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Problems and Possibilities in Controlling Oxygen Inhibition of Photosynthesis

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This review considers the different ways, direct or indirect, by which oxygen affects photosynthesis and aims at evaluating various possibilities of controlling the oxygen-inhibition of photosynthesis. Two major ways in which oxygen affects photosynthesis are: (a) oxygenase reaction of the enzyme ribulose 1, 5-bisphosphate (RuBP) carboxylase-oxygenase leading to the production of glycolate which through photorespiratory pathway results in loss of carbon as CO_2; (b) direct oxidation of photo-produced reductant in a Mehler type of reaction thereby resulting in loss of net photosynthesis.

The analysis of results obtained with various inhibitors used to check glycolate metabolism, glycine decarboxylation suggests that this type of chemical control is of limited value and cannot be used as a method of controlling photorespiratory losses. A different group of chemical inhibitors have been used with an aim at differentially affecting the oxygenase reaction of the bifunctional enzyme RuBP carboxylase-oxygenase. Analysis of the results obtained by these types of chemical modifiers suggests that it is probably difficult to alter the oxygenase function without affecting carboxylase activity. However, possibilities remain that the enzyme shows microheterogeneity for these two functions and thus further basic studies on the biochemistry and genetics of the active and the activator site are necessary before a differential alteration in enzyme function can be made. The review focuses at the possibility of the occurrence of a direct oxidation of photo-products of photosystems I and II resulting in depletion of reduced nicotinamide adenine dinucleotide phosphate (NADPH). It is however difficult, at present, to evaluate the extent of such auto-oxidation under in vivo conditions. The survey of the literature is quite indicative of the existence of such a possibility. Hence this mode of oxygen inhibition ought to be controlled in order to increase net photosynthesis. It is suggested that further studies on elucidating the mechanisms of O_2 inhibition of photosynthesis are required for any long-term practical solution in order to prevent losses.

Key Words: Photosynthesis, Photorespiration, Ribulose 1, 5-bisphosphate carboxylase, Glycolytic pathway, Mehler reaction

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Introduction

It is a well established fact that about 90% of plant dry mass comes from photosynthetically fixed CO_2 and only around 10% from nutrients and minerals present in the soil. Therefore, any amount of fertilizer input, with best kind of agronomic practices, cannot increase the crop productivity beyond a certain limit. Thus, a very important way to achieve higher dry mass production, and if possible a proportional increase in economic yield, is to increase the photosynthetic efficiency of crop plants.

The very purpose of solar energy conversion through photosynthesis by green plants is to produce low potential reductants which can reduce atmospheric CO_2 to highly-reduced components like carbohydrates and fatty acids, against the challenge offered by high-oxidizing power of oxygen present in the atmosphere in a greater proportion than CO_2. The oxidation and reduction reactions in the photosynthetic electron-transport chain are fast and they do not permit the O_2 produced during oxidation of water to oxidise the reduced components of the electron-transport chain. However, the process in the electron-transport chain is not completely protected against oxygen attack since the reductant generated at the terminal step of photosystem I (PSI) reaction may be prone to oxidation by O_2, before it is enzymatically oxidised in a relatively slow process. Also, the reducing components of the photosynthetic carbon reduction cycle, generated through enzymatic reactions, are very susceptible to oxygen attack and are easily oxidized to some other components, leading to the depletion of the intermediates of carbon reduction cycle and thus resulting in a consequent reduction of the rate of photosynthesis.

The oxygen inhibition of photosynthesis can be broadly divided into three major types. Firstly, the competition of O_2 with CO_2 for the catalytic site of the enzyme ribulose 1,5-bisphosphate (RuBP) carboxylase leads to the oxidation of reduced carbohydrate compounds and causes a big loss of intermediates of carbon reduction cycle resulting in the reduced rate of photosynthesis. This process is termed as "photorespiration". Secondly, oxygen brings about the oxidation of the reductant generated by the photoelectron transport and competes with physiological electron acceptor nicotinamide adenine dinucteotide phosphate (NADP). This process is called 'Mehler reaction'. We have designated it as "direct oxygen inhibition of photosynthesis". Thirdly, the irreversible photooxidative damage to photosynthetic machinery by O_2 which is chiefly expressed under CO_2 depletion conditions. It has been suggested that a direct attack of active oxygen species may affect permeability of the cells and cause sensitivity to photoinhibition (Foyer & Hall 1980 and Kaplan 1981).

In the present article, we do not intend to review the mechanism of oxygen-inhibition of 'photosynthesis' but wish (1) to catalogue and evaluate the current approaches that are being made to control photorespiration (the first component of oxygen-inhibition of photosynthesis), and (2) to establish the existence and locations of any Mehler reaction (second component) of O_2 inhibition of photosynthesis in the intact plants.

Photorespiration

Photorespiration refers to a light-dependent consumption of O_2 with a concomitant production of CO_2 (Decker 1955 and Tregunna et al. 1961, 1964). Glycolate, the substrate for photorespiration (Zelitch

1966), is derived from intermediates of the Calvin cycle in the chloroplasts-involving reactions which also requires the participation of molecular O_2. The main route of glycolic acid production is through an oxygenase reaction catalyzed by fraction-I protein (Bowes et al. 1972, Lorimer et al. 1972, 1973, Takabe & Akazawa 1973, Krik & Heber 1976 and Krause et al. 1977). Phosphoglycolate produced during the RuBP oxygenase reaction is dephosphorylated by phosphoglycolate phosphatase localized in the chloroplasts to form glycolate (Richardson & Tolbert 1961).

The balance between photosynthesis and photorespiration is based on the dual activities of RuBP carboxylase. The competition between CO_2 and O_2 for RuBP at the catalytic site of carboxylation determines the relative rates of photosynthesis and photorespiration, high CO_2 or low O_2 favour carboxylation and, therefore, photosynthesis; while low CO_2 or high O_2 favour oxygenation and, therefore, glycolate synthesis and photorespiration.

The other possible route of getting glycolate can be the formation of peroxide in a Mehler-type reaction between O_2 and reduced ferredoxin and subsequent peroxidation of pentose phosphate to form glycolic acid (Coombs & Whittingham 1966). Such a synthesis of glycolate in the chloroplast might represent the first step in photorespiratory metabolism.

The glycolic acid thus formed in the chloroplast migrates to peroxisome, (single membrane bound organelle found in close spatial association with chloroplasts and mitochondria where it is oxidised to glyoxylate by glycolate oxidase (Tolbert 1971). Glyoxylate so formed may be partially oxidised to form formate and CO_2 (Zelitch 1966) or may be aminated by glutamate/glyoxylate aminotransferase to make glycine (Kisaki & Tolbert 1969). Glycine then migrates to mitochondria where two moles of glycine is decarboxylated by a tetrahydrofolate (THF)-mediated reaction to form serine and CO_2. This reaction is catalysed by glycine decarboxylase and serine hydroxymethyl transferase and is accompanied by the formation of ATP (Kisaki & Tolbert 1969,1970, Kisaki et al. 1971a, b, Bird et al. 1972). This conversion of glycine to serine in mitochondria is the major source of photorespiratory CO_2 evolution. Serine, once formed, can be further metabolised in the peroxisome to glycerate, which upon phosphorylation can re-enter the Calvin cycle and be converted to sucrose (*see* Tolbert 1971) (fig. 1).

Thus, there is net loss of carbon from the plant as the photorespiratory efflux of CO_2. This release of CO_2 also decreases the size of diffusion gradient for CO_2 into the leaf. Loss of carbon from the photosynthetic carbon reduction cycle (Calvin cycle) in the form of glycolate depletes the pools of carboxylation substrate (RuBP) and other intermediates of the carbon cycle (Coombs 1976).

