Chapter 15

THE BIOCHEMISTRY OF PHOTOSYNTHESIS

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INTRODUCTION

The unique and important feature of plants is their ability to grow using sunlight as the source of energy and CO_2 from the air as the carbon source with water and elements coming from the environment or soil. The process of photosynthesis with light capture, coupled with O_2 evolution and CO_2 fixation, occurs in the chloroplast. This organelle operates in a semi-autonomous fashion with many of its metabolic processes apparently independent of direct cytoplasmic control. In the light the chloroplast generates its own ATP and reducing power with which CO_2 is assimilated. The carbon is either exported as triose phosphates to the cytoplasm or stored in the chloroplast as starch. In the dark, reducing power can be generated by a hexose monophosphate shunt in the chloroplast.

MORPHOLOGY OF HIGHER PLANT CHLOROPLASTS

The chloroplast, as it appears in most published electron micrographs, usually has a characteristic lens shape with a length of 4 to 10 picometers (pm). There are three major structural regions of the chloroplast: the double outer membrane or envelope; the mobile stroma containing the soluble enzymes for metabolism, protein synthesis and starch storage; and the highly organized internal lamellar membranes containing chlorophyll and involved in the biophysical reactions of energy capture and conversion (Figure 1). The internal membranes are shaped like discs and are often stacked together like a pile of coins to form a granum. Each disc is vesiculated or saclike and is termed a thylakoid. If sectioned in a plane parallel to the thylakoid membrane, both the chloroplast and the membranes appear disc-shaped. The outer envelope is a selectively permeable double membrane that regulates the movement of carbon intermediate products, reducing power and adenylates in and out of the chloroplast, while retaining starch for degradation at night. The stroma is mostly protein, consisting of about 50 percent of "fraction 1 protein" or ribulose-1,5-P_2 carboxylase/oxygenase.

The thylakoid membranes of most higher plants such as cotton are structurally

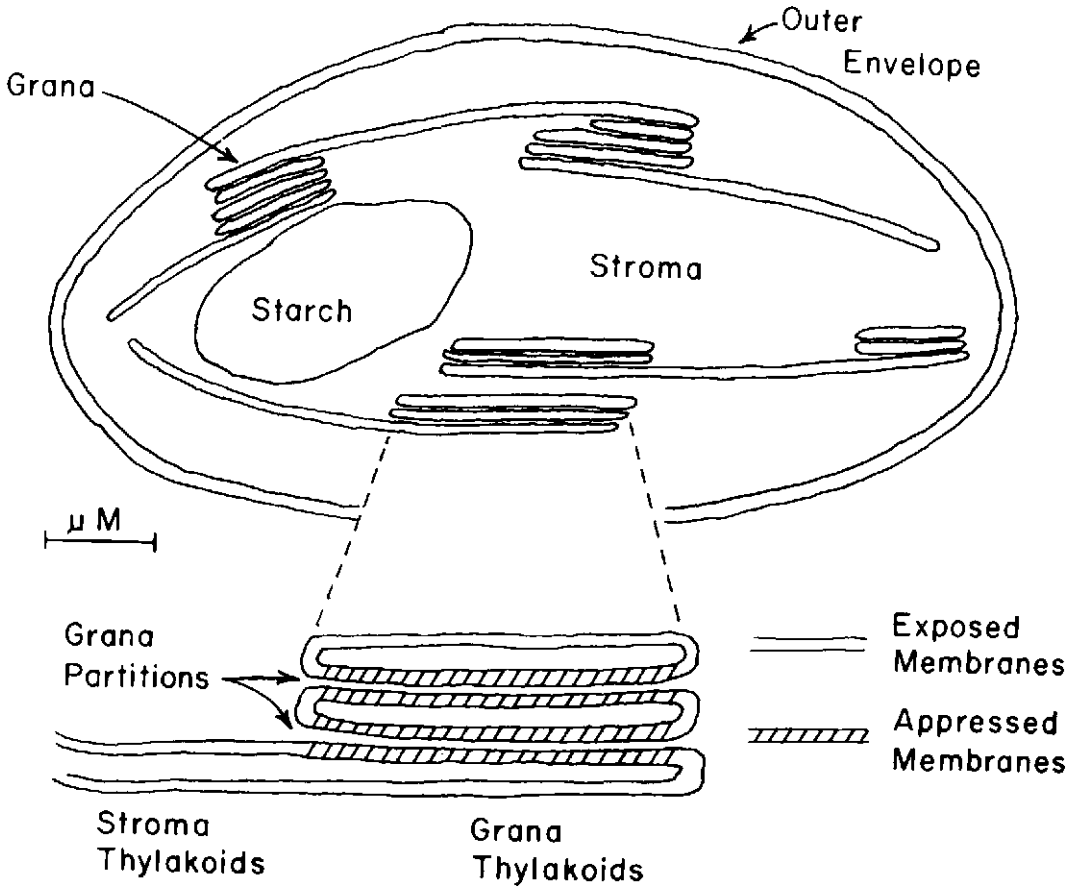


Figure 1. Diagram of the compartments typical of a higher plant chloroplast. An enlargement is shown to depict the grana and stroma thylakoid membranes. Photosystem I particles are mostly located in the exposed membrane regions (stroma thylakoids and exposed ends of the grana thylakoids) while photosystem II particles are enriched in the oppressed membrane regions (grana partitions). (Anderson, 1981).

organized into a network of closely contacting appressed membranes, the grana thylakoids, which are interconnected with single, unstacked membranes, the stroma thylakoids (Figure 1). The innersurface of these thylakoid membranes encloses a space which is continuous between the grana and stroma thylakoids. The thylakoids have two distinct membrane regions called exposed and appressed membranes. The exposed thylakoids whose outer surfaces are in direct contact with the stroma, include stroma thylakoids and the end membranes of the grana

stacks. In contrast the outer surfaces of the appressed membranes of the grana partitions have limited access to the stroma.

FUNDAMENTAL ENERGY PROCESSES IN PHOTOSYNTHESIS

Photosynthesis as it operates in the chloroplast has two phases, the light reactions, which are directly dependent on light energy, and the dark reactions, which can occur without the direct influence of light. Research over the last 30 years has heavily concentrated on the light reactions of photosynthesis. They are primarily responsible for converting light energy into chemical energy in the form of ATP and NADPH. These compounds in turn bring about the reduction of carbon dioxide to sugar and other products. The light reactions require the cooperative interactions of two kinds of photosystems, known as photosystem I and photosystem II.

Early observations indicated that the rate-limiting step in plant photosynthesis takes place in the dark (Myers, 1971). When photosynthetic organisms are subjected to intermittent illuminations with short flashes of light (milliseconds or less) followed by dark intervals of varying duration, evolution of O_2 after a single flash of 10^{-5} s was maximal, if it was followed by a much longer dark period (greater than 0.06 s). The term "dark reactions" does not mean that they take place only in the dark; in living plants they function together with the light reactions in light. At night while the leaf respires many of the dark reactions of photosynthesis are inoperative. As explained later, the "dark reactions" communicate with the action of the light reactions not only by utilization of ATP and NADPH but by light-generated pH and Mg^{2+} gradients in the stroma in the presence of a reducing environment.

ROLE OF THE PIGMENT SYSTEMS

The various photosynthetic pigments involved in light absorption from higher plants can be classified into two main groups: chlorophyll and carotenoids. The function of these pigments is to provide the plant with an efficient system of absorbing light throughout the visible spectrum (Vernon and Seely, 1966). This energy is then transferred to reaction centers where it is utilized in a photochemical reaction. The bulk of the pigments are light-harvesting pigments involved in the process of light absorption and subsequent energy transfer.

There are two kinds of chlorophyll in higher plants, chlorophyll *a* and chlorophyll *b*. Chlorophyll *a* (Chl *a*) is the major pigment and is found in all photosynthetic organisms that evolve oxygen. In the plant, Chl *a* has various forms with different absorption maxima, due to unique environments, e.g., Chl 660, 670, 680, 685, 690 and 700-720 nanometers (nm). The evidence for the existence of these various forms comes from derivative spectrophotometry, low-temperature absorption measurements and the action spectra of various photochemical reac-

tions. The short-wavelength Chl *a* forms are fluorescent and are predominantly present in photosystem II. The long-wavelength forms are weakly fluorescent and are mostly present in photosystem I.

Chlorophyll *b* (Chl *b*), also present in higher plants, has a major absorption maxima at 650 nm, with a minor component in some species at 640 nm. The major portion of Chl *b* is present in photosystem II.

Chlorophyll *in vivo* is noncovalently bound to protein in the thylakoid membrane. Upon treatment with organic solvents, the weak interactions between chlorophyll and the membrane components are eliminated, and its absorption maximum shifts to a lower wavelength, depending on the solvent-chlorophyll interactions.

The carotenoids are the yellow and orange pigments found in most photosynthetic organisms. The two classes of carotenoids are (1) carotenes absorbing blue light, of which β -carotene is the most common; and (2) carotenols or alcohols, commonly called xanthophylls. Most of the carotenes are present in photosystem I while the xanthophylls are located in photosystem II. Both of these carotenoid pigments function by absorbing light, mostly in the regions of the spectra not absorbed by chlorophyll, and transferring the energy to Chl *a*. They also help protect chlorophyll from photo-oxidation.

