regain control. These are particularly marked when concentrations of P_i in the cytosol are limiting, implying that the ability of electron transport to supply ATP at optimal rates may be constrained in these circumstances (Walker, 1992b).

The autocatalytic nature of the Calvin cycle is also apparent when maximum rates of CO_2 fixation by isolated chloroplasts are measured at different temperatures. The rates of other metabolic processes, such as respiration, double for every 10 degrees rise in temperature between about 1 C and 30 C, whereas increasing temperatures does not increase the rates of purely photochemical events rates. Photosynthetic carbon assimilation falls into neither category. At temperatures close to 0 C, its rate may increase eight- or ten-fold. This has been attributed to the fact that, whereas individual reactions within the Calvin cycle may double for every 10 C increase in temperature, the amount of substrate made available for fixation by autocatalysis will also increase so that the effects will be multiplicative (Baldry *et al.*, 1966).

5.6 Photorespiration

In order for plants to exist as autotrophic organisms,³ assimilation of inorganic matter including C, N, S, P is required by anabolic processes (in which biomolecules are synthesised from simpler components). However, plants also have metabolic processes that produce inorganic matter in catabolic (degradative) processes. For example, in non-photosynthetic tissue (*e.g.* roots and seeds), respiration and some loss of CO_2 occurs out of the necessity for converting soluble carbohydrates and amino acids into other forms of organic matter, including starch, protein and lipids. In general terms, the net rate of gain of organic matter equals the rate of assimilation of

³ Autotrophic organisms are those able to synthesise organic materials from inorganic sources.

inorganic matter minus the rate of production of inorganic matter. Respiration, a catabolic process, is known to occur in the light in photosynthetic tissue. Unlike dark mitochondrial-type respiration, additional types of respiration occur in photosynthetic tissue in the light. Besides considering the extent of occurrence of photorespiration, there are questions about the purpose of different types of photorespiration.

5.6.1 Photorespiration via the Mehler-peroxidase reaction

One type of photorespiration occurs when O_2 acts as an alternative to NADP by accepting electrons from Photosystem I (PSI) in the so-called Mehler-peroxidase reaction. This results in the production of the superoxide radical O_2^- , which is subsequently reduced to water. The reductant is ascorbate, and the overall process is called the Mehler-peroxidase reaction (MPR). This reaction occurs through the following combined steps: (1) the transfer of electrons from splitting of water at Photosystem II (PSII) through the photosynthetic electron transport chain to oxygen to form superoxide at PSI; (2) the dismutation of the superoxide radical by superoxide dismutase (SOD) to form H_2O_2 ; (3) the reduction of H_2O_2 to water by ascorbate via the enzyme ascorbate peroxidase (APO), resulting in the formation of monodehydroascorbate (MDHA); and (4) the regeneration of ascorbate. The regeneration of ascorbate from MDHA occurs by donation of electrons from the electron transport chain via ferredoxin, by an NAD(P)-dependent MDHA reductase. Alternatively, if the disproportionation reaction $2 \text{MDHA} \rightarrow \text{ascorbate} + \text{DHA}$ generates dehydroascorbate (DHA), then ascorbate can be regenerated through the combined actions of DHA reductase and glutathione reductase (Miyake and Asada, 1994). In the process, O_2 is evolved at PSII and consumed at PSI, resulting in no net change in O_2 levels. Obviously, the net rate of O_2 evolution from plants will be lower than the true rate of O_2 evolution from PSII when the MPR is functional. In this form of photorespiration, there is no release of CO_2 in the process. There are two potential benefits of the MPR. One is to provide additional ATP (via the proton-motive force generated from the associated electron flow) when there is insufficient ATP generated from linear electron flow associated with NADP reduction (Ivanov and Edwards, 1997). The other is to provide a means of safely dissipating energy under high irradiance, when the energy absorbed is in excess of what can be used for carbon assimilation (Osmond and Grace, 1995). The magnitude of the MPR reaction in plants continues to be investigated; during C_3 photosynthesis under non-stressful conditions, its contribution to photorespiration is considered to be very low (Badger *et al.*, 2000).