However, in contrast to this picture, Krause et al. (1977) and Powels and Osmond (1978) advocate the thesis that normal dissipation of excess energy absorbed during the harvest of solar energy by chlorophyll (Chl) molecules can not be achieved without carbon assimilation and photorespiration. In the absence of photorespiration, accumulated energy during illumination cannot be dissipated harmlessly. This is postulated as leading to the inhibition of CO_2-fixing capacity of photosynthesis. The suggestion needs confirmation. As we will see later in this review, the quantum yield in terms of CO_2-fixed per einstein is high at low O_2 content in C_3-plants and also

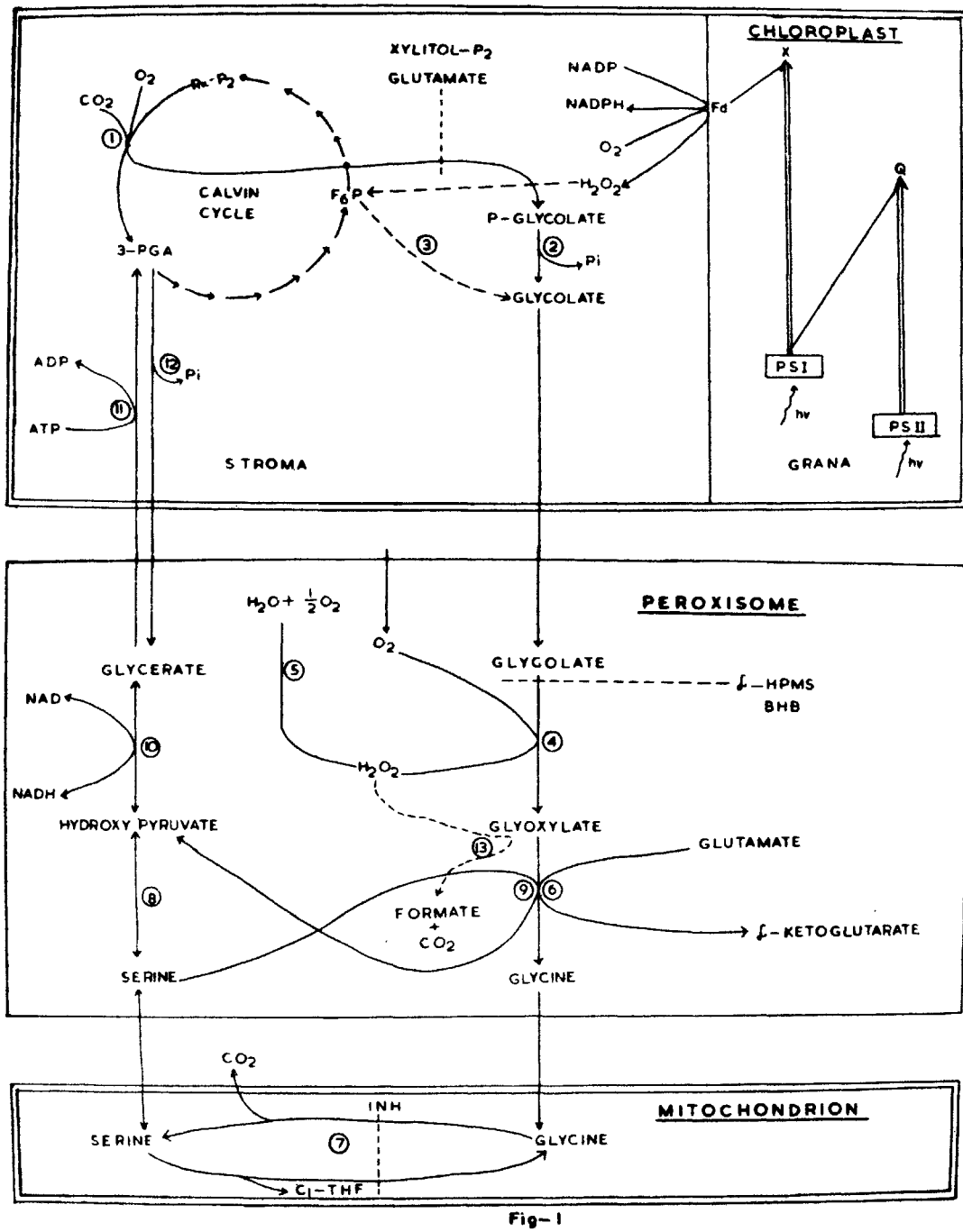

Fig-1

Figure 1 Pathways of photorespiration. The specific reactions are: *1*, Ru-P_2 carboxylase-oxygenase; *2*, Phospho-glycolate phosphatase; *3*, Non-enzymatic oxidation by H_2O_2; *4*, Glycolate Oxidase; *5*, Catalase; *6*, Glutamate-glyoxylate aminotrasferase; *7*, Glycine decarboxylase and serine hydroxy methyl transferase; *8*, Amino transferase, *9*, Serine-glyoxylate aminotransferase; *10*, NADH-hydroxypyruvate reductase; *11*, Glycerate kinase; *12*, PGA phosphatase; *13*, Non-enzymatic oxidation of glyoxylate

we note that the rate of CO_2 fixation at rate saturating intensity is high. The very process of photorespiration appears to be harmful for photosynthesis and thus this process should be checked. The C_4 cycle is able to overcome the disadvantages of photorespiratory pathway.

The C_4-Photosynthesis

Some plants (the C_4-plants) have evolved a biochemically and anatomically complex variant for the photosynthetic assimilation of CO_2 (Hatch & Slack 1966). This modification increases the potential for photosynthesis.

The primary assimilation of CO_2 in C_4-plants occurs via the phosphoenolpyruvate (PEP) carboxylase catalysing irreversible carboxylation and the product, oxaloacetate, is then converted to two other C_4 (4 carbon) acids, namely, malate and aspartate, via NADP malate dehydrogenase and aspartate aminotransferase, respectively (Black 1973, Chollet & Ogren 1975). These latter acids are then transferred to the bundle sheath cells, probably via simple diffusion through the cell cytoplasm (Hatch 1977). The precise steps that follow the intercellular transfer of these acids vary in individual species. However, the net result is the same. In each case, CO_2 released by decarboxylation of the C_4-carboxyl group to form pyruvate, is refixed via Calvin cycle, which is specifically located in the bundle sheath cells. The C_3 product remaining after decarboxylation of C_4 acids (i.e., pyruvate) is transferred back to mesophyll cells, where it serves as a precursor of the primary CO_2 acceptor, PEP. The final step in the regeneration of PEP is catalyzed via pyruvate phosphate dikinase, an enzyme specific amongst plants to those utilizing the C_4 pathway. This enzyme is located in the mesophyll chloroplasts and it catalyses the above reaction. Since the initial steps in the recycling of adenosine monophosphate (AMP) and pyrophosphate are catalyzed by adenylate kinase and pyrophosphatase respectively, the effective energy cost for PEP formation from pyruvate is 2 ATP.

Plants utilising the C_4-pathway have a unique leaf anatomy (Kranz-anatomy), which may be critical for the operation of the biochemical processes responsible for photosynthesis. In most C_4 species, there are two layers of photosynthetic cells in the leaf, the outer mesophyll cells and the inner bundle sheath layers. Earlier, studies were carried out using species of economic plants such as maize, sorghum, sugarcane, in which bundle sheath chloroplasts differ conspicuously from those of mesophyll cells, in that the grana are much reduced or absent.