SPATIAL ORIENTATION OF THE PHOTOSYSTEMS

Anderson (1981) has proposed that there is a large heterogeneity in the distribution of photosystem I and photosystem II in thylakoids of higher plants. Freeze-fracture electron microscopy has revealed a difference in the size, shape and density of particles located in appressed and exposed membranes (Figure 1) (Arntzen, 1978; Arntzen and Briantais, 1975; Staehelin, 1976). This suggests a difference in the distribution of macromolecular complexes of thylakoid membranes in the two regions. This striking difference of the structural organization of the thylakoids is also substantiated by a differentiation of function. The fractionation of thylakoids into grana and stroma thylakoid fractions either by detergent or mechanical methods has yielded fractions derived from the appressed membranes enriched in photosystem II while small vesicles derived from the stroma thylakoids are enriched in photosystem I. Few if any photosystem I complexes are present in the appressed membranes at the grana partitions. In this model of the spatial separation of photosystem I and II, it appears that plastoquinone, as part of the electronic transport chain, is the most likely candidate for the mobile electron carrier between the two photosystems.

As each photosystem is supplied by about 300 antenna Chl, each electron transport chain may pass a pair of electrons once every 20 ms in well-operating chloroplasts. Were a single Chl molecule to drive the reaction, there would not be enough light quanta to suffice, even if the molecule were exposed to bright sunlight. An average Chl molecule absorbs one quantum of light per 100 ms under bright sunlight, one per second under diffused daylight, and only one per 10 s

on a cloudy day. An organized pool of Chl with several energy transfers occurring simultaneously is essential to match the rather low absorption rate of quanta per Chl to the higher rates of electron transport. A typical thylakoid disc from a mature spinach chloroplast contains at least 10^5 Chl molecules and its membrane is covered by at least 200 electron transfer chains.

FLOW OF ELECTRONS IN LIGHT

Light quanta absorbed by the chlorophyll and carotenoid pigments are funneled into specific photochemical reaction centers. The efficiency of this energy transfer is high, implying that the probability for transfer of a quantum between two neighboring pigments is higher than the probability for any competing process such as fluorescence emission, formation of metastable states, wasteful photochemistry and radiation-less deactivation. As these processes usually occur within nanoseconds, the transfer through the whole light-harvesting antenna pigment system to a reaction center must occur in a much shorter time. Rapid transfer of energy occurs via dipolar coupling between pigments which are tightly packed and communicate by resonance. Because energy transfer is enhanced when the absorption spectra of neighboring pigments overlap, it is not unusual to note that the reaction centers have absorption maxima at longer wavelengths (lower energy).

The end result of photosynthetic electron flow in the chloroplast is the evolution of oxygen and the formation of ATP and NADPH necessary for the assimilation of CO_2. The currently accepted representation of photosynthetic electron transport is one of a cooperative interaction of two light reactions. This model originated with Hill and Bendall (1960). A representative of their hypothesis, as it has evolved today, is presented in Figure 2 (Barber, 1977; Govindjee, 1975; Trebst and Avron, 1977). Their formulation was proposed primarily to account for three major experimental observations: (1) the decline in efficiency of photosynthesis at long wavelengths (greater than 685 nm) and the synergistic effect of shorter wavelengths on the photosynthetic action of far red illumination; (2) the presence in green tissues of two cytochromes, cytochrome f (Cyt f) and Cyt b_6, whose characteristic potentials differed about 400 mV, as did their light-induced absorption changes; and (3) the stimulation of electron flow to $NADP^+$ when ATP formation occurred concurrently. According to the model (Figure 2), photosystem II oxidizes water to free O_2 and reduces Q, while photosystem I reduces a low potential electron acceptor X and oxidizes P-700. Q may be equivalent to a component producing an absorbance change at 550 nm, referred to as C-550. Similarly, X appears to be a pigment having an absorption change at 430 nm and is referred to as P-430. Oxidized P-700 is reduced by reduced Q via exergonic electron transport reactions that are coupled to the phosphorylation of ADP to ATP. The oxidation of water also provides protons and a membrane potential to run a second phosphorylation of ADP. These two steps of ATP production occur during noncyclic electron flow and are called noncyclic photophosphorylation.

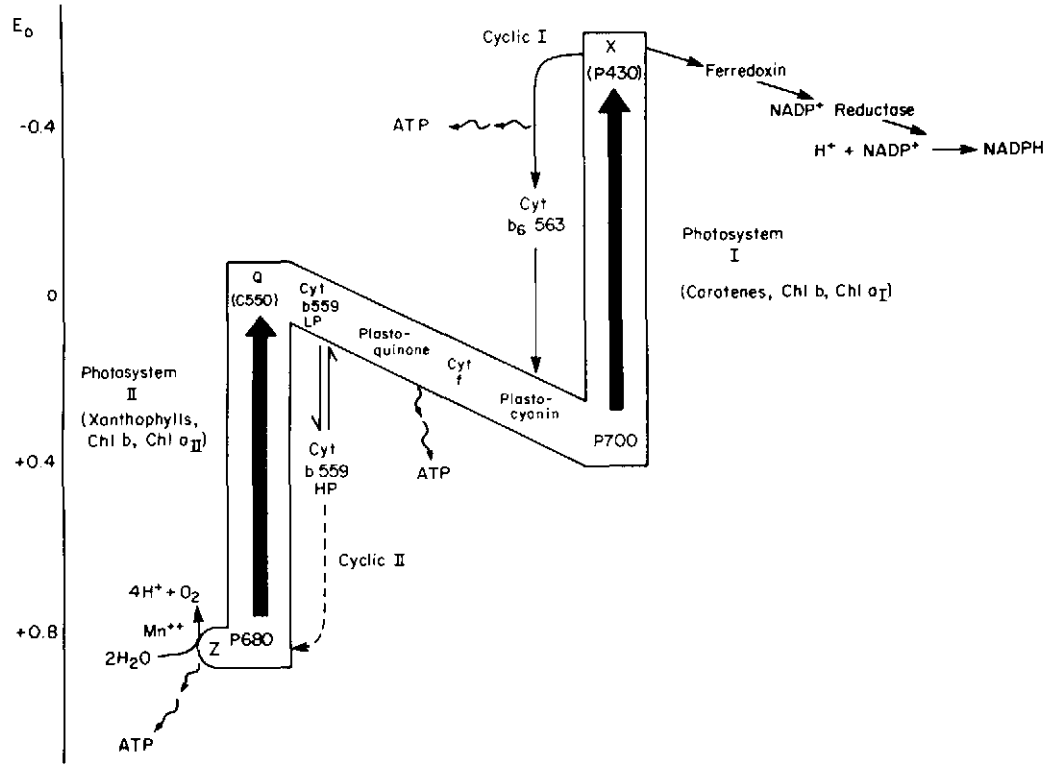


Figure 2. The Z-scheme for photosynthetic electron transport including sites of coupling for photophosphorylation. This model originated with Hill and Bendall (1960).

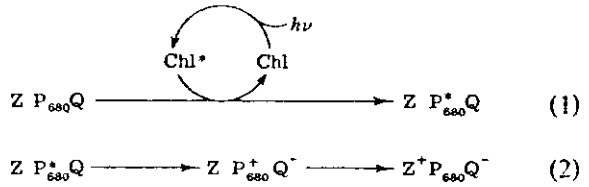
The carriers catalyzing the electron flow reactions are Cyt b-559 (low potential), plastoquinone (PQ), Cyt f, and plastocyanin (PC), in that order. The site of phosphorylation is probably between PQ and Cyt f.

The low potential electron acceptor X for photosystem I can transfer energy to form NADPH via a ferredoxin reducing substance, ferredoxin (Fd) and the ferredoxin-NADP+ reductase. Alternatively, energy from the primary acceptor X can cycle back to Cyt f or PC by way of Cyt b_6. In this latter instance the electron transport traces a closed circuit utilizing only photosystem I. It is referred to as cyclic electron transport and the accompanying formation of ATP is designated cyclic photophosphorylation. The amount of cyclic photophosphorylation that occurs *in vivo* is still uncertain.

PHOTOSYSTEM II AND EVOLUTION OF OXYGEN

Photosystem II of higher plants is associated with oxygen evolution to provide

electrons for the subsequent reductive processes mediated by the electron transport chain and photosystem I. The pigment-enzyme complex of photosystem II is located mostly on the appressed membranes of the grana thylakoids where it exhibits a high degree of structural and organizational integrity. The trap Chl of photosystem II exists in a reaction center complex with a primary electron donor Z and a primary acceptor Q (Velthuys, 1980). When the reaction center complex is in the proper redox state, the trapping of an exciton by the chlorophyll pool is funnelled to the trap Chl *a* (P-680). This creates a photochemical product by separation of charge between Z and Q. Oxidized $Z(Z^+)$ can then receive an



electron by oxidation of water comprising a multistep procedure. The chemical nature of the primary Z complex is unknown at present. Tightly bound Mn^{2+} appears to be associated with Z, with Cl^- also shown to be essential.