5.6.2 Photorespiration via RuBP oxygenase

Another unique form of respiration in the light occurs as a consequence of the reaction of RuBP with O₂. This leads to formation of glycolate and metabolism in the PCO (Photosynthetic Carbon Oxidation) cycle (Fig. 5.9). Metabolism of products of this form of photorespiration results in release of CO₂ and ammonia. The relation between the carboxylase and oxygenase activity of rubisco is given by (Jordan and Ogren, 1984)

$$v_c/v_o = S_{rel} [CO_2]/[O_2] \quad (5.14)$$

where v_c is the velocity of reaction with carboxylase, v_o the velocity of reaction with oxygenase, and S_{rel} the specificity factor, which indicates the specificity of the enzyme for reaction with CO₂ rather than O₂. A specificity factor of 100 (a typical value for rubisco from a terrestrial C₃ plant) indicates that, at equal concentrations of CO₂ and O₂ around rubisco, the rate of carboxylation (v_c) would be 100 times faster than the rate of oxidation (v_o). Obviously the enzyme is reasonably effective as a carboxylase at equivalent gas concentrations. However, under current atmospheric conditions, the v_c/v_o ratio is only about 2.5 in C₃ plants because the concentration of O₂ is so much higher than CO₂; the exact value is affected by leaf diffusive resistance to CO₂ and temperature. The greater the stomatal limitation to photosynthesis (*e.g.* as may occur under water stress), the lower the supply of CO₂ to rubisco. This decreases the v_c/v_o ratio and increases partitioning of RuBP into the PCO cycle. The v_c/v_o ratio decreases with increasing temperature, partly because S_{rel} decreases as the kinetic properties of rubisco change in response to a large decrease in affinity for CO₂, and partly because the solubility of CO₂ decreases more than that of O₂ (Jordan and Ogren, 1984; Leegood and Edwards, 1996). The 2,3-enediol intermediate has a higher free energy of activation for reaction with O₂ than with CO₂, making oxygenase activity increase more with temperature than carboxylation activity, and causing S_{rel} to decrease (Chen and Spreitzer, 1992). Under atmospheric CO₂ concentrations, C₃ photosynthesis has a relatively flat response to temperature, in part because the rate of photorespiration increases with increasing temperature. Also with increasing temperature, k_c (the Michaelis constant for CO₂ as substrate) increases, and this limits carboxylation under atmospheric levels of CO₂ (Farquhar and von Caemmerer, 1982; Jordan and Ogren, 1984). Thus, in C₃ plants, CO₂-saturated photosynthesis has a steep response to temperature because RuBP oxygenase activity is low and high CO₂ levels overcome the limitation of decreasing affinity for CO₂.

When RuBP reacts with O_2 , the products are P-glycolate and 3-PGA, as shown in Fig. 5.9. 3-PGA is converted to triose-P through the reductive phase, the same as when 3-PGA is produced as a result of carboxylation. The P-glycolate is metabolised through the glycolate pathway, resulting in the synthesis of PGA. Thus RuBP carboxylase and RuBP oxygenase have in common the synthesis of PGA. The difference is in the number of PGA molecules synthesised. When 2 CO_2 react with RuBP in the carboxylase reaction, 4 PGA are synthesised. When 2 O_2 react with 2 RuBP, 3 PGA molecules are synthesised, as shown in Fig. 5.10. Carboxylation is an assimilatory pathway, while oxygenation is a respiratory pathway. Through carboxylation, CO_2 and P_i are assimilated into organic-P. Through the oxygenase reaction, inorganic matter— CO_2 , P_i and NH_3 —is produced. Since CO_2 and O_2 compete for reaction with RuBP, the relative rates of these two processes depends on the relative concentrations of CO_2 and O_2 at the catalytic site. Since for each 2 O_2 reacting with 2 RuBP, one CO_2 is produced in the glycolate pathway, the net rate of CO_2 assimilation is $(v_c - 0.5v_o)$, in the absence of dark-type mitochondrial respiration in the light.

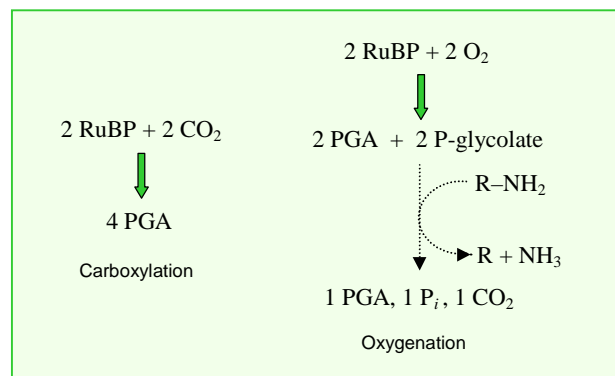
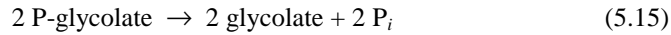
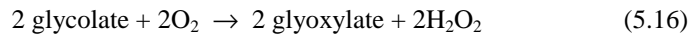


Figure 5.10 Fixation of CO_2 via RuBP carboxylase and generation of inorganic matter via respiration using RuBP as substrate. $R-NH_2$ is an amino acid and R is a keto-acid.