On the basis of a wide range of studies, the C_4-plants, can be divided into three distinct groups. These groups are distinguished primarily on the basis of most active enzyme capable of decarboxylating the C_4 organic acid which occurs in a given species. Also, the three groups of plants may also differ at the morphological and ultrastructural level. The C_4-plants in which malate is preferentially formed during photosynthesis (malate-formers), generally have chloroplasts with reduced grana in the bundle sheath cells. Those species in which aspartate is formed (aspartate-formers) can be further divided on the basis of the position of the chloroplasts within the bundle sheath cells, whether they occur in their centripetal or centrifugal arrangement. Species with centrifugal arrangements of chloroplasts may have higher levels of phosphoenol pyruvate (PEP) carboxykinase, whereas, those with centripetal arrangement have a higher level of NAD specific malic enzyme (Coombs & Green-

wood 1976). The importance of Kranz-anatomy in C_4-photosynthesis has also been questioned.

Thus, the C_4 pathway serves as a 'biochemical CO_2 pump' for increasing CO_2 concentration at the site of RuBP carboxylase in the bundle sheath cells above that of CO_2 in free equilibrium with the leaf. This elevated level of CO_2 in C_4-plants is in marked contrast to the situation in C_2 species, in which CO_2 concentration at the site of RuBP carboxylase is in more direct equilibrium with the external atmosphere (Bjorkman 1973).

Control of Photorespiration

Attempts have been made to control photorespiration and, thereby, increase net photosynthesis by different methods. These include: (i) altering atmospheric oxygen or carbon dioxide concentration, (ii) plant breeding, (iii) inducing mutations, (iv) cell culture experiments, and (v) chemical control of photorespiration. Despite the fact that it is possible to increase photosynthesis by high concentration of CO_2 and low concentration of O_2, it is impracticable to achieve this in field conditions. Similarly, because of the complexity of higher plants with their multicellular organisations and biochemical variation, extensive studies are needed before mutations or plant breeding can be expected to be useful even though there are a few optimistic reports which have appeared on this subject (Rice & Carlson 1975, Bjorkman et al. 1970 and Day 1977). Our intention here is to present a detailed account of chemical control of photorespiration, an area which may prove more promising.

Chemical Control

(a) Site-specific Inhibition of the Photorespiratory Pathway

Chemical control of photorespiration has apparently been achieved for a short period of time. Addition of hydroxypyridenemethanesulphonic acid (α-HPMSA), an inhibitor of glycolate oxidase (see figure 1), increases CO_2-fixation by 50% in tobacco leaf discs (Zelitch 1966) (table 1). However, this chemical cannot be used in long-term experiments, as it closes stomates and penetrates the chloroplast membrane (Khavari-Nazad 1977) inhibiting the photosynthetic process itself.

Another chemical, 2-hydroxy-3-butynoic acid (HBA) is an irreversible inhibitor of glycolate oxidase once it destroys the co-enzyme flavin mono nucleotide (FMN) (Jewess et al. 1975). Exposure of illuminated leaf discs to this compound causes accumulation of glycolate, but there is no observed increase in net CO_2 assimilation (Halliwell 1976). Kumarasinghe et al. (1977) have demonstrated that at 150 ppm of $^{14}CO_2$, the treatment of wheat leaf segments with sodium-2-hydroxy-3-butynoic acid (SHB) for 40 min resulted in an accumulation of ^{14}C in glycolic acid and inhibition (7%) of photosynthesis. However, when the above treated leaves were subsequently allowed to photosynthesize at 1000 vpm of CO_2, no inhibition of photosynthesis was observed (table 1) and there was a rapid increase in the radioactivity of sucrose, with a simultaneous decrease in radioactivity in glycine.

A very sucessful chemical may be one, which inhibits or checks the synthesis of glycolic acid. Glycidate, an inhibitor of glycolate formation, stimulates net photosynthetic CO_2 incorporation into leaf discs of tobacco (Zelitch 1974). Zelitch (1978) and Lawyer and Zelitch (1978) have studied the effect of glycidate on various photorespiratory enzymes of tobacco leaf discs and callus cells. Of the many

Table 1 *Effect of different compounds on photorespiratory and photosynthetic processes*

Compound	Site of action	Nature of action	Plant part/ preperation used	Effect on CO_2 fixation in air of high pO_2	Effect on CO_2 fixation in low pO_2 or high pCO_2	References
Glycidate	Glycolate synthesis	Inh.	Leaf discs	Aug.	—	Zelitch (1974)
-do-	-do-	Stim.	Isolated intact chloroplasts	Aug.	—	Chollet (1976a)
-do-	-do-	Inh.	Leaf segments	Inh.	Inh.	Kumara-Singhe, Keys and Wlttting-ham (1977)
-do-	-do-	Stim.	Attached leaf	Aug.	—	Poskuta and Kochanaska (1978)
-do-	-do-	Stim.	Chromatium	Aug.	Aug.	Asami and Akazauna (1976)
L-Glutamate	-do-	Inh.	Leaf discs	Aug.	—	Oliver and Zelitch (1977a)
-do-	-do-	Inh.	Maize bundle sheath strands	Aug.	—	Oliver (1978a)
L-Aspartate	-do-	Inh.	Leaf discs	—	—	Oliver and Zelitch (1977a)
-do-	-do-	Inh.	Maize bundle sheath strands	Aug.	—	Oliver (1977a)
Phosphoenol pyruvate	-do-	Inh.	Leaf discs	—	—	Oliver and Zelitch (1977a)
Glyoxylate	-do-	Inh.	Leaf discs	Aug.	—	Oliver and Zelitch (1977b)
-do-	-do-	Inh.	Leaf discs	Aug.	—	Oliver (1978b)
-do-	-do-	NE	Leaf discs	NE	NE	Chollet (1978a)

(continued on page 278)

(**Table 1** *continued*)

Compound	Site of action	Number of action	Plant part/ preperation used	Effect on CO_2 fixation in air of high pO_2	Effect on CO_2 fixation in low pO_2 or high pCO_2	References
α-HPMS	Glycolate oxidation	Inh.	Leaf discs/ enzyme preparation	Aug.	—	Zelitch (1966, 1971)
-do-	-do-	Inh.	Leaf discs	Inh.	Inh.	Khaveri-Nejad (1977)
-do-	Glycolate synthesis	Inh.	Mesophyll cells	Reduced or no effect depending on the cornce of HCO_3 used	—	Bauman, Guido and Gottfied Guenther (1979)
2-Hydroxy -3-butanoic acid	Glycolate oxidase	Inh.	Leaf discs/ enzyme preparation	NE	—	Jewess, Ker & Wittaker (1975)
Butyl-2-Hydroxy-3-butanoic acid	-do-	Inh.	Leaf cells	Slightly Inh.	NE	Servaites and Ogren (1977)
Sodium bisulphite	-do-	Inh.	Leaf discs/ enzyme preparation	Inh.	—	Corbett and Wright (1970) Tripathy & Murthy (1976)
Isonicotinic acid hydrazide	Glycine decarboxylation	Inh	Leaf discs/ enzyme preparation	Inh.	—	Kisaki and Tolbert (1970) Kisaki, Imai and Tolbert (1971), Kumarasinghe et al. (1977)
-do-	-do-	Inh.	Leaf discs	Inh.	NE	Servaites and Orgen (1977a)
Sodium bisulphate	-do-	Inh.	Leaf discs	Inh.	—	Tripathy & Murthy (1976)
Zinc Sulphate	-do-	Inh.	Mitochondrial preparation	—	—	Kisaki, Yoshide & Imai (1971)
Zinc Sulphate	-do-	Aug.	Leaf discs	Inh.	—	Tripathy & Murthy (1976)
-do-	—	Inh.	Chloroplast PS II	—	—	Tripathy & Mohanty (1978)