Little is known about the biochemical mechanism of O_2 evolution. One of the functional problems is to understand how four photoreactions, which correspond to the transfer of four electrons, are able to cooperate to produce one O_2 molecule. Most of our present knowledge comes from kinetic studies. When dark-adapted chloroplasts were submitted to a series of short saturating flashes (10^{-5} s), the amount of O_2 evolved per flash oscillated with a periodicity of four. From these experiments Joliot and Kok (1975) concluded that the cooperation between four photoreactions occurs at the same photocenter with each photocenter being independent of the other.

PHOTOSYSTEM I AND THE REDUCTION OF NADP+

Photosystem I has a characteristically longer absorption maximum than photosystem II. It is involved in moving electrons to reduce NADP+ or to provide for cyclic electron flow to give extra ATP without net electron transport. It has been hard to identify the primary electron acceptor for photosystem I. In 1971, a spectroscopic component, P-430, was discovered that exhibited properties necessary for the primary acceptor (Hiyama and Ke, 1971). The chemical identity of this component has been speculated to be bound Fd. At least one to two bound nonheme iron sulfur centers exist at the reducing end of photosystem I, and a large amount of nonheme iron or Fd is bound to the thylakoid membrane. Studies with enriched photosystem I particles indicate a large pool of iron-sulfur protein of four to five times the amount of P-700, PC or Cyt f. The rest of the reducing side of photosystem I is one of the best understood segments of the photosynthetic electron transport pathway (see Malkin, 1982). There are two soluble proteins involved in the direct transfer of electrons from the reducing membrane-bound P-

430 to $NADP^+$. The first is soluble Fd which has been shown to be photoreduced by isolated chloroplasts. Ferredoxin is a reddish brown protein having a potential of -430 mV. It contains 2 moles of nonheme iron and acid-labile sulfur per mole of protein and functions as a one-electron carrier, as shown by the ability of 1 mol of $NADP^+$ to oxidize 2 moles of reduced Fd. A second protein, ferredoxin-$NADP^+$ oxidoreductase, is necessary for the collection and transfer of electrons, one at a time, from ferredoxin to the two-election reduction of $NADP^+$. This enzyme contains one bound FAD per molecule. Reduced X and/or reduced ferredoxin also appear to regulate the activities of some of the enzymes involved in carbon flow during photosynthesis.

P-700 is the primary donor of photosystem I. Its concentration in the chloroplast is about 1 per 400 Chl. Redox titrations have established it as a single electron carrier with a potential of 450 mV. Most likely P-700 itself is a chlorophyll localized in a special environment. Because its absorbance band is at a slightly longer wavelength than the bulk light-harvesting chlorophylls, most of the excitation energy captured by the bulk chlorophyll will be funneled to P-700. P-700 has been isolated in a Chl *a*-P-700 protein complex with 40 Chl *a* per P-700 and 90,000 MW.

INTERMEDIATES OF ELECTRON TRANSPORT

Identification of the primary electron acceptor of photosystem II (Q or C-550) has been complicated partially by the confusion concerning the roles of Cyt b-559 and P-680. It appears that Cyt b-559 can exist as two forms: a low potential form Cyt b-559, with a potential of about 80 mV interconvertible to a high potential form of 350 mV. The exact physiological role of these two forms is not clear, but it seems likely that the low potential form interacts with the electron transport chain between the two photosystems via plastoquinone, while the high potential form is involved in a cyclic flow of electrons around photosystem II.

Plastoquinone (PQ) is the name given to a mixture of related electron transport quinone intermediates—the principle component is PQ A. The concentration of plastoquinone is much higher than that of the other electron transport intermediates. It is normally present in a concentration equal to 5-10 percent of the total chlorophyll or 10-14 molecules per photosystem I or photosystem II unit. The rate-limiting step for photosynthetic electron transport is the oxidation of reduced plastoquinone by plastocyanin through Cyt f with a half-time of 20 ms (Witt, 1975). At least 10 photosystem II reaction centers are interconnected by the plastoquinone pool.

Cytochrome f is the best known of the photosynthetic cytochromes. It can be released by gentle procedures from the photosynthetic membranes of several higher plants. It has a characteristic absorption peak for the reduced form of 554 nm, with a redox potential of 365 mV. Although called Cyt f, it is actually a c-type cytochrome. Because of its association with the green part of the plant, f, for the latin *folium* (leaf), was used.

Plastocyanin (PC) is the electron donor to P-700. It is thought to mediate electron transfer from Cyt f, plastoquinone and photosystem II to feed photosystem I. It is a copper protein with a characteristic blue color in the oxidized form. Chloroplasts contain a Chl/plastocyanin ratio of about 300 with plastocyanin accounting for one-half the total copper in the chloroplast. The redox potential of spinach plastocyanin is 370 mV.

The second b-type cytochrome in photosynthetic tissue is Cyt b_6 or b-563. It has a potential of about zero volts and is auto-oxidizable. Although originally proposed to function in the main electron transport flow, it has now been shown to mediate cyclic flow between X and the electron transport chain. Its interaction is probably with Cyt f or plastocyanin, or possibly even through the large plastoquinone pool.

PHOTOPHOSPHORYLATION

Part of the absorbed light energy is conserved in the formation of ATP. Chloroplasts in the light are capable of high rates of ATP formation. This process is very similar to the coupled conservation of energy during respiration with electron transport in mitochondria. In plants this light-activated process is called photophosphorylation.

Both electron transport and photophosphorylation are said to be "coupled." No phosphorylation will occur unless electron transport is proceeding. Conversely, there should be no electron transport unless ADP and Pi are present to permit simultaneous phosphorylation. Actually, isolated chloroplasts always have a small amount of electron transport in the absence of added ADP or inorganic phosphate due to leaks in the system. Investigations with isolated chloroplasts suggest that the coupling of phosphorylation to electron transport may not be tight (Krause and Heber, 1976). The photosynthetic apparatus appears to adjust itself to lower values when lower stoichiometric amounts are needed for photoreduction, *i.e.*, the chloroplast *in vivo* operates with a flexible $P/2e^-$ ratio. The control of this flexible ratio may involve the concentration of ADP or the breakdown of the proton gradient across the thylakoid.

Photophosphorylation occurs in intact thylakoids with an aqueous phase inside and out and whose membrane is relatively impermeable to protons or hydroxide ions. The electron transport intermediates are embedded anisotropically across the membrane. The hydrogen carriers are believed to be so oriented in the membrane that when an electron is passed the necessary proton to complement it comes from outside the vesicle. In turn, when giving up the electron it is to an electron acceptor on the inside with a complementary proton released on the inside. In this way, due to the geometry, electron transport through the chain is coupled obligatorily to vectorial proton translocation across the membrane. The first internal protons are those released from water splitting, and this constitutes site II for energy conservation. The second coupling site, site I, operates during the sequential reduction, with the reoxidation of plastoquinone (PQ). Because PQ

is a pool of molecules, the shuttle may actually involve transfer between several or many molecules before complete oxidation and reduction across the pool has occurred.

The membrane-bound enzyme that is involved directly in photophosphorylation is called chloroplast coupling factor one (CF_1). It sits on the exposed surface of the thylakoid membrane and can be released by dilute EDTA. With an approximate 325,000 MW, it is composed of five different polypeptide subunits. It can move laterally in the membrane since antibodies make the knobs clump together. There appears to be one CF_1 per 500-850 Chl. The CF_1 acts as a proton translocator and, as well, as an ATP synthetase. There is no indication that CF_1 protein penetrates all the way through the membrane although the protons must move completely through. Apparently, there are highly hydrophobic proteins in the membranes which, as intrinsic components of the membrane, serve as a point of attachment of the CF_1 to facilitate ATP synthesis.

For many years when the maximum $P/2e^-$ ratios in intact chloroplasts were thought to be below 1.5, additional electron transfer steps for synthesis of extra ATP were considered. One of the most important has been cyclic phosphorylation supported by cyclic electron flow in photosystem I (Figure 2). There is no compelling evidence to show that cyclic photophosphorylation plays a significant role in higher plants under natural aerobic conditions. Rather, in intact leaves or in chloroplasts, O_2 does readily react with an electron carrier beyond photosystem 1. In an N_2 atmosphere intact leaves exhibit strong chloroplast shrinkage under far-red illumination (which excites preferentially photosystem I) indicative of photophosphorylation by cyclic electron transfer. As very low levels of O_2 (0.1 percent) reversed this effect, it seems that O_2 can easily drain electrons from the cyclic pathway and thereby inhibit cyclic photophosphorylation. Yet, oxygen does support photophosphorylation in light using both photosystems. Apparently, electrons transferred through the two photosystems will either move to $NADP^+$ or, if this is reduced, can be transferred to molecular O_2 (Mehler reaction). In this reaction, H_2O_2 could leave the chloroplast by diffusion and be decomposed by catalase in the peroxisomes. Such a system would be self regulating with respect to photophosphorylation, as $NADP^+$ has a much greater affinity as the terminal electron acceptor than O_2. There are several indications that this regulation occurs in intact plants. During induction of CO_2 fixation, ATP can become limiting whereupon NADPH accumulates and less $NADP^+$ is available for reduction. Electrons are then diverted to oxygen which results in additional photophosphorylation without $NADP^+$ reduction. This process is termed pseudocyclic electron transport, or Mehler reaction, as oxygen evolution and oxygen uptake balance each other and no net O_2 change is observed.