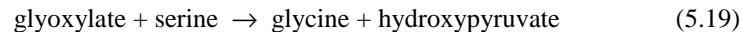
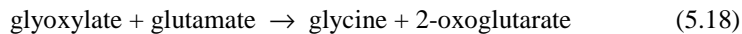
The metabolism of P-glycolate to PGA has its origin and termination in the chloroplast, but the pathway is very circuitous and involves metabolism in other organelles, namely peroxisomes and mitochondria. The sequence of reactions whereby two molecules of P-glycolate are metabolised in the glycolate pathway is as follows: in the first step phosphate is removed from phosphoglycolate (reaction catalysed by a phosphatase located in the chloroplast) and the product glycolate is exported from the chloroplast and imported by the peroxisomes.



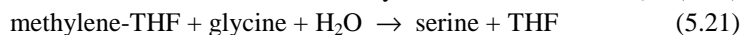
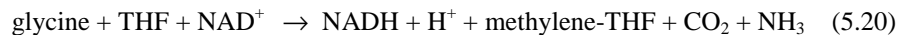
In the peroxisomes the glyoxylate is aminated to form glycine. First, glycolate is oxidised to glyoxylate by glycolate oxidase and the hydrogen peroxide formed is converted to water and O₂ by catalase.



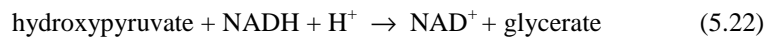
Then, by two transaminase reactions glyoxylate is aminated to form glycine which exported from the peroxisomes and imported by the mitochondria.



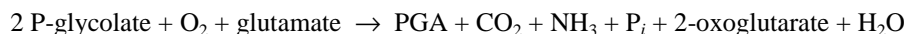
In the mitochondria, two molecules of glycine are converted to serine, CO₂ and NH₃ by two reactions which are catalysed by glycine synthase and serine hydroxymethyltransferase respectively.



The serine is imported to the peroxisomes, where it is converted to hydroxypyruvate (eq. 5.19), and then the hydroxypyruvate reduced to glycerate (5.22). The glycerate is imported to the chloroplast and phosphorylated to form PGA.



The sum of reactions 5.15–5.24 is



What is clear is that the relative partitioning between CO₂ fixation and O₂ reaction with RuBP is dependent on the relative concentration of the two gases around rubisco. It is also evident that the rate of CO₂ assimilation can be limited under current atmospheric levels of CO₂ because of competition by O₂ and photorespiration. During PCO cycle activity CO₂ is produced and energy is required to reduce the PGA and to assimilate the NH₃ and P_i that are formed. However, it is also evident that decreasing the level of PCO cycle activity, *e.g.* by CO₂ enrichment, does not guarantee that there

will be an increase in photosynthesis and growth. Obviously plants cannot assimilate triose-P at a rate above which it can be utilised. Thus, in situations where the rate of photosynthesis is limited by the rate of triose-P utilisation, for example in synthesis of starch and sucrose, then the rate of photosynthesis will not increase by eliminating photorespiration (*e.g.* by increasing CO₂ level or lowering O₂). Cases in which transient increases in growth of C₃ plants under elevated levels of CO₂ have been observed may reflect lack of ability for sustained use of the additional photosynthate. PCO cycle activity is least likely to limit plant growth under conditions where the rate of production of photosynthate (triose-P) nearly matches or exceeds the capacity to utilise it. This condition is favoured under conditions where stomatal conductance and access to atmospheric CO₂ is high. It is favoured by low temperatures which increases the solubility of CO₂, increases S_{rel} , and decreases the K_c for CO₂. As plants face periods of stress which limits the supply of CO₂ to the chloroplast, increased PCO cycle activity may be beneficial in providing a mechanism to safely dissipate excess energy (Osmond and Grace, 1995).

5.7 CO₂-concentrating mechanisms

It is clear that CO₂ is limiting for photosynthesis in certain environments since some species have evolved mechanisms for active accumulation of CO₂. CO₂-concentrating mechanisms have been discovered in a range of organisms from microalgae to higher plants. Although there are differences in the mechanisms, there are certain common features, most notably the ATP-dependent accumulation of CO₂ at the site of rubisco (Fig. 5.11). In microalgae, including green algae and cyanobacteria, a CO₂-concentrating mechanism is induced when grown under low (CO₂). Growth of microalgae under low CO₂ induces synthesis of the enzyme carbonic anhydrase (catalyses the interconversion between CO₂ and bicarbonate) and the appearance of new proteins in the plasmamembrane and chloroplast envelope some of which are believed to be essential components of the CO₂ uptake mechanism.