Inh. = Inhibits; Stim. = Stimulates; Aug = .Augments; NE = No Effect

photorespiratory enzymes investigated—like phosphoglycolate phosphatase, NADH-glyoxylate reductase, NADPH-glyoxylate reductase, glutamate, glyoxylate aminotransferase and glycine decarboxylase—glycidate only effectively inhibits activities of glutamate; glyoxylate aminotransferase by 80% and NADPH-glyoxylate reductase by 30%, whereas it had negligible effect on other enzymes. The inhibition is dependent on glycidate concentration and to a less extent on the substrate concentration. Glycidate treatment of leaf discs also brings about a 40% increase in glyoxylate pool size. A 3-fold increase in glutamate pool size in presense of glycidate in photosynthesizing leaf discs of tobacco has also been reported (Zelitch 1978). As will be discussed later, an increase in either glyoxylate or glutamate concentrations in tobacco leaf discs inhibits glycolate synthesis and photorespiration and also increases net photosynthesis (Oliver & Zelitch 1977 a,b). Therefore, the effect of glycidate in decreasing glycolate synthesis is probably indirect, resulting from the inhibition of glutamate: glyoxylate aminotransferase activity which causes accumulation of both the substrates (glutamate and glyoxylate). If NADPH-glyoxylate is active in normal cell metabolism, its inhibition by glycidate may also result in accumulation of glyoxylate in the tissues. Thus, accumulation of glutamate and glyoxylate in the presence of glycidate can lead to a reduced rate of photorespiration and an enhanced rate of photosynthetic CO_2 fixation.

On the other hand, glycidate has been shown (Chollet 1976a) to enhance both glycolate synthesis and CO_2 fixation in isolated intact chloroplasts. The increased rate of glycolate formation in the presence of above epoxide in intact chloroplasts appears to be a function of enhanced overall photosynthetic activity of chloroplasts in that the ratio of glycolate formation to $^{14}CO_2$ fixation remains constant, as does the percentage of total fixed carbon entering into the glycolate pool. Thus it is probable that in Zelitch's leaf disc experiments, glycidate might have regulated the synthesis of some other metabolite in the leaf discs of tobacco which, in turn, might have checked glycolate biosynthesis leading to higher rate of CO_2 uptake, since, as will be discussed later, glycidate has no effect on the RuBP carboxylase-oxygenase activity assayed in vitro (Zelitch 1976). In contrast to the experiments of Zelitch (1974), Kumarasinghe et al. (1977) have demonstrated that glycidate inhibits photorespiratory metabolism and also CO_2 fixation of wheat leaf segments in an atmosphere of air and 100 vpm of CO_2. Such conflicting reports are serious setbacks to the progress of research seeking the control of photorespiration. However, a pertinent question can be raised, i.e., whether the above discrepancies are due to selection of different plant species, tobacco or wheat used for experimental studies.

Chollet (1978) has reported that preincubation of illuminated tobacco leaf discs in glycidate inhibits photorespiration by about 40%. This is determined by the ratio of $^{14}CO_2$ evolved into CO_2 free air in light and in darkness according to the method used by Zelitch (1968). However, under identical preincubation conditions used for the above kind of assay, glycidate fails to reduce photorespiration or stimulate net photosynthesis in tobacco leaf discs based on other studies on CO_2 exchange parameters, including CO_2 compensation concentration in 21% O_2 and Warburg inhibitory effect on photosynthesis. Thus, the effect of glycidate on photorespiration measured as the ratio of

$^{14}CO_2$ evolved into CO_2-free air in light and in darkness is inconsistent with other measures of photorespiratory CO_2 exchange in tobacco leaf discs. Hence the data of Chollet even challenges the validity of light to dark ratio of $^{14}CO_2$ efflux as an assay of relative rates of photorespiration; although such a use of this ratio has been advocated by Zelitch (1968). Thus the techniques adopted by the two workers for measurement of net photosynthesis and photorespiration were different, and perhaps it is one of the reasons for the contradicting results.

Poskata and Kochanska (1978), while treating attached leaves of bean with potassium glycidate, have observed that the epoxide at 5mM concentration stimulates the rates of both apparent photosynthesis and photorespiration, but it has little effect on dark respiration. The magnitude of stimulations is also related to the concentration of oxygen in the atmosphere. Whereas in an atmosphere of air the stimulation of apparent photosynthesis and photorespiration by glycidate is in the range of 50 and 30%, it is in the range of 200 and 300% respectively in an atmosphere of almost pure O_2. The above findings while support the contention of Zelitch (1974) that glycidate stimulates CO_2 uptake by C_3-plants, are contradictory to Zelitch's observation that this compound inhibits photorespiration. The above work however corroborates the observation of Chollet (1976) that glycidate stimulates both CO_2 fixation and glycolate synthesis in isolated intact chloroplasts. One intriguing feature of the above report is that duration of the epoxide treatment is 2 hr while stimulation of CO_2 uptake appears only after 1 hr treatment with above substance. This indicates that permeability of glycidate into leaf cells may have an important bearing in the above experiment. It also seems that concentration of glycidate used is also an important factor in bringing about the desired effect. Hence, the contradictory reports on glycidate action just mentioned above may be due to problem of permeability of the above epoxide through the cell membrane and the concentration used to bring about desired effect.

Wildner et al. (1979) have reported almost 100% stimulation of net photosynthesis in isolated spinach chloroplasts with the glycidate which had no effect on glycolate formation, RuBP oxygenase or any of the constituents of photochemical reactions of photosynthesis. Therefore, the explanation given by these workers is that glycidate exerts its role by neutralizing the negative membrane potential developed by exchange of PGA^{3-} with Pi^{2-}, with the consequence that exchange of Pi^{2-} with $3PGA^{3-}$ is facilitated.

Glycolate accumulation, in the presence of α-HPMS, is inhibited in leaf discs previously floated on 30mM solution of L-glutamate (Oliver & Zelitch 1977, 1978). The glutamate inhibition (about 40%) of glycolate synthesis continues for more than 4 hr after the glutamate solution is removed. The inhibition of glycolate synthesis is accompanied by a marked decrease in the rate of photorespiratory CO_2 release and a maximal increase of net photosynthesis by 25% (table 1). The products of $^{14}CO_2$ fixation in leaf discs previously treated with glutamate shows a decrease in glycine (26%) in comparison with discs floated in water. The actual mechanism by which glutamate slows glycolate synthesis is yet uncertain. The inhibition is probably brought about either by glutamate itself or by one of its catabolites acting directly on one or more of the enzymes involved in glycolate synthesis. Glutamate (10mM) has no effect on RuBP carboxylase-oxygenase activity. In the above experiments, these workers

have found that aspartate, phosphoenolpyruvate and glyoxylate can consistently inhibit the rate of glycolate accumulation. As the photosynthetic rate and the glutamate dependent stimulation in the rate were found to be sensitive to a photosynthetic electron transport inhibitor, the stimulation could not be attributed to any other factor than to the decrease in the formation of photorespiratory CO_2.