CARBON METABOLISM DURING PHOTOSYNTHESIS

The light-produced intermediates, ATP and NADPH, are utilized in the chloroplasts to fix CO_2 and reduce it to the level of carbohydrates. Parts of the reduced carbon remains as starch in the chloroplast for utilization at night, while the rest is transported to the cytoplasm to form sucrose and organic and amino acids. Those products, which are formed in the chloroplast and transported to the cytoplasm, are two and three-carbon compounds: glycolate, 3-phosphoglycerate (glycerate-3-P), and the triose phosphates (triose-P), dihydroxyacetone phosphate and glyceraldehyde 3-phosphate (glyceraldehyde-3-P). Depending on needs of the plant, 25-50 percent of the fixed carbon is stored as starch in the chloroplast.

PHOTOSYNTHETIC CARBON REDUCTION PATHWAY (CALVIN CYCLE)

The only pathway for net CO_2 fixation resulting in carbon incorporation into hexoses is the reductive photosynthetic carbon cycle (Bassham and Calvin, 1957). Even though C_4 plants initially fix CO_2 into oxaloacetate which is converted into malate and aspartate, these must be decarboxylated so that the CO_2 released can be refixed by way of the photosynthetic carbon cycle. Many of the reactions of the photosynthetic carbon cycle are similar to steps of the glycolytic pathway and the hexose monophosphate shunt and consist of three different phases of carbon metabolism (Figure 3). The first phase is the production of ribulose-1,5-bisphosphate (ribulose-P_2) and its carboxylation, steps that are unique to the photosynthetic carbon cycle. The second is the reduction of glycerate-3-P to the level of an aldehyde, glyceraldehyde-3-P. The third phase involves the disproportionation of triose-P to produce pentose monophosphates, the precursors for ribulose-P_2, by way of tetrose, hexose and heptose phosphates.

CO_2 is incorporated by carboxylation of ribulose-P_2, catalyzed by ribulose 1,5-bisphosphate carboxylase/oxygenase (ribulose-P_2 carboxylase). The initial products are two molecules of glycerate-3-P. The ribulose-P_2 carboxylase catalyzes a second important reaction whereby molecular O_2 reacts with ribulose-P_2 to form phosphoglycolate (P-glycolate) and glycerate-3-P. P-glycolate phosphatase releases glycolate which diffuses from the chloroplast to be the substrate for photorespiration.

In the presence of ATP, glycerate-3-P is phosphorylated to glycerate-1,3-P_2 by action of glycerate-3-P kinase. Glycerate-1,3-P_2 is reduced by NADP-glyceraldehyde-3-P dehydrogenase and NADPH with release of inorganic phosphate to give glyceraldehyde-3-P. Glyceraldehyde-3-P quickly equilibrates with dihydroxyacetone phosphate by triose-P isomerase. These two triose phosphates are combined by aldolase to form fructose 1,6-bisphosphate (fructose-P_2). Then, fructose 6-phosphate (fructose-6-P) is formed by the action of fructose-P_2 1-phosphatase, a regulatory enzyme which controls the flow of carbon to subsequent pathways.

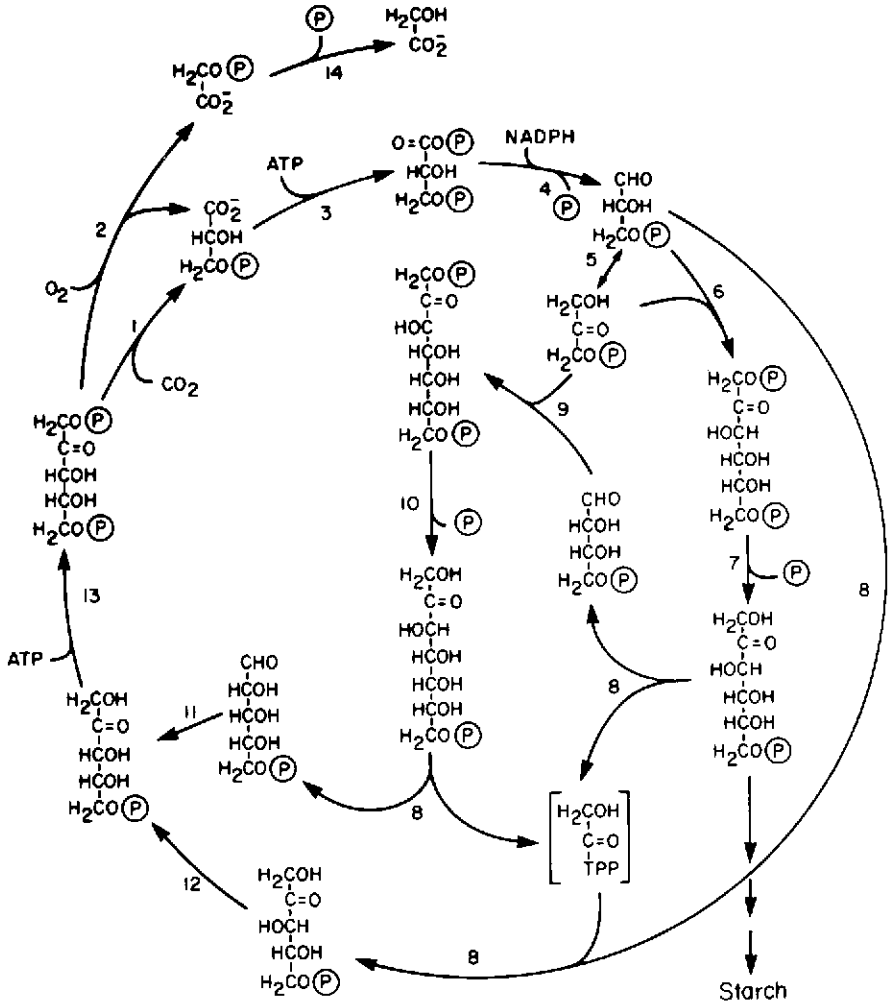


Figure 3. Photosynthetic carbon reduction pathway or Calvin cycle. The enzymes involved are (1,2) ribulose-P_2 carboxylase/oxygenase; (3) glycerate-3-P kinase; (4) NADP-glyceraldehyde-3-P dehydrogenase; (5) triose-P isomerase; (6) aldolase; (7) fructose-P_2 1-phosphatase; (8) transketolase; (9) aldolase; (10) sedoheptulose-P_2 1-phosphatase; (11) ribose-5-P isomerase; (12) ribulose-5-P epimerase; (13) ribulose-5-P kinase; (14) P-glycolate phosphatase.

The reverse step in glycolysis, by way of phosphofructokinase is also present in the chloroplast, but probably does not operate in the light. Fructose-6-P can be converted to glucose 6-phosphate (glucose-6-P), glucose 1-phosphate (glucose-1-P), and eventually starch. For continuation of the photosynthetic carbon cycle, transketolase cleaves fructose-6-P into erythrose 4-phosphate (erythrose-4-P) and an enzyme-bound glycoaldehyde-thiamine pyrophosphate adduct. Erythrose-4-P and dihydroxyacetone phosphate combine to produce sedoheptulose 1,7-bisphosphate (sedoheptulose-P_2) which is split by sedoheptulose-P_2 1-phosphatase to form sedoheptulose 7-phosphate (sedoheptulose-7-P). In a second reaction catalyzed by transketolase, sedoheptulose-7-P is split to ribose-5-phosphate (ribose-5-P) and another bound glycoaldehyde-thiamine pyrophosphate. This activated aldehyde is transferred by transketolase to glyceraldehyde-3-P to produce xylulose 5-phosphate (xylulose-5-P). Ribose-5-P, by way of an isomerase, and xylulose-5-P, by way of an epimerase, are both converted to ribulose 5-phosphate (ribulose-5-P). Lastly, the cycle is completed by the ribulose-5-P kinase forming ribulose-P_2 from ATP and ribulose-5-P.

The net result of the photosynthetic carbon cycle is to fix CO_2 to the oxidation level of a carbohydrate. This requires 3 ATP and 2 NADPH per CO_2 reduced. Thus, to produce one hexose from 6 CO_2, 18 ATP and 12 NADPH are required.

REGULATION OF CO_2 FIXATION

Many factors are involved in regulating the rate of photosynthetic carbon assimilation. Under saturating irradiance, CO_2 is limiting for photosynthesis in the atmosphere, and this condition is corrected by growing plants in enriched concentrations of CO_2. The usual atmospheric level of CO_2 is about 330 ppm, while more than 600 ppm CO_2 with cotton is necessary to saturate photosynthesis (Radin and Ackerson, 1981). The level of CO_2 at the site of carboxylation may be considerably less than 320 ppm, but equal or greater than the CO_2 compensation point (about 50 ppm with C_3 plants). This is due to limitations on CO_2 diffusion by the stomata and mesophyll cell resistances.

Recently it has been noted in this laboratory that photosynthesis with plants using air levels of CO_2 and O_2 is often not limited to regeneration of the carboxylation substrate, ribulose-P_2. Indeed a comparison of the effects of irradiance on CO_2 exchange rate show that at low, limiting irradiances, the amount of available ribulose-P_2 is high and saturating even though the CO_2 exchange rate is only 10 to 25 percent of maximal. The increase in photosynthesis at higher irradiances is due to increased activity of the carboxylation enzyme, ribulose-P_2 carboxylase/oxygenase, rather than increased concentration of the substrate, ribulose-P_2, as provided by NADPH and ATP of the light reactions and the Calvin cycle.