In higher plants, through C₄ photosynthesis two types of CO₂-concentrating mechanisms exist, namely CAM plants and C₄ plants. Although differing in mechanism, these two photosynthetic groups have in common a C₄ pathway of photosynthesis, composed of enzymes which are orders of magnitude more active than in leaves of C₃ plants, which is responsible for capture of atmospheric CO₂.

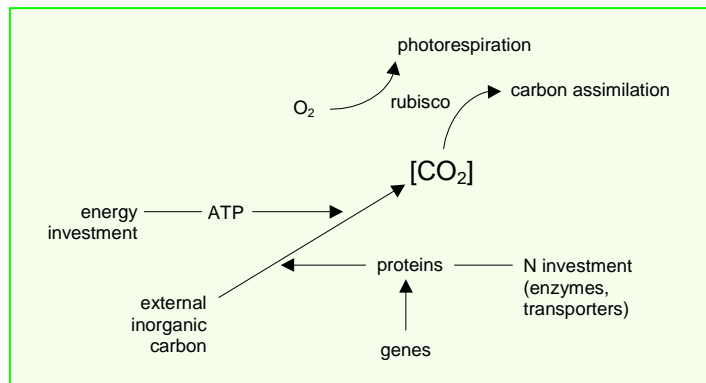


Figure 5.11 Illustrations of common features of CO₂-concentrating mechanisms in cyanobacteria, microalgae and higher plants. External inorganic carbon refers to a limiting concentration available to higher plants in the atmosphere and to photosynthetic microorganisms in aquatic environments.

5.7.1 CAM plants

CAM, an abbreviation for Crassulacean acid metabolism, is named after the family Crassulaceae since the mechanism was first identified in this family. Terrestrial CAM plants have been found within 33 plant families (Smith and Winter, 1996). In CAM plants, the CO₂-concentrating mechanism functions by fixing atmospheric CO₂ in the dark into organic acids (primarily malate) which are stored in the vacuole. This is a type of C₄ photosynthesis in which the primary initial product of fixation of atmospheric CO₂ is malate, a C₄ acid. On the following day malate is exported from the vacuole to the cytoplasm where it is enzymatically decarboxylated resulting in the production of CO₂. The production of CO₂ in the leaf causes the stomata to close, so that the leaf becomes relatively gas-tight. As a result there is a rise in CO₂ in the leaf, which allows for efficient fixation by RuBP carboxylase with minimal photorespiration. Thus, there is temporal separation of fixation of atmospheric CO₂ into C₄ acids and their utilisation to provide a high level of CO₂ to rubisco. This mechanism in land plants is thought to be more important for its conservation of water than for its reduction in photorespiration since it allows stomata to be closed during the day when the potential for water loss is highest. However, in aquatic CAM plants (*e.g. Isoetes howellii*), no one can argue that water is limiting; rather CAM allows the plant to assimilate CO₂ in the dark, when the supply of CO₂ is higher than in the day, and thus avoid day-time competition with other photosynthetic organisms for CO₂ (see Keeley review in Winter and Smith, 1996).

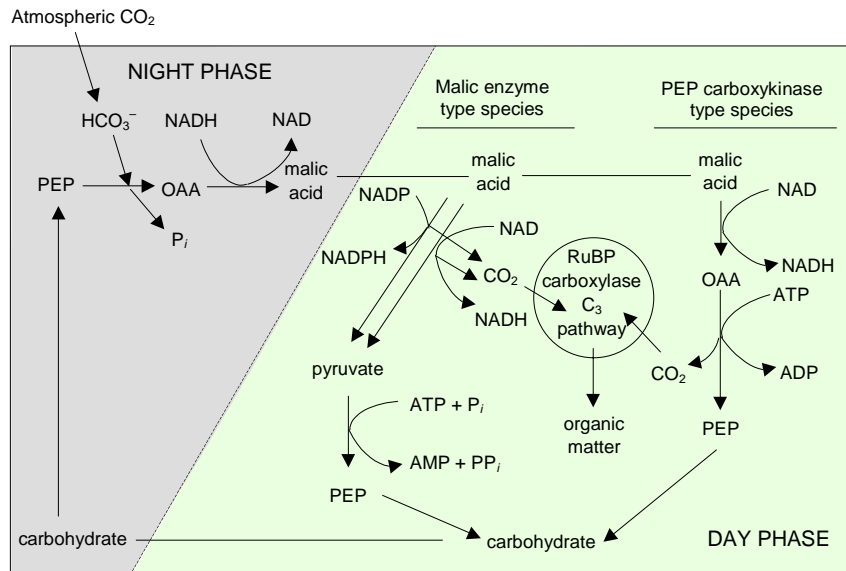
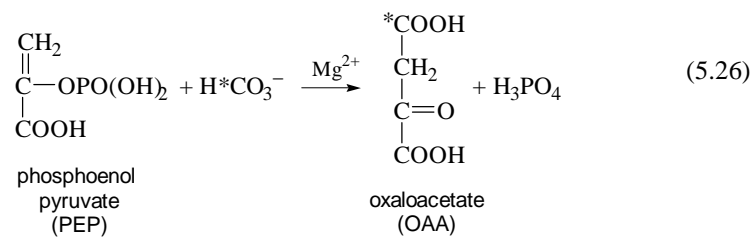
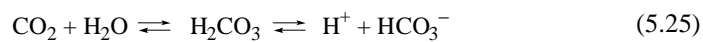