Attempts have been made to increase net photosynthesis by using different concentrations of glyoxylate, one of the metabolite of glycolate pathway. Oliver and Zelitch (1977) and Oliver (1978) reported an increase in net photosynthesis by a factor of 2.0 caused by floating tobacco leaf discs in 5.0mM potassium glyoxylate. This is caused by an inhibition of photorespiratory CO_2 release which is accompanied by a decrease in glycolate synthesis and is rapidly achieved if glyoxylate solution is replaced with water. It differs from glutamate inhibition of glycolate where synthesis of glycolate is not retained even after 4 hr of replacement with water. This clearly indicates the different mechanism by which glyoxylate and glutamate inhibit glycolate synthesis. Since the first product of glycolate metabolism is glyoxylate, the latter may inhibit glycolate synthesis in vivo by a feedback mechanism early in the pathway to block photorespiration. These workers have suggested alternatively that a metabolic product of glyoxylate may be regulating glycolate synthesis. Similarly when isolated soyabean mesophyll cells were treated with glyoxylate before $NaHCO_3$ was added, stimulation was observed. This shows that glyoxylate stimulates net photosynthesis either solely by decreasing photorespiration or by increasing gross photosynthesis (Oliver 1980). Thus these results indicate that chemical or genetic regulation of compounds which inhibit photorespiration and produce higher rates of photosynthesis can be achieved.

According to Baumann et al. (1979) the effect of glyoxylate and other metabolites of the glycolate pathway depends on the different concentration of bicarbonate used. Their experiments with mesophyll cells of *Chenopodium album* did not show increase in photosynthesis with glyoxylate. Therefore, the role of HCO_3^- must be understood thoroughly prior to any further work with glyoxylate.

Control of photorespiration at the level of glycine decarboxylation has also been tried. Isonicotinylhydrazide (INH) inhibits glycine conversion to serine and carbon dioxide (Kisaki & Tolbert 1970) and it has been reported to increase photosynthetic CO_2 fixation (Zelitch 1971). Kumarasinghe et al. (1977) on the contrary have demonstrated that INH inhibits photosynthesis by 50% in wheat leaf segments.

Smith et al. (1976) have reported that INH, which blocks the glycolate pathway with the resultant accumulation of glycine, increases the CO_2 compensation point by 21% in bean leaves, while it has no effect on the dark respiration. From the above observation, they suggest that it is unlikely that photorespiratory CO_2 arises from the conversion of glycine to serine, since the inhibition of this step should have lowered the CO_2 compensation point. However, Servaites and Ogren (1977) have shown that INH inhibits photosynthesis at 21% O_2 and not at 2% O_2 (see below), this might have led to an increased CO_2 compensation point in the above experiments.

$ZnSO_4$ has been shown to inhibit glycine decarboxylation in mitochondrial preparations of tobacco (Kisaki et al. 1971). However, later it was reported that $ZnSO_4$ enhanced the rate of glycine decarboxylation in leaf segments of rice

(Tripathy & Murthy 1976) (table 1). Further studies on stimulatory and inhibitory effects of the above chemical in vivo and in vitro systems are required to account for the above discrepancy. $ZnSO_4$ at 1mM concentration also inhibits photosynthesis of barley and rice leaves. It acts at the oxidising side of photosystem II (PS-II) (Tripathy & Mohanty 1980).

Grodzinski and Butt (1976) have shown that the rate of O_2 uptake of intact peroxisomes increases, but less than double of that when azide, the inhibitor of catalase function is added to the raction mixture. The low amount of release of $^{14}CO_2$ from glycolate-1-^{14}C, in the presence of azide, suggests that a non-enzymatic degradation of glyoxylate to CO_2 and formate may account for a part of photorespiration, the other part might be coming from glycine decarboxylation. Therefore, any attempt to completely inhibit photorespiration at the level of glycine decarboxylation may not be feasible.

Servaites and Ogren (1977a) have treated isolated soyabean mesophyll cells with three inhibitors of the glycolate pathway in order to evaluate the potential of such inhibitors for increasing photosynthetic efficiency. Preincubation of cells under acidic conditions with α-HPMS increases $^{14}CO_2$ incorporation into glycolate, but severely inhibits photosynthesis. INH increases the incorporation of $^{14}CO_2$ into glycine and reduces incorporation into serine, glycerate and starch. Butyl-2-hydroxy-3-butynoate (BHB) completely and irreversibly inhibits glycolate oxidase activities and increases the accumulation of ^{14}C into glycolate. Concomitant with glycolate accumulation, there is a reduction in the levels of serine, glycerate, glyoxylate and starch and the elimination of label in glycine. The inhibitors INH and BHB do not eliminate serine synthesis, suggesting that some serine is synthesized by an alternate pathway. However, the rate of photosynthesis is not affected by INH or BHB in the absence of O_2, while these compounds increase the O_2 inhibition of photosynthesis with increasing concentration of O_2.

Having made these observations, Servaites and Ogren argue that since INH and BHB do not enhance photosynthesis at 21% O_2 but rather inhibit it and do not have any effect on photosynthesis at 2% O_2 where glycolate synthesis itself gets inhibited, it follows that the INH and BHB inhibitions of photosynthesis at atmospheric oxygen concentration is brought about by the blocking of glycolate oxidation. They suggest that photorespiration has an essential role to play, i.e., by recycling carbon of glycolate, synthesized from the destruction of RuBP at atmospheric O_2 concentrations, back to the Calvin cycle. This recycling is achieved (only three carbons are recycled) by the conversions of two molecules of glycerate and subsequent phosphorylation of glycerate by glycerate kinase to form 3-PGA, the essential intermediary of Calvin cycle (see figure 1, steps 11 & 12). Their arguements suggest that CO_2 lost during photorespiration i.e., during glycine decarboxylation is inevitable, and this photorespiratory glycolate pathway is essential for the recycling of glycolate, wastefully synthesized at atmoshperic oxygen tension. Hence the photorespiratory pathway is evolved to check carbon losses from the carbon reduction cycle and it is only partially successful as loss of carbon also takes place during serine synthesis.

Thus, they conclude that chemicals which inhibit glycolate metabolism do not reduce photorespiration or increase photosynthetic efficiency, but rather

inhibit the latter due to accumulation of intermediates of the pathway leading to the suppression of their back flow into the Calvin cycle. Accumulation of glycolate constitutes a loss equal to the loss from its further oxidation, besides posing major problems for the plants.

Inhibition of glycolate oxidation pathway at atmospheric conditions of CO_2 and O_2 is severely deleterious to photosynthesis and it may be necessary that glycolate, once synthesized, be metabolized through the complete photorespiratory pathway, if normal photosynthesis rates are to be maintained. This means that temporarily increasing photosynthetic productivity by inhibition of the glycolate oxidation is not desirable and that the search for chemical or genetic control of photorespiration must focus on reducing or preventing the diversion of carbon from the photosynthesis cycle into the glycolate pathway. That is by inhibiting the primary event initiating photorespiration, the RuBP oxygenase reaction.

To summarize, it appears obvious from the literature that efforts aimed at chemically controlling photorespiration are full of contradictions. This may be due to the variations in plant specimens chosen, duration of chemical treatment applied, concentration of the chemical used and differential permeability of the chemical through cell membranes and experimental designs. Surprisingly, contradictory reports exists on the effects of certain chemicals on CO_2 fixation by plant leaves. Although such experiments can be carried out very easily, they are undesirable in that the data may hinder the progress of research aiming at controlling photorespiration. However, from the literature discussed above, it appears that there are two main schools of thought pertaining to chemical control of photorespiration. (i) Zelitch and coworkers hold that inhibition of photorespiration at any one of the steps i.e., starting from glycolate synthesis to glycolate catabolism to serine and CO_2, is capable of increasing the rate of photosynthetic CO_2 fixation. Hence photorespiration which is a completely wasteful process can be controlled and photosynthesis increased by changing the concentration of some intermediary metabolites. If stable changes in the intracellular concentration of such metabolites can be incorporated into higher plants, phenotypes showing large increases in net photosynthesis can be expected in species with high rates of photorespiration; and (ii) in contrast, Ogren and coworkers contend that attempts to check photorespiration at any intermediary step of glycolate metabolism are futile excercises, since such attempts lead to accumulation of metabolites and depletion of Calvin cycle intermediates such as RuBP and a consequent decrease in photosynthetic CO_2 fixation. Thus photorespiration is essential to recycle glycolate to Calvin cycle via serine to 3-PGA pathway; as glycolate is inevitably synthesized at normal atmospheric O_2 and CO_2 concentrations. Therefore, inhibition of the glycolate oxidation pathway at atmospheric conditions of CO_2 and O_2 is deleterious to photosynthesis and it may be necessary that glycolate once synthesized, be metabolized through the complete photorespiratory pathway, if normal photosynthesis rates are to be maintained. Besides, all the proposed inhibitors are at the cost of essential reactants—essential for the overall metabolism of the plant. Therefore effort may be useful for short-term but may prove futile in the long run agricultural practices. This means that increasing photosynthetic