RIBULOSE-P_2 CARBOXYLASE/OXYGENASE

The ribulose-P_2 carboxylase often comprises more than 50 percent of the protein in the chloroplast stroma. It is a protein of high molecular weight (560,000) existing as an aggregate of two types of subunits, large and small

(L_8S_8). The larger of the two subunits has a molecular weight of 51,000 to 58,000 while the smaller subunit is 12,000 to 18,000 daltons. The large subunit is catalytically active even in the absence of the small subunit. The function of the small subunit might well be regulatory, but how this is accomplished in the molecule remains to be determined (see Jensen and Bahr, 1977). The structure of crystalline tobacco ribulose-P_2 carboxylase consists of a two-layered structure each having four large and four small spherical masses. The two layers are arranged about a fourfold axis with four twofold axes perpendicular to it. When viewed down the fourfold axis, the molecule is square (Baker *et al.*, 1977).

The problem of how the ribulose-P_2 carboxylase operates and is regulated at air levels of CO_2 *in vivo* is now of considerable research interest. From earlier kinetic studies, it was apparent that the activity of the isolated enzyme, as exhibited by its apparent affinity for CO_2, was too low to account for the observed rates of photosynthetic CO_2 fixation. The $K_m(CO_2)$ for the purified ribulose-P_2 was reported to be high, between 70 and 600 μM. In intact isolated spinach chloroplasts, the apparant $K_m(CO_2)$ for CO_2 fixation is of the order of 10-20 μM. Water, in equilibrium with 1 atm air with 0.03 percent CO_2, has 10 μM CO_2 at 25C.

The $K_m(CO_2)$ of the ribulose-P_2 carboxylase assayed upon lysis of chloroplasts was 11-18 μM at pH 7.8, which is comparable to that for light-dependent CO_2 fixation by intact chloroplasts. At low CO_2 concentrations, the kinetics of the ribulose-P_2 carboxylase is not stable after release from the chloroplast. During assays up to 10 min a lower steady rate is obtained after 3 min, which eventually displays a Km (CO_2) value of about 500 μM, comparable to that seen in most previous work with the purified carboxylase. Incubation of the enzyme in buffer alone or buffer plus ribulose-P_2 before adding CO_2 and Mg^{2+} hastens the decline in activity. In addition it appears that the ribulose-P_2 carboxylase, while still in the intact chloroplast, is not fully activated, and this degree of activation can be altered by incubating chloroplasts with various CO_2 concentrations.

As the rate of carboxylation of ribulose-P_2 in the plant defines the rate of gross photosynthesis, the rate of ribulose-P_2 oxygenation is the major determinant for glycolate production for photorespiration. O_2 has been shown to be a competitive inhibitor of carboxylation, as CO_2 is of oxygenation. The relative rates of the two reactions are regulated by the concentration of O_2 and CO_2. Where both reactions have been measured under the same conditions, effector metabolites such as NADPH, gluconate-6-P and glycerate-3-P activate or inhibit both reactions to the same extent.

The time-dependent and order of addition-dependent kinetics of ribulose-P_2 carboxylase are a result of the activating effects of Mg^{2+} and CO_2 and the inactivating effects of ribulose-P_2, in addition to their roles as substrates (Figure 4). The initial activity of the enzyme responds to the concentration of CO_2 and Mg^{2+} during preincubation, indicating the reversible formation of an active enzyme-CO_2-Mg^{2+} complex. Kinetic analyses have indicated that the enzyme is activated by a slow, but reversible, initial binding between enzyme and CO_2 followed by a rapid reaction with Mg^{2+}. The amount of activation is a function of

both CO_2 and Mg^{2+} concentration and the final degree of activation at fixed CO_2 and Mg^{2+} is sharply pH dependent with a distinctly alkaline pK_A. The relationship between CO_2 and Mg^{2+} concentrations and pH during activation suggests that the protonated enzyme does not react with CO_2. When the enzyme is first incubated with ribulose-P_2 and the reaction is initiated with Mg^{2+} and CO_2, a marked lag is observed in the course of product formation.

Activation of the carboxylase followed by reaction with either CO_2 to give two molecules of glycerate-3-P or O_2 to give P-glycolate and glycerate-3-P is shown in the scheme of Figure 4. Mg^{2+} and CO_2 are necessary for enzyme activation with the first CO_2 separate from the CO_2 involved in catalysis. When spinach chloroplasts are lysed in the presence of saturating amounts of ribulose-P_2, Mg^{2+} and

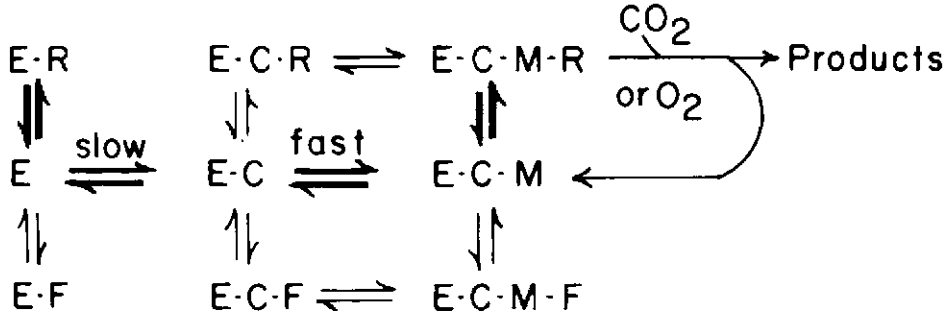


Figure 4. Mechanism of activation of the ribulose-P_2 carboxylase/oxygenase (E) by CO_2 (C) and Mg^{2+} (M). The active form, E•C•M, when bound to ribulose-P_2 (R) reacts with either CO_2 or O_2 to form products. Other effector metabolites (F) can also bind with the enzyme to influence its kinetics.

CO_2, the initial rate of CO_2 fixation can be about 200 μmoles/mg Chl•h. If upon lysis the extract is allowed to activate in the presence of Mg^{2+} and bicarbonate, then ribulose-P_2 added, the rate of fixation can be 300-400 μmoles/mg Chl•h. The amount of activation measured corresponds to the amount of the enzyme that exists as the E•C and E•C•M forms.

The activity of ribulose-P_2 carboxylase can be variable while the enzyme is in the chloroplast. Incubation of chloroplasts in high CO_2 levels activates the enzyme, as does illumination. This can be reversed by removing the CO_2 and/or by dark. The light activation is inhibited by electron transport inhibitors, such as DCMU or uncouplers for photophosphorylation such as CCCP. The chloroplast carboxylase is inactivated in the dark under conditions where the E•C complex can dissociate in a CO_2-deficient media. Light activation and dark inactivation can be explained by light-dependent changes of Mg^{2+} and pH in the chloroplast (Bahr and Jensen, 1978).

As stated above, with higher plants growing with air levels of CO_2, the levels of the substrate, ribulose-P_2, can be high and saturating under most irradiances. Under these conditions the rate of photosynthetic CO_2 fixation appears to be

controlled by the activity of the ribulose-P_2 carboxylase. In dark, ribulose-P_2 drops to less than 10 percent of the amount in light which stops photosynthesis. The mechanisms which control the activity of the ribulose-P_2 carboxylase in light most likely involves light mediated changes in the Mg^{2+} and pH in the chloroplast stroma. In this manner the light reactions exert control over the rate of CO_2 fixation by regulating the activity of the ribulose-P_2 carboxylase (Perchorowicz *et al.*, 1981).

OTHER ENZYMES REGULATED BY LIGHT

Many other reactions of the Calvin cycle are influenced in the chloroplast by light. This influence is not a direct effect of light on the enzyme but is an effect of changing conditions in the chloroplast stroma to cause enzyme activities to increase or decrease. The enzymes which appear to be increased in activity in the light include: NADP-glyceraldehyde-3-P dehydrogenase, glycerate-3-P kinase, fructose-P_2 1-phosphatase, sedoheptulose-P_2 1-phosphatase and ribulose-5-P kinase. Although not involved in the Calvin cycle, glucose-6-P dehydrogenase becomes inactive in the light. The ribulose-5-P kinase has been shown to become practically inactive in the dark, whereas the other regulated enzymes are stimulated two to eight fold in the light.

The major mechanism for light activation of these enzymes involves transfer of photosynthetically generated reducing power from photosystem I to the reduction of exposed thiol groups on the enzyme protein. One attractive mechanism involves the transfer of electrons from photosystem I to ferrodoxin which then reduces a small molecular weight intermediate, thioredoxin, by way of the ferredoxin-thioredoxin reductase. The reduced thioredoxin then interacts with the thiol groups of the enzymes to modulate activity of the enzyme during photosynthesis (Buchanan, 1980). A number of different forms of thioredoxin, each relatively specific for the enzyme being regulated, have been reported. Of interest, not all of these forms are located in the chloroplast.

Complementary to the soluble system described above, activation of these enzymes by thylakoid membrane-bound components has been proposed. These membranous reductants (designated "light effect mediators" or LEMS) are thiol containing components that occur in the oxidized (disulfide) state in the dark and in the reduced (sulfhydryl) state in the light. There are two different LEM components. Both are reduced photochemically by noncyclic electron transport at different sites on the oxidizing side of photosystem I. LEM I is reduced directly by the electron acceptor of photosystem I while LEM II receives reducing power through ferredoxin from photosystem I (Anderson, 1979).