Figure 5.12 Key metabolic steps in Crassulacean acid metabolism.

Figure 5.12 shows the key metabolic steps in CAM plants. During the night, carbohydrate reserves (*e.g.* starch and sucrose) are metabolised via glycolysis to produce phosphoenolpyruvate (PEP). Atmospheric CO_2 entering the leaf is converted to bicarbonate through carbonic anhydrase.

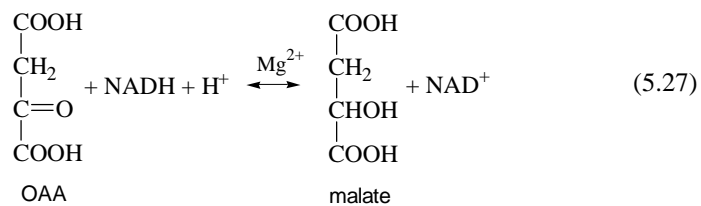


Phosphoenolpyruvate, like RuBP, is a high-energy substrate in the sense that the addition of CO_2 to it, which gives rise to the formation of oxaloacetate and P_i leads to a large decrease in free energy. About 25 kJ is needed to drive the carboxylation but the decrease in free energy associated with the overall reaction is sufficiently large to give a net change of about -29 kJ. Accordingly the equilibrium position of the

reaction is such that it is, like rubisco, virtually irreversible. Once fixed, the CO₂ stays fixed (Walker, 2000). Unlike RuBP carboxylase, PEP carboxylase utilises HCO₃⁻ as substrate rather than CO₂, and O₂ does not affect catalysis. The reaction that combines HCO₃⁻ and PEP to yield the C₄ acid oxaloacetate (OAA) is catalysed by PEP carboxylase. The high affinity of the enzyme for bicarbonate results in an apparent *k_c* value for CO₂ well below current atmospheric levels (He and Edwards, 1996). The catalytic mechanism starts with binding of metal²⁺ (Mg²⁺ or Mn²⁺), PEP and HCO₃⁻ in this order to the active site. The chemical steps are summarised as follows: (1) phosphate transfer from PEP to form carboxyphosphate and enolate of pyruvate; (2) carboxyphosphate decomposes to form enzyme-bound CO₂ and phosphate; (3) CO₂ combines with the metal-stabilised enolate; and (4) the products oxaloacetate and phosphate are released from enzyme (for details, see Chollet *et al.*, 1996).

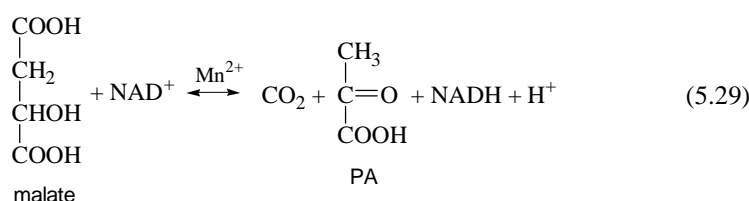
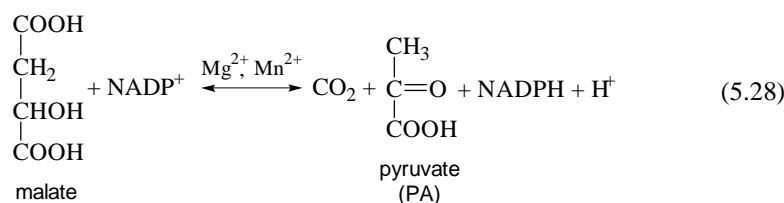
Diurnal changes in activity of PEP carboxylase in CAM plants and light activation in C₄ plants and associated changes in kinetic properties were shown to be via phosphorylation/dephosphorylation at a Ser residue near the C terminal of the protein by a specific protein kinase/phosphatase system. The phosphorylated enzyme is the active form occurring in the dark in CAM plants and in the light in C₄ plants (Carter *et al.*, 1996; Chollet *et al.*, 1996). Unlike rubisco, where O₂ competes with CO₂ for reaction with the substrate RuBP, O₂ does not compete with bicarbonate for reaction with PEP in the reaction catalysed by PEP carboxylase. Thus, atmospheric levels of O₂ do not inhibit PEP carboxylase activity.