efficiency by inhibition of glycolate oxidation is not desirable and thus the search for chemical or genetic control of photorespiration must focus on reducing or preventing the diversion of carbon from photosynthetic cycle to glycolate pathway, i.e., only checking glycolate synthesis which occurs at normal CO_2 and O_2 concentrations. This might be achieved by imposing alterations in kinetic characteristics of RuBP carboxylase-oxygenase, which will lead to increased rate of carboxylation relative to oxygenation.

(b) *Alteration of the Oxygenase Activity of RuBP Carboxylase (Oxygen Stimulated Formation of Glycolate).*

There are detailed reviews which have appeared on the complex enzyme RuBP carboxylase-oxygenase (Tolbert & Ryon 1976 and Jensen & Bahr 1977). These reviews mainly deal with its biogenesis, regulation and enzymic properties. Although a recent report (Lorimer 1981) describes the catalytic site of this enzyme, however, considerable efforts are to be made to determine the location, dimension and properties of catalytic and activator sites or to determine the role of the amino acid residues involved in establishing these properties in bifunctional enzyme.

Lorimer et al. (1976, 1981) have predicted that the above bifunctional enzyme binds an activator CO_2 that is distinct from the CO_2 ultimately fixed in the carboxylation reaction. The CO_2 binding as well as cation bindings are essential for conversion of the enzyme from inactive to active form, and activation is required for maximal expression of either carboxylase or oxygenase activities (Badger & Lorimer 1976).

Recent advances on the studies of the properties of the above enzyme based on pyridoxal -5′-phosphate binding and subsequent stabilization of above binding by $NaBH_4$, indicate that there are two different ∈-aminolysyl groups of RuBP carboxylase-oxygenase, both of which are involved in the catalytic site and one at the activator site. Both sites are probably located in the same area on the large subunit of the enzyme. In addition, one sulfhydryl group is proposed to be at the catalytic site and may represent the group which abstracts the proton at C-3 of RuBP to initiate the enediol formation during catalysis (Paech & Tolbert 1978). However, there are contradictory reports which state that the activator site is located in the small subunit (Akazawa 1978).

Miziorko (1979) carrying out studies of the binding of the enzyme with carboxyribitol bisphosphate, has also confirmed that RuBP carboxylase has separate CO_2 activator and substrate sites. While a dual role for CO_2 seems certain, the precise role of divalent cations in the RuBP carboxylase reaction is unclear. In the presence of CO_2 and in absence of RuBP there is only one cation bound per enzyme active site and this cation also appears to function in the process of activation. However, it is conceivable that if only one cation is involved, it plays a dual role in activation and catalysis. Further experiments have to be carried out to resolve this issue.

In order to investigate the amino acid residues or groups involved in the catalytic site of the enzyme, Schloss et al. (1978) have studied the binding properties of RuBP carboxylase-oxygenase with N-bromoacetylethanolamine which inactivates the enzyme. They have come to the conclusion that at least one sulfhydryl group is located in the ribulose-bisphosphate binding site of the enzyme. With the recent development of use of

α-dicarbonyl compounds, including 2, 3-butanedione and phenylglyoxal as selective reagents for the chemical modification of arginyl groups under mild conditions, an ever-increasing awareness of the importance of arginine residues in binding phosphorylated substrates, coenzymes and effectors in a wide variety of enzymes has emerged (Roirdan et al. 1977 and Chollet 1981). Since RuBP carboxylase not only acts on a phosphorylated substrate but is also modulated by anionic effectors including NADPH, the possible importance of arginyl residues in catalytic and regulatory properties of this bifunctional enzyme by modification with 2, 3-butanedione and phenylglyoxal have been investigated. Chollet (1978b) has shown that treatment of crystalline tobacco ribulose-bis-phosphate carboxylase with arginine selective 2, 3-butanedione results in a time and concentration dependent loss of activity. Inactivation is markedly enhanced by borate buffer and alkaline *p*H. Only the phosphorylated substrat RuBP is capable of protecting this bifunctional enzyme against inactivation by 2, 3-butanedione. These results suggest that the essential role of arginyl residues in the enzymic mechanism of RuBP, is to provide positively charged binding site, for the negatively charged phosphate groups of the substrate, RuBP. Schloss et al. (1978) have also explored the essentiality of arginyl residues in the enzyme by modification with phenylglyoxal. Amino acid analyses of the inactivated enzyme reveal the modification of 2-3 arginyl residues out of 35 present per native protomer. In their experiments, the substrate RuBP reduces the degree of inhibition of the enzyme RuBP carboxylase by phenylglyoxal, whereas Mg^{2+} and CO_2 have little effect. However, the protective effect is not reflected by clear differences in levels of incorporation of RuBP between protected and non-protected enzyme. Therefore, they conclude that the inactivation of RuBP carboxylase by phenylglyoxal probably results from modification of an arginyl residue outside the binding domain for RuBP. Whatsoever the differences may arise from the studies reported above, it appears very likely that arginyl residues of the enzyme are involved in binding with negatively charged phosphate groups of the substrate RuBP.

The mechanism of the oxygenase reaction is still uncertain. It has been shown in case of several mono and dioxygenases that the reactive oxygen species involved in the reaction is superoxide anion (O_2^-) (Hirata & Hayaishi 1975). In the case of RuBP oxygenase, Wildner and Henkel (1976) have proposed that superoxide anions may be involved in the oxygenase reaction.

Bhagwat and Sane (1978) have claimed that inclusion of superoxide dismutase in the assay mixture results in strong inhibition of oxygenase reaction. RuBP was found to compete for superoxide anions with superoxide dismutase. These may suggest that in case of RuBP, oxygenase, superoxide radical anions are involved in the oxygenation reaction. The mechanism of O_2^- production is not clear, but it is likely that Cu^{2+} is involved in the process.

Brandon (1978) has claimed that the enzyme RuBP oxygenase from parsley leaves is different from RuBP carboxylase. He holds that he has been able to separate the enzymes by gel filtration at a *p*H of 8.3 and that RuBP oxygenase contains Cu^{2+} like many other oxygenases. However, McCurry et al. (1978) later working on RuBP-carboxylase-oxygenase from the same parsley leaves, following the same method of Brandon, could not confirm that the

carboxylase and the oxygenase are two separate enzymes. Rather RuBP carboxylase-oxygenase from parsley leaves seems to be a single protein.