The significance of regulation of photosynthesis by light-mediated activation is self-evident. When light is on there is need to produce ribulose-P_2 for carboxylation. However, once the level of this substrate is saturating, modulation of these enzymes still regulates photosynthesis by regulating the products. Modulation at the fructose-P_2 and sedoheptulose-P_2 1-phosphatases regulates whether carbon

leaves the chloroplast as triose-P or remains in the chloroplast to be stored as starch. Regulation of the NADP-glyceraldehyde-3-P dehydrogenase and glycerate-3-P kinase control whether glycerate-3-P leaves the chloroplast or the triose phosphates leave the chloroplast as carbon sources to support sucrose biosynthesis in the cytoplasm.

STORAGE OF ENERGY BY STARCH ACCUMULATION

The rate of leaf photosynthesis appears to respond proportionately to the rate at which the photosynthetic products are transported and utilized. CO_2 fixation during photosynthesis is often reduced when high accumulations of starch occur in the leaf chloroplast. This led to the hypothesis of product inhibition on photosynthesis. If a cold night prevents the breakdown and translocation of starch, photosynthesis the next day is decreased. If the plants have a greater need for utilization or greater sink requirements, then little starch will accumulate during the day, and the photosynthetic rates will be higher than those with less sink requirements (see Chapter 16). This relationship suggests that photosynthesis may be indirectly inhibited by accumulation of starch, if translocation is limited. However, endogenous sucrose in leaves appears to have little adverse effect on the photosynthetic rate, most likely because sucrose is not formed nor located in the chloroplast where photosynthesis occurs. However, if leaves suspended in solution are fed sugars such as mannose or glucose, the starch content does increase in the light. This increase does not appear to be due to direct glucose incorporation into starch, but rather the results of sequestering cytoplasmic inorganic phosphate (Walker, 1976; Herold, 1980).

The drop in photosynthesis with high starch content in the chloroplast is proposed to be due to the physical distortion of the chloroplast by the starch grains. A chloroplast, largely free of starch grains, is an extremely thin organelle in the living cell. The accumulation of starch between the thylakoids can distort the chloroplast so that it approaches the shape of a sphere. This could increase the effective path length of CO_2 diffusion or tend to bind Mg^{2+}, and thus reduce the activity of the ribulose-P_2 carboxylase. The actual regulatory mechanisms by which chloroplast starch accumulation limits photosynthesis are still quite speculative and require more investigative research.

Starch biosynthesis in the chloroplast operates by the following reaction:

$$\text{ATP} + \alpha\text{-glucose-1-P} \longrightarrow \text{ADP-glucose} + \text{pyrophosphate}$$

$$\text{ADP-glucose} + \alpha\ 1\text{-}4\ \text{glucan} \longrightarrow \text{ADP} + \alpha\ 1\text{-}4\ \text{glucosyl-glucan}$$

The first step catalyzed by ADP-glucose pyrophosphorylase primes the synthetic route by formation of ADP-glucose. Metabolic regulation of starch formation apparently occurs by control of this allosteric enzyme. The second step is catalyzed by an α 1-4 glucan (starch) synthetase and adds a glucosyl residue to the glucan primer. Usually, the enzyme is intimately associated with the starch granule (Preiss and Levi, 1980).

Another enzyme, starch phosphorylase, is also capable of synthesizing starch:

$$\alpha\text{-glucose-1-P} + \alpha\ 1\text{-}4\ \text{glucan} \rightleftharpoons \alpha\ 1\text{-}4\ \text{glucosyl-glucan} + \text{phosphate}$$

The phosphorylase is most likely involved only in the degradation or breakdown of starch rather than in synthesis because of the high ratio of inorganic phosphate to glucose-1-P in the chloroplast. With isolated chloroplasts, the levels of inorganic phosphate have been measured from a low value of around 4 mM to over 100 mM.

Most studies on the path of starch synthesis and breakdown have been deduced from experiments with purified chloroplast enzymes. They suggest that regulation resides with the ADP-glucose pyrophosphorylase. This enzyme is allosterically affected by intermediates of the photosynthetic carbon cycle, with positive activation by glycerate-3-P and inhibition by inorganic phosphate. Indeed, with isolated chloroplasts, higher amounts of inorganic phosphate in the suspending media do reduce the amount of carbon going to starch. If glycerate-3-P is increased, then there is an increase in starch synthesis. Photosynthesis is also inhibited by high orthophosphate and reversed by glycerate-3-P and triose phosphates. The phosphate inhibition could be caused by a loss of intermediates of the carbon cycle including glycerate-3-P with depletion of the ribulose-P_2 pool. When triose phosphates are added, the phosphate-induced loss of sugar phosphates from the chloroplast is reversed, glycerate-3-P increases, and starch synthesis is enhanced (Heldt *et al.*, 1977).

The control of starch metabolism by levels of inorganic phosphate in the cytoplasm appears to be operating in the leaf. Processes which increase inorganic phosphate in the cytoplasm, such as the hydrolysis of sucrose phosphate to sucrose, facilitate export of triose phosphates from the chloroplast in exchange for inorganic phosphate. When mannose was added to leaf discs of spinach beet, phosphate was sequestered in the cytoplasm as mannose-6-phosphate. Starch formation in these leaf discs was increased tenfold, yet the starch formed was not synthesized from the mannose carbon but from CO_2 (Herold *et al.*, 1976).

Starch stored in the chloroplast during the day is mobilized to soluble products and exported from the chloroplast at night. Isolated chloroplasts loaded with ^{14}C-starch in the light will remobilize this starch in the dark into glycerate-3-P and maltose as the major products (Peavey *et al.*, 1977; Stitt and Heldt, 1981). This mobilization is promoted by phosphate and inhibited by glycerate-3-P. Comparisons of enzyme activities of cytoplasm with chloroplast fractions of pea suggest that maltose comes from action of maltose phosphorylase rather than β-amylase during starch degradation. The results are consistent with phosphorylytic mechanism of chloroplast starch breakdown.

PHOTORESPIRATION AND ITS REQUIREMENTS

Photorespiration is the uptake of oxygen and the formation of CO_2 in the light during the metabolic processes associated with photosynthesis. The exchange of oxygen and CO_2 and energy loss from photorespiration occur simultaneously with photosynthesis. Therefore, net photosynthesis is measured as the difference be-

tween the gross or true CO_2 fixation rate minus the rate of photorespiration. The magnitude of photorespiration as estimated from CO_2 gas exchange varies among plants from a low and almost immeasurable value for C_4 plants to high values of 25 to 50 percent of the photosynthetic rate in C_3 plants. Photorespiration increases with increasing light intensity, temperature and oxygen concentration and with decreasing CO_2 availability. Gaseous measurements of CO_2 and O_2 exchange during photorespiration are generally underestimations since the opposite exchange of photosynthesis masks the true magnitude of the metabolic turnover of photorespiration. At the CO_2 or O_2 compensation point, no net CO_2 or O_2 exchange between the leaf and it environment is observed because of equal and counterbalancing rates of gas diffusion between photosynthesis and photorespiration. Respiration during the light also adds somewhat to the value estimated as photorespiration. The term "photorespiration" as used today is mostly equivalent to the "Warburg" effect as described for the inhibition of photosynthesis by oxygen (Gibbs, 1969). A few of the many reviews about photorespiration are by Zelitch (1971), Chollet and Ogren (1975), Schnarrenberger and Fork (1976) and Tolbert (1980).

A great number of factors regulate total photosynthetic activity, but when most of these such as water, temperature, nutrient availability and light are optimum, photosynthesis is still limited by the low CO_2 and high O_2 content in air (0.33 percent CO_2 and 21 percent O_2). The light intensity over the upper part of a leaf canopy during the middle of a summer day generally exceeds that which can be used for CO_2 fixation. Under these optimum conditions the rate of photosynthesis by a cotton plant is limited by the availability of CO_2. The rate can be nearly doubled by increasing the CO_2 content until CO_2 is no longer limiting or by decreasing the O_2 content to 2-5 percent to reduce photorespiration. Under this low oxygen the measured rate of CO_2 gas exchange of photosynthesis is approximately the gross CO_2 fixation rate.

The low CO_2 content in the atmosphere provides a severe limitation on the rate of photosynthesis. With high light and high oxygen the plant is quite sensitive to photoinhibition and appears to be protected by the photorespiratory process. Photorespiration consumes or eliminates the excess photosynthetic assimilatory power (ATP, $NADPH_2$ or reduced ferredoxin). The main function of photorespiration seems to protect the photosynthetic apparatus and regulate the growth by utilizing excess energy. Photorespiration appears to serve as a protective mechanism against light and oxygen toxicity.

The C_4 plants utilize a large part of their excess photosynthetic energy to trap and concentrate the CO_2 in the bundle sheath cells where carboxylation takes place under much higher CO_2 levels. In a C_4 plant the true and apparent rate of photosynthesis are about the same, even though there is some internal photorespiration. The photosynthetic rates observed in C_4 plants are not stimulated by decreasing the O_2 concentration but they may be increased by higher CO_2 and light.