OAA, which is formed via PEP carboxylase, is reduced to malate via NAD-malate dehydrogenase using NADH generated in glycolysis and the malate is stored in the vacuole.

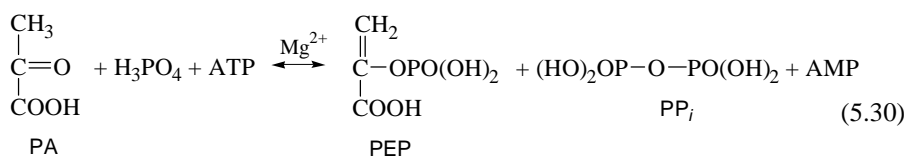


During the day the malate is exported from the vacuole and decarboxylated by C₄ acid decarboxylases. Two subgroups of CAM species exist, those which primarily use malic enzymes and those which primarily use PEP carboxykinase as the decarboxylation mechanism.

Malic enzyme type CAM species have high activities of malic enzymes; malate is decarboxylated by NADP-malic enzyme located in the cytoplasm and by NAD-malic enzyme located in the mitochondria.

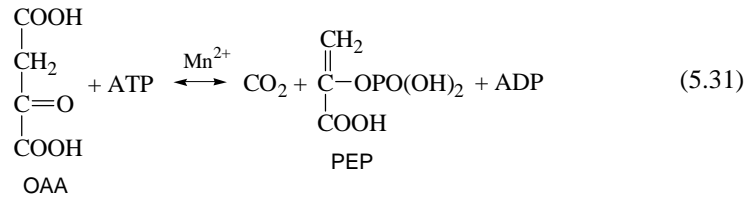


CO₂ generated from decarboxylation of malate enters the chloroplast, where it is assimilated into organic matter by the C₃ pathway and the pyruvate formed is converted to carbohydrate by gluconeogenesis. A key enzyme in this process is pyruvate, P_i dikinase which converts pyruvate to PEP.



This enzyme, which was first reported as 'PEP synthetase' by Hatch and Slack in C₄ plants in 1967, was identified as pyruvate, orthophosphate dikinase, a new key enzyme in CAM and C₄ plants which is activated by illumination (See Edwards *et al.*, 1985). Although the reaction itself is reversible, it proceeds to form PEP *in vivo* because of the active pyrophosphatase (PP_i → 2P_i) and adenylate kinase (AMP + ATP → 2ADP) in the chloroplasts consume the products AMP and PP_i. The reaction mechanism includes phosphorylation of P_i to form pyrophosphate, and histidine residues at the active site of the enzyme with (- and $\bar{\epsilon}$ -P of ATP, respectively, and then pyruvate reacts with the His-P residue to form PEP. As for light/dark regulation, the active enzyme having His-P is inactivated by phosphorylation of a specific threonine residue with $\bar{\epsilon}$ -P of ADP, and it is reactivated by phosphorolysis of Thr-P to form pyrophosphate. Interestingly, a single regulatory protein mediates both phosphorylation and dephosphorylation of the Thr residue (Edwards *et al.*, 1985).

In PEP-CK type CAM species, the malate is converted to OAA by NAD-malate dehydrogenase during the day (eq. 5.27). The oxaloacetate is then decarboxylated by PEP carboxykinase.



PEP carboxykinase is located in the cytoplasm of CAM and C_4 plants and phosphorylation/dephosphorylation of the enzyme may play a role in its regulation (Walker and Leegood, 1996).