Thus with the recognition that RuBP carboxylase-oxygenase plays a pivotal role in regulating O_2 effects on C_3 photosynthesis (Andrews et al. 1971, Ogren & Bowes 1971, Krause & Heber 1976 and Krause et al. 1977), one potential solution has become apparent; that the above enzyme must be altered either mutagenically or chemically, so that carboxylation is increased, oxygenation is decreased or both. Recent in vitro studies with the purified protein indicate that under most assay conditions, the carboxylase and oxygenase functions of the catalytic site are tightly coupled, rather than independent of each other (Badger & Lorimer 1976 and Chollet & Anderson 1976). Biochemical studies (Keck & Ogren 1976) with *Panicum milioides* show that the plant has reduced the O_2 inhibition of net photosynthesis accompanied by an altered RuBP carboxylase with an increased affinity for CO_2 relative to O_2. Again further work (Quebedeaux & Chollet 1977) on the above plant grown at altered pO_2 and pCO_2 shows that *P. milioides* represents the first well documented example of C_3 plant associated with kranz type anatomy (Brown 1976) having reduced photorespiration, high photosynthetic uptake and high plant growth and dry matter production. Therefore, the above in vitro behaviour of RuBP carboxylase-oxygenase in *P. milioides*, correlating with growth and dry matter production should encourage us to further research on mutagenic or chemical alteration of the above enzyme. Recently, Rathnam and Chollet (1978, 1979) have reported that CO_2 donation by C_4 acids like malate and aspartate, when added to isolated mesophyll protoplasts and bundle sheath protoplasts, reduced photorespiration in *P. milioides*. The study provided clue to the C_3-C_4 intermediate nature of *P. milioides*. According to these workers, reduced photorespiration and O_2 inhibition of photosynthesis in *P. milioides* is due to its ability to perform C_4-like PEP-carboxylations and-C_4-acid decarboxylations thus providing an increase in pCO_2 at the site of bundle sheath RuBP carboxylase-oxygenase: an altered nature of this enzyme is not necessary in their interpretation.

Sugar phosphates have been used in an attempt to regulate carboxylase oxygenase activity in vitro. The carboxyribitol bisphosphate, which acts at the RuBP substrate site, exhibits non-competitive and irreversible inhibition of both carboxylase and oxygenase reactions (Siegel & Lane 1972, table 2).

An allosteric group of effectors is sugar monophosphate, of which ribose-5-phosphate is the most active. When assayed at optimal *p*H, 1mM ribose-5-phosphate increases the carboxylase activity and inhibits the oxygenase (Ryan & Tolbert 1975). However, ribose-5-phosphate is an inhibitor of phosphoglycolate phosphatase, the accumulation of which inhibits triose phosphate isomerase with a Ki of 10^{-7}M, (Tolbert & Ryan 1976). Therefore, ribose-5-phosphate cannot be used as an effective regulator.

Fructose bisphosphate is another effector which, it is claimed at right *p*H acts as an inhibitor of carboxylase and a stimulator of oxygenase activity. However, this compound does not completely inhibit or grossly enhance either reaction (Tolbert & Ryan 1976). To the extent that the differential effect applies, the accumulation of fructose bisphosphate which might occur in the presence of excess

Table 2 *Comparative effects of various compounds on RuBP carboxylase and RuBP oxygenase activity.*

Compound treated	Effect on RuBP carboxylase	Reference	Effect on RuBP oxygenase	Reference	Remarks
Carboxyribitol-1, 5-diphosphate	Inh	Siegel and Lane (1972)	Inh	Ryan and Tolbert (1975)	A potent inhibitor of both reactions
Xylitol-1, 5-diphosphate	Inh (*p*H 9.0)	Ryan, Barker and Tolbert (1975)	Inh (*p*H 9.0)	Ryan, Barker and Tolbert (1975)	A powerful inhibitor of both the reaction $<$*p*H 9.0
	NE (*p*H 9.0)		NE (*p*H 9.0)		
6-phosphogluconic acid	Stim (preincubated with enzyme)	Buchanan and Schrumann (1973) Ryan and Tolbert (1975) and Chollet & Anderson (1976)	Stim (preincubated with enzyme)	Buchanan and Schrumann (1973), Ryan and Tolbert (1975), Chollett and Anderson (1976)	Inhibitor of both the reactions when incubation period is less
	Inh (No preincubation)		Inh (No preincubation)		
Ribose—5-phosphate	Stim	Buchanan & Schrumann (1973)	Inh	Ryan and Tolbert (1975), Chollett and Anderson (1976)	Reports contradictory
	Inh	Chollett and Anderson (1976)			
Fructose—6-phosphate	Stim	Buchanan and Schrumann (1973)	Inh	Ryan and Tolbert (1975)	Reports Contradictory
	NE	Chollett and Anderson (1976)	NE	Chollett and Anderson (1976)	
Glucose—6-phosphate	Stim	Buchanan and Schrumann (1973)	Inh	Ryan and Tolbert (1975)	Reports contradictory
	NE	Chollett and Anderson (1976)	NE	Chollett and Anderson (1976);	

(continued on page 288)

(Table 2 *continued*)

Compound Treated	Effect on RUBP carboxylase	Reference	Effect on RuBP Oxygenase	Reference	Remarks
Fructose—1-6-diphosphate	Stim	Chollet and Anderson (1976)	Stim	Chollett and Anderson (1976),	Reports contradictory
	Inh	Buchanan and Schrumann (1973)		Ryan and Tolbert (1975)	
3-PGA	Stim	Chollett and Anderson (1976)	Stim	Chollet and Anderson (1976)	Reports contradictory
	Inh	Buchanan and Schrumann (1973)			
NADPH	Stim	Chollet and Anderson (1976)	Stim	Chollet & Anderson (1976)	Stimulator of both the reactions
Glycidate	NE	Zelitch (1976) Windler and Henkel (1976)	Inh	Windler and Henkel (1976)	Reports contradictory
	NE	Zelitch (1978)	NE	Zelitch (1978)	
Hydroxylamine	NE	Bhagwat et al. (1978)	Inh	Bhagwat et al. (1978)	The report needs further confirmation

Inh.=Inibits; Stim.=Stimulates; NE=No effect

sugars, may inhibit more CO_2 fixation but may increase the oxygenase activity and photorespiration.

Phosphogluconate is another kind of sugar phosphate regulator of RuBP carboxylase-oxygenase (table 2). This intermediate of oxidative pentose phosphate pathway is formed in the dark and may either inhibit or stimulate between 0.1 to 1mM concentrations carboxylase as well as oxygenase in a parallel manner. Phosphogluconate may act at a regulatory site for activation or inactivation of the enzyme (Chu & Bassham 1973 and Ryan & Tolbert 1975).

Unlike phosphogluconate, which may act as a regulator for the enzyme RuBP carboxylase-oxygenase, Xylitol, 1.5-bisphosphate has been claimed to be an inhibitor of both carboxylase and oxygenase activities. When the molar ratio of inhibitor to enzyme is less than 2, xylitol bisphosphate inhibits RuBP carboxylase and oxygenase activities by 50%, which constitutes a considerable extent of inhibition in view of the fact that RuBP carboxylase-oxygenase contains eight active sites on its eight large subunits (Jensen & Bahr 1977). Xylitol bisphosphate also induces a sigmoidal concentration

dependence for bicarbonate suggesting that the xylitol bisphosphate induced inhibition are those of allosteric effectors. Furthermore, the inhibitory effect of xylitol bisphophate is *p*H-dependent with a *pKa* for inhibition of 8.6 and it is uneffective at *p*H above 9. Since phosphate group of xylitol bisphosphate gets completely ionized at *p*H 8, the group exhibiting a *pKa* of 8.6 such as that of an amine appears to be involved in the effector site. Thus xylitol bisphosphate may be useful for studying the composition of regulator site of RuBP carboxylase/oxygenase but cannot be used as differential inhibitor for oxygenase.

p-Chloromercury benzoate (PCMB) has also been shown to inhibit differentially the carboxylase and oxygenase activities (Gnanam 1978).