The biochemistry and carbon metabolism of photorespiration and photosynthesis are intimately related so that photosynthesis does not occur in air without photorespiration. While the metabolic processes of photosynthesis occur only in the chloroplast, different parts of the photorespiratory pathway occur in the chloroplasts, peroxisomes, mitochondria and cytoplasm. Although photorespiration has no direct photochemical involvement, the process is called photorespiration because it only occurs in the light as a consequence of the excess reducing capacity generated during photosynthetic electron transport.

The pathway of carbon during photorespiration begins with the biosynthesis of glycolate and its eventual metabolism with loss of CO_2. This complex pathway, as detailed in Figure 5, can be divided into five different segments which occur in the chloroplast, peroxisomes and the mitochondria. These processes have collectively been referred to as the "glycolate pathway", the "C_2 pathway", or the "photorespiratory carbon oxidation cycle". The photorespiratory pathway has been elaborated along with the Calvin photosynthetic carbon cycle by the use of $^{14}CO_2$ tracers and by isolation of enzymes and identification of their compartmentation at each step (Tolbert, 1973). Later experiments using $^{18}O_2$ analyzed by mass spectrophotometry indicated that one atom of $^{18}O_2$ is incorporated into the carboxyl group of glycolate (Berry *et al.*, 1978). This incorporation occurs during activity of the ribulose-P_2 carboxylase/oxygenase.

Glycolate Biosynthesis in the Chloroplast—Carbon is shunted to the photorespiratory pathway by action of the ribulose-P_2 carboxylase/oxygenase and oxygen. The product formed is P-glycolate which comes from carbon atoms 1 and 2 of ribulose-P_2 and O_2 attacks the bound ribulose-P_2 on the carboxylase/oxygenase enzyme. P-glycolate phosphatase hydrolyzes P-glycolate to glycolate which then is transported to the peroxisomes. Other mechanisms for glycolate biosynthesis have been proposed in the chloroplast, but none seem to fit the complete requirements of high oxygen and low CO_2 to support photorespiration.

Glycolate Oxidation and Formation of Glycine in Peroxisomes--In the peroxisome, glycolate is irreversibly oxidized to glyoxylate by glycolate oxidase. This reaction takes in O_2 to form glyoxylate plus H_2O_2, which is broken down to water and O_2 by catalase. The glyoxylate is converted to glycine through the action of several physiologically reversible aminotransferases. Two aminotransferases in leaf peroxisomes are highly specific for glyoxylate and/or the respective amino donor, glutamate or serine. The serine-glyoxylate aminotransferase is a more active enzyme and allows the serine formed to go on to hydroxypyruvate while converting glyoxylate to glycine. However, the formation of one serine requires a conversion of two glyoxylates to two glycines. Thus, a second glyoxylate aminotransferase is necessary, and it is linked to glutamate in the peroxisome. The glutamate is the eventual acceptor of the released NH_3 in the mitochondria during the conversion of two glycines to one serine.

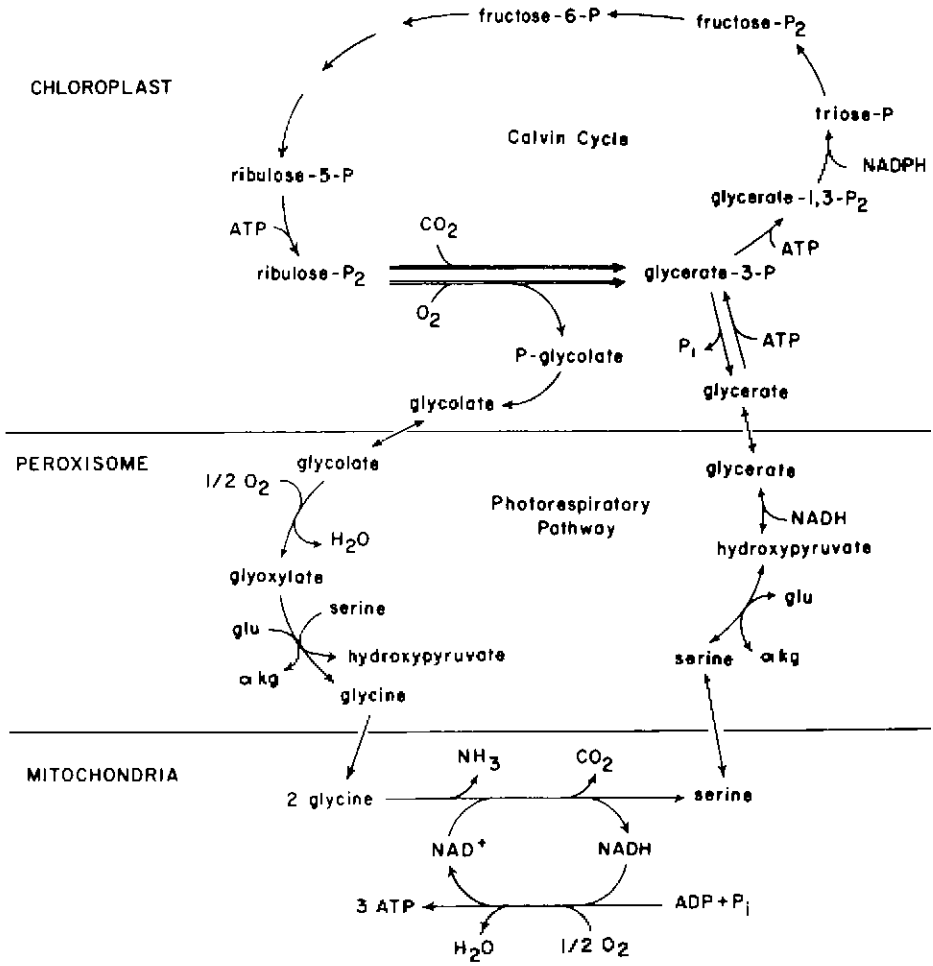


Figure 5. Integration of carbon metabolism between the Calvin cycle of photosynthesis and the photorespiratory pathway.

Two Glycines Oxidized to CO_2, NH_3 and Serine in Mitochondria—Upon transfer of glycine to the mitochondria, serine is formed. This conversion is catalyzed by glycine decarboxylase and serine hydroxymethyl transferase which produces the major source of CO_2 lost during photorespiration. Woo and Osmond (1976) showed that glycine decarboxylation occurs in the intermembrane space of the mitochondria and that rates of glycine decarboxylation approach those required for rates of CO_2 evolution during photorespiration. The oxidative decarboxylation of glycine is coupled to the cytochrome electron transport and oxidative phosphorylation system of the mitochondria. Indeed, the mitochondria of C_3 leaves are

especially adept at oxidizing glycine with rates of O_2 uptake being equal to those with malate or succinate. The mechanism involves the relase of CO_2 and ammonia with the trapping of the carbon two of glycine as N^5, N^{10}-methylenetetrahydrofolate. This is transferred to a second glycine to form serine. During the action of these two enzymes two electrons are available, which after passing through the electron transport pathway, produce ATP by oxidative phosphorylation with the uptake of O_2. The released NH_3 is retrapped by the GOGAT system which forms glutamine and eventually glutamate. As much NH_3 is released as CO_2 so that an effective recycling of NH_3 is operative. The formation of two glycines from two glyoxylates requires two amino donors, a serine and a glutamate. An amino group from the serine is conserved within the glycolate pathway by the formation of one serine from two glycines.

Transamination of Serine and Reduction to Glycerate in Peroxisomes—This pathway between serine and glycerate is a reversible pathway which can include glycerate-3-P during photorespiration. In plants and animals serine can be produced from glycerate-3-P. During photorespiration serine is converted to hydroxypyruvate by the serine-glyoxylate aminotransferase with glyoxylate generated during photorespiration. This aminotransferase is irreversible, and since the glyoxylate is only formed during photorespiration, other serine aminotransferase reactions are also prevelant in leaf proxisomes. The reversible interconversion between glycerate and hydroxypyruvate is catalyzed by a NAD-linked dehydrogenase known as NADH-hydroxypyruvate reductase. This reaction requires a source of NADH which probably occurs through a shuttle of reducing power originating in the chloroplast. This shuttle may well involve malate being shuttled in and oxidized to oxalacetate to produce NADH. During photorespiration this shuttle of reducing power results in additional energy loss to the chloroplast during photosynthesis.

Phosphorylation of Glycerate to Glycerate-3-P in Chloroplast—To reenter the Calvin cycle, glycerate coming from the peroxisome is phosphorylated by glycerate kinase, an enzyme observed in isolated chloroplasts.

Note then, that of the four carbons from the two glycolates which produce one serine, one carbon is lost as CO_2. If the rate of photorespiratory CO_2 release is one fourth of the rate of apparent photosynthesis, it is easy to see that the rate of carbon flowing through glycolate equals the rate of carbon flow during apparent photosynthesis under conditions prevailing in the atmosphere.