5.7.2 C_4 plants

C_4 plants have been found among at least 18 families of higher plants (Edwards and Walker, 1983; Sage and Monson, 1999). Photosynthesis in C_4 plants consists of the coordinated function of two cell types in the leaves, usually designated mesophyll cells (MC) and bundle sheath cells (BSC). In C_4 plants, atmospheric CO_2 enters leaves through stomata and is first accessible to MC, where it is fixed by phosphoenolpyruvate (PEP) carboxylase to form oxaloacetate, and then malate and aspartate. These C_4 dicarboxylic acids are transported to BSC where they are decarboxylated, the released CO_2 concentrated, and refixed by RuBP carboxylase and assimilated through the Calvin cycle to form sucrose and starch. Thus, in C_4 plants there is a spatial separation of CO_2 fixation into malate (occurring in MC) and utilisation of C_4 acids as donors of CO_2 to the C_3 pathway (occurring in BSC), whereas in CAM, there is a temporal separation of CO_2 fixation into malate (occurring at night) and utilisation of C_4 acids as donors of CO_2 to the C_3 pathway (occurring during the day). In C_4 plants, although anatomical differentiation is apparent in BSC, they are functionally similar to C_3 MC in carbon assimilation except for the presence of enzymes concerned with decarboxylation of C_4 acids. For reviews of the biochemistry of C_4 photosynthesis, see Edwards and Walker (1983) and Hatch (1987).

The physiological significance of separate, but coordinate, function of the two cell types in C_4 plant photosynthesis is the specialisation of MC towards generation of a high concentration of CO_2 in BSC in order to reduce the oxygenase activity of rubisco, and consequential reduction of photorespiration. Photosynthesis in C_4 plants

can be visualised as a mechanism to provide rubisco with near saturating CO_2 when C_4 plants can afford a high stomatal conductance, or to provide sufficient CO_2 for survival and growth when stomatal conductance is low.

C_4 photosynthesis functions in both C_4 and CAM plants to capture atmospheric CO_2 , and to supply it to rubisco. Even though the mechanism in CAM and C_4 plants is very different, as noted earlier some of the key enzymes are the same. Three subgroups have been found in C_4 plants, distinguished by differences in the decarboxylation step in BSC. These are the NADP-malic enzyme (NADP-ME), NAD-malic enzyme (NAD-ME) and PEP carboxykinase (PEP-CK) types. As noted earlier, the same decarboxylases function in CAM in two types: malic enzyme-type species and PEP-CK-type species. In Fig. 5.13, the key metabolic steps during C_4 photosynthesis are illustrated in one of the C_4 subtypes, a NADP-ME species.

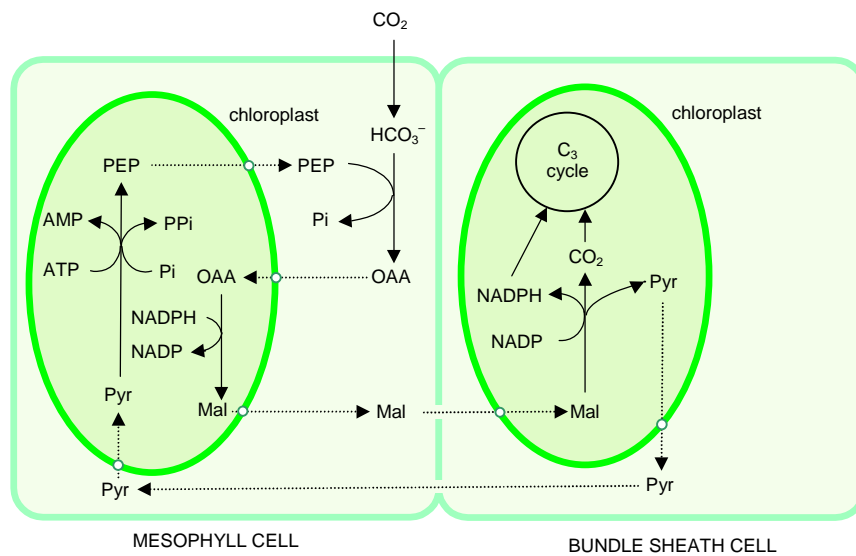
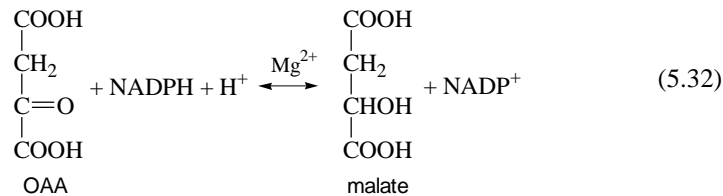


Figure 5.13 Key metabolic steps during C_4 photosynthesis in NADP-malic enzyme-type C_4 species. Members of this subgroup include maize, sugarcane and sorghum. Illustration provided by Dr. R. Kanai.

C_4 plants are considered to have evolved from C_3 plants. Common to all C_4 plants is the initial fixation of HCO_3^- by PEP carboxylase (eq. 5.26) to form oxaloacetate in the MC cytoplasm. Carbonic anhydrase (eq. 5.25) in the same compartment helps to equilibrate atmospheric CO_2 , entering MC via stomata, to HCO_3^- . Malate is formed from oxaloacetate in MC by the enzyme NADP-malate dehydrogenase (eq. 5.32).



In the course of evolution of C_4 plants from C_3 plants, the MC developed a high level of carbonic anhydrase and PEP carboxylase in the cytosol for initial CO_2 fixation in the cytoplasm, and pyruvate, orthophosphate (P_i) dikinase (eq. 5.30) in the chloroplasts for provision of PEP, the HCO_3^- acceptor. It is equally important that the synthesis of some key photosynthetic enzymes in carbon metabolism of C_3 photosynthesis is repressed in MC of C_4 plants. This includes rubisco and phosphoribulokinase of the Calvin cycle in MC chloroplasts, and enzymes of glycine decarboxylation in the PCO cycle in MC mitochondria.

5.8 Survival and efficiencies of photosynthesis

The obvious advantage for C_4 photosynthesis is to supply CO_2 to rubisco under any condition where it would be most limiting for photosynthesis. The solubility of CO_2 in photosynthetic tissue decreases with increasing temperature, and the carboxylase to oxygenase specificity of rubisco decreases with increasing temperature, all of which increases the relative RuBP oxygenase/carboxylase activity and photorespiration via the PCO cycle in C_3 plants. Thus, C_4 plants have an advantage at higher temperatures and they have a much higher temperature optimum for photosynthesis than C_3 plants.

Under water stress caused by drought or high salinity, low stomatal conductance provides resistance to CO_2 uptake, which favours PCO cycle activity. CAM and C_4 plants have adaptations in photosynthesis, which along with modifications in life forms (*e.g.* leaf forms, leaf succulence, rooting systems) enable certain species to survive in very harsh environments where C_3 plants are not found. CAM plants (*e.g.* cacti) use water efficiently, and can survive in arid conditions. CAM appears most advantageous when the differential between day and night temperature is high since the potential for water loss at a given water vapour concentration in the air increases with increasing temperature of photosynthetic tissue. Low stomatal conductance of CAM plants helps conserve water in arid environments but is unfavourable for direct fixation of atmospheric CO_2 by C_3 photosynthesis.

C₃ plants and C₄ plants can survive and coexist in moderate environments, although C₄ photosynthesis may be advantageous under warm temperatures and high-light conditions. In cool climates, where the potential for photorespiration is minimal, it is more effective for the plant to invest in the components for C₃ photosynthesis. There are clear trade-offs: plants which invest in CAM or C₄ have lower levels of components of the C₃ pathway, *e.g.* rubisco protein, and these trade-offs can result in a similar capacity for photosynthesis under moderate environmental conditions.

The efficiency of use of solar energy for CO₂ assimilation (quantum yield, mole CO₂ fixed or O₂ evolved per mole quanta absorbed by the leaf) depends on light intensity, other environmental factors and the photosynthetic mechanism of carbon assimilation. Under high light, where the capacity to produce assimilatory power is high (ATP and NADPH), it is more likely that photosynthesis will be limited in C₃ than in C₄ plants due to the limiting supply of CO₂. Under limiting light, where the rate of photosynthesis is limited by the production of assimilatory power, the rate of CO₂ assimilation will depend on the efficiency of use of ATP and NADPH. This efficiency under limiting light can be calculated as the maximum quantum yield. In C₃ plants, the maximum quantum yield is limited due to photorespiration; and for that reason the quantum yield decreases with increasing temperature, with decreasing CO₂, or with increasing O₂, all of which causes increased PCO cycle activity. In C₄ plants, the rate of photorespiration is low, but there is an additional investment in supply of energy (ATP) to accumulate CO₂. Under moderate temperatures, and adequate water, the quantum yield is similar in C₃ and C₄ plants, but under CO₂ limitation (higher temperature and decreased stomatal conductance), C₃ plants have a lower quantum yield. Whether considering ability to survive, maximum capacity for photosynthesis under higher light, or maximum quantum yield under limiting light in C₃ versus C₄ photosynthesis, environmental conditions must be carefully considered. Since plants are prone to CO₂ limitation in hot, dry, and/or saline habitats, C₄ plants are expected to have an advantage over C₃ plants for conversion of solar energy into biomass in such conditions. CAM plants can utilise solar energy and grow under extremes of high temperature and drought.

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