Photorespiration is an exothermic, irreversible process whereby energy is lost. A mole of O_2 uptake occurs during ribulose-P_2 oxidation in the chloroplast for each P-glycolate formed. Net uptake of another 0.5 mole of O_2 occurs during glycolate oxidation in the peroxisomes. How much O_2 uptake occurs in the mitochondria during glycine oxidation depends upon how the NADH formed is used. If it supplies electrons to the electron transport pathway to form ATP by

oxidative phosphorylation, another 0.5 moles of O_2 would be utilized. If the NADH were used to refix the NH_3 by the GOGAT system no O_2 uptake would occur in the mitochondria. During serine conversion to glycerate, the NADH consumed for reduction of hydroxypyruvate may have been the result of photosynthetic O_2 evolution. Thus, during photorespiration for each two glycines converted to one serine, one CO_2 would be evolved and at least three O_2 would be taken up with this ratio depending upon the origin of the electrons. There is no net energy conservation as net ATP or NADH formation in any part of photorespiration; there is only energy loss. When one considers the evolution of O_2 during photosynthesis and the oxidative utilization of O_2 during photorespiration, a rapid internal cycling of O_2 occurs.

Since photorespiration occurs because of insufficient CO_2, much of this CO_2 evolved by photorespiration is refixed photosynthetically by ribulose-P_2 carboxylase. The measured amount of CO_2 evolved in the light is thereby an underestimation of photorespiration. Several methods have been used to estimate the magnitude of photorespiration. The degree of inhibition of photosynthesis by 21 percent O_2 (air) is about 20-25 percent of the rate in N_2. Thus, it has been estimated that the rate of photorespiration is 20-25 percent of the rate of photosynthesis. That means that 20-25 percent of the CO_2 fixed is released during photorespiration. Using a value of 25 μmoles CO_2 fixed/m^2•s, the photorespiratory rate of CO_2 release is about 5 μmoles CO_2 released/m^2•s. Dark repiration rates are about 1 to 2 percent or about .05 μmoles/m^2•s. Although an estimate, it appears that photorespiration has a potential of being about 10 times faster than dark respiration.

Photorespiration, by wasting the assimilatory power of photosynthesis, competes with and reduces secondary synthetic processes. Oxygen in the atmosphere at levels above ambient, in addition to decreasing CO_2 fixation, changes the main products of photosynthesis from sucrose to glycine and serine. Since sucrose is the product of photosynthesis used by other parts of the plant, the reduction of its synthesis, even in air by photorespiration, means that the growth of plants is also inhibited in air by photorespiration. Bjorkman *et al.* (1968) and others have grown young plants in 2-5 percent O_2 and found an increase in mass about twice as fast as those grown in air. Two to five percent is sufficient to saturate the mitochondrial requirement for oxygen, but as the ribulose-P_2 carboxylase/oxygenase and the glycolate oxidase have a much lower affinity for O_2, they are not even saturated by 100 percent O_2. When plants are grown at higher levels of O_2 than air, their growth rate slows and ceases around 30-40 percent O_2, depending on the plant. Increasing the O_2 concentration increases photorespiration until a level of O_2 is reached with air levels of CO_2 where photorespiration and photosynthesis are equal.

The main factor regulating photorespiration is the competition between photosynthesis and photorespiration by the competitive substrate availability of CO_2 and O_2 for the carboxylase versus oxygenase activities with bound ribulose-P_2. This competition was well demonstrated *in vivo* as well as with the isolated

enzyme by Bowes and Ogren (1972). With whole plants the competition is shown upon determining the CO_2 compensation point. This is the condition when the net exchange of CO_2 due to photosynthesis and photorespiration is zero. Most often CO_2 compensation points are determined by putting a plant or leaf in a closed chamber with light and temperature control and measuring the final steady state level of CO_2 in the chamber.

Photorespiration increases with increasing light, temperature and pH. All of these factors can be explained by the avilability of CO_2 versus O_2 for the ribulose-P_2 carboxylase/oxygenase in the leaf. Photorespiration increases more rapidly as temperature increases than does photosynthesis so that at elevated temperatures (between 35 and 45C) the CO_2 compensation point is approached even in air. This phenomenon can be attributed, at least in part, to a faster decrease in CO_2 solubility than O_2 solubility in water with increasing temperature (Ku and Edwards, 1977). Other than temperature effects which affect solubility of CO_2 and O_2, there does not appear to be methods to regulate oxygenase activity versus carboxylase activity. Increased carboxylase activity will give also increased oxygenase activity. Because carbon during metabolism is committed to either photorespiration or photosynthesis by action of the ribulose-P_2 carboxylase/oxygenase, the regulation of photorespiration is most difficult and elimination appears impossible and not safe for the plant.

BIOCHEMICAL LIMITATIONS OF WHOLE PLANT PHOTOSYNTHESIS

Many conditions in the environment affect the role of photosynthetic CO_2 uptake. One approach for considering the cellular limitations is to ask what limits the action of the ribulose-P_2 carboxylase. Enzyme studies as well as chloroplast and leaf measurements show that, if the substrates, CO_2 and ribulose-P_2, are present and the ribulose-P_2 carboxylase is active, then CO_2 fixation must occur. Those conditions which result in limiting the availability of CO_2, the regeneration of ribulose-P_2 or the amount of active ribulose-P_2 carboxylase can be considered as the biochemical limitations of whole plant photosynthesis.

For the level of 0.032-0.035 percent CO_2 in the atmosphere, photosynthesis is limited by CO_2 availability. The internal CO_2 is still less as it is restricted by stomatal aperature. The presence of O_2 also competes with CO_2 for the bound ribulose-P_2 on the carboxylase. Limits on photosynthesis by limiting CO_2 occurs also under high temperature (solubility of CO_2) as well as increases in atmospheric O_2.

The action of light on the photosynthetic apparatus provides energy to regenerate the CO_2 acceptor, ribulose-P_2. If ribulose-P_2, which is only found in the chloroplast, is not available, then CO_2 fixation stops. This occurs in the dark where ribulose-P_2 drops to almost zero. When light is present, ATP and NADPH are used to form ribulose-P_2 from other sugar phosphates. The lack of carbon to

regenerate ribulose-P_2 occurs following high rates of photorespiration or after long periods of darkness where starch and other carbohydrate reserves have been exhausted. Other sugar phosphates are known to compete with ribulose-P_2 for binding on the carboxylase further reducing fixation especially when ribulose-P_2 is low. Regeneration of ribulose-P_2 is also limited by the availability of inorganic phosphate to produce ATP.

When CO_2 and ribulose-P_2 are available, photosynthetic CO_2 assimilation can still be limited by the amount of active ribulose-P_2 carboxylase/oxygenase (Perchorowicz *et al.*, 1981). Under some conditions the amount of the available enzyme protein is limiting, or the amount of active enzyme compared to inactive enzyme is low. In low light the enzyme becomes inactive due to suboptimal levels of Mg^{2+} and pH in the chloroplast stroma. This parameter can be measured and a good approximation made of the availability of activated ribulose-P_2 carboxylase (Perchorowicz *et al.*, 1982). Other environmental conditions such as heat and water stress can indirectly limit activity of the ribulose-P_2 carboxylase in the chloroplast. Under limiting cell water many of the binding sites of the ribulose-P_2 carboxylase may not be available or may already be bound with other intermediates.

Laboratory research to directly determine the factors limiting photosynthesis is becoming more prevalent. One can easily determine CO_2 availability by measuring stomatal resistance with a dew point hygrometer. Techniques for measuring the substrate, ribulose-P_2, are well developed, although they usually underestimate the amount by at least 10 percent (Perchorowicz *et al.*, 1981). As activation of the carboxylase involves a slow conformational change in the protein, this can apparently be determined by kinetic measurements after chilling the leaves to ice temperature and making leaf extracts (Perchorowicz *et al.*, 1982). The use of these approaches should help to define the actual biochemical limitations on photosynthesis as caused by different environmental stresses.

SUMMARY

Photosynthesis is the essential process whereby plants utilize radiant energy to synthesize their biomass from CO_2, while O_2 is evolved. Most of this process operates in the chloroplast where both the pigmented photosystems and the required enzymes are located. The pigment systems, which are organized as two photosystems, are imbedded in lamellar membranes and contain mostly chlorophyll and some carotenoids. Light radiant energy is trapped by the pigments and, with the assistance of electron transfer mediators (mostly quinones and cytochromes), is converted to chemical energy as ATP and NADPH to drive the photosynthetic carbon reduction pathway. Ribulose 1,5-bisphosphate is generated by this metabolic pathway and serves as the initial acceptor for CO_2 during photosynthesis. This carboxylation produces 3-phosphoglycerate which, upon reduction in the chloroplast, serves eventually as the carbon and energy source for

sucrose production in the cytoplasm. Some of the fixed carbon remains in the chloroplast as starch.

A major control of carbon assimilation during photosynthesis occurs at the carboxylation step where the activity of the ribulose 1,5-bisphosphate carboxylase is regulated. Further controls regulate the regeneration of ribulose 1,5-bisphosphate by modifying the activity of other enzymes of the photosynthetic carbon reduction pathway. Because the carboxylase also catalyzes a competing reaction with oxygen, a second pathway for carbon flow occurs called photorespiration. This process returns three of the four carbons which are diverted into photorespiration back to the photosynthetic pathway. The fourth is evolved as CO_2.

Biochemical limitations on photosynthesis can be viewed as limitations on CO_2 fixation. Reduction in photosynthesis is caused by either limiting CO_2 availability or ribulose bisphosphate regeneration or by reduced activity of the carboxylase enzyme. Current research promises to eventually increase photosynthetic productivity by determining how these limitations can be overcome.

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