Wildner and Henkel (1976) have claimed that glycidate has no effect on RuBP carboxylase activity, whereas it inhibits RuBP oxygenase reaction. Zelitch (1978) has not been able to confirm this report and has advocated that glycidate inhibits neither carboxylase nor oxygenase.

Chollet and Anderson (1976) working with the crystalline tobacco enzyme have observed that, within the limits of experimental error, low levels of NADPH, 6-phosphogluconate, fructose-1, 6-bisphosphate and 3-phosphoglyceric acid stimulate both carboxylase and oxygenase reactions to the same extent, ribose-5-phosphate inhibits both the reactions, whereas glucose-6-phosphate and fructose-6-phosphate are without effect (table 2). These results contradict the earlier work of Ryan and Tolbert (1975) (reported above) which claimed that several plastid metabolites including ribose-5-phosphate stimulate carboxylase and inhibit oxygenase activities. Chollet and Anderson conclude that RuBP carboxylase oxygenase activity of crystalline tobacco enzyme are not differentially regulated by chloroplast metabolites.

Recently Bhagwat et al. (1978) have claimed the specific inhibition of oxygenase activity of RuBP carboxylase by hydroxylamine without affecting the carboxylase activity of the enzyme. Kinetic and preincubation studies suggest that hydroxylamine reacts with RuBP oxygenase only in the presence of RuBP to form a catalytically inert complex. The plausible explanation of the specific inhibition of oxygenase by hydroxylamine is that in presence of RuBP it generates a site on the enzyme molecule which is susceptible to nucleophilic attack by hydroxylamine and once hydroxylamine is bound to the site, it prevents oxygenation. However, it is difficult to conceive that under such conditions the carboxylation reaction will continue. Furthermore, it is possible that hydroxylamine reacts with RuBP and affects carboxylase reaction. As discussed before, participation of–SH groups in the active site region of the enzyme has been suggested. However, hydroxylamine may not be inactivating sulfydryl groups, because carboxylase activity is not inhibited. It is possible that hydroxylamine binding to the protein brings about conformational change with the result that SH groups cannot participate in the reaction mechanism. Since in the above investigation, both carboxylase and oxygenase reactions were similarly activated by $NaHCO_3$ and Mg^{2+} at *p*H 8.5 and also both the assays were carried out at same *p*H (8.5); it appears that this report of specific inhibition of oxygenase by hydroxylamine may be correct. However, with unconfirmed claim of differential inhibition confirmation of the above report from other laboratories is essential. Although hydroxylamine (NH_2OH) is an inhibitor of PS-II (Izawa et al. 1969, Bennoun &

Joliot 1969 and Mohanty et al. 1972) and thus cannot be used to study the in vivo CO_2 fixation at levels inhibitory to oxygenase action, this compound may still be useful in further research aimed at elucidating the mechanism of carboxylase oxygenase inhibitory functions.

Many contradictory reports on the role of various effector molecules on carboxylase or oxygenase functions of the enzyme are discussed in preceding paragraphs. There is no unanimity of views on the role of most of the effector molecules studied. Such discrepancies are due to: (1) in many cases carboxylase or oxygenase functions have been analyzed separately in different laboratories, (2) varying degrees of activation have been followed for carboxylase or oxygenase, and both of the enzyme have been activated at different *p*H's. The effectors activity should be measured at same *p*H for both and not at *p*H 7–8 for carboxylase and *p*H 9.1 for oxygenase, as effector molecules behave differently at different *p*H's. Under activated conditions the broad *p*H optima for both the carboxylase and oxygenase are similar and around 8.1 to 8.3. There are also contradictory opinions on the ratios of RuBP carboxylase and RuBP oxygenase activities. The ratio has been reported to vary from 1:1 in tobacco leaves to about 12:1 in marine seed weeds (Tolbert 1977). As these ratios of relative specific activities are meaningful, at specified concentration of CO_2 and O_2, these differing reports also need re-evaluations in the light of above suggestions.

However, inspite of above contradictory reports, our contention is that, as the molecular configurations and charge distributions of O_2 and CO_2 are different, they (O_2 and CO_2) may be acting with different charged groups even in the same active site. Therefore, the possibility of preferential regulation of CO_2-binding over O_2 in the one active site by making chemical changes in that site remains very real.

Contrary to the popular belief that glycolate is the only source of photorespiratory CO_2, it has been recently predicted (Daley & Bidwell 1977) that a substantial amount of photorespiratory CO_2 might arise from the spontaneous or enzymatic decarboxylation of phosphohydroxypyruvate, sometimes one of the early products of photosynthetic carbon fixation, not withstanding the fact that further proof must await additional evidence in favour of above proposition. Another alternative pathway suggested by Naik and Singh (1980) is that during light, succinate which accumulates in wheat leaves could inhibit oxidation of photorespiratory substrates (i.e., inhibition of $^{14}CO_2$ release from glyoxylate, glycolate and glycine). In mitochondria, the high concentration of succinate and glyoxylate results in reversal of the isocitrate-lyase reaction which could be the source of photorespiratory CO_2.

Therefore, attempts to control photorespiration by chemical means or otherwise at any individual step of the complex photorespiratory process should be carefully analysed and critically studied. Without a thorough understanding of the mechanism of photorespiration, any attempts to control it may prove futile.

Regulation of RuBP carboxylase-oxygenase activity by changing the *p*H of the suspending medium of intact chloroplasts has also been tried (Robinson et al. 1977). Servaites and Ogren (1977) have examined the effect of *p*H on the kinetics of photosynthesis, O_2 inhibition of photosynthesis and photorespiration in the isolated mesophyll cells of soyabean. At

a constant subsaturating bicarbonate concentrations (0.5mM), O_2 inhibition of photosynthesis increased with increasing *p*H, because high *p*H shifts the CO_2-bicarbonate equilibrium towards bicarbonate thereby reducing the CO_2 concentration. On the other hand at constant subsaturating CO_2-concentrations, cell photorespiration decreases with increasing *p*H. This is indicated by decrease in CO_2 compensation point, O_2 inhibition of photosynthesis and glycine synthesis. $Km(CO_2)$ values for isolated cell photosynthesis and in vitro RuBP carboxylase activity decrease with increasing *p*H while Ki (O_2) for both systems is similar at all *p*H values.

Very often the concentration of CO_2 and O_2 in leaves remain nearly constant, because these two gases are nearly in equilibrium with the atmosphere. Thus in leaves increasing cytoplasm *p*H showed decrease in photorespiration and increase in photosynthesis. Therefore, there is a considerable potential for low photorespiratory activity i.e., reduced rate of glycolate synthesis by increasing *p*H of stroma of chloroplasts. However, it is difficult to artificially increase chloroplast stromal *p*H in intact leaves. The fact remains, however, that natural changes in cytoplasmic *p*H may effect carboxylase oxygenase ratios.

Temperature has also been shown to differentially regulate the leaf photosynthesis and RuBP carboxylase-oxygenase activity (Bjorkman 1968). Laing et al. (1974) have measured the kinetic properties of soyabean net photosynthetic CO_2 fixation and of the RuBP carboxylase-oxygenase activities of purified enzyme as functions of temperature, CO_2 concentration and O_2 concentration. With leaves, O_2 inhibition of net photosynthetic CO_2 fixation increased when the ambient leaf temperature was increased. The inhibition of CO_2 fixation at higher temperature is caused by a reduced affinity of the leaf for CO_2 and an increased affinity of the leaf for O_2. With the purified RuBP carboxylase, O_2 inhibition of CO_2 incorporation and the ratio of oxygenase activity to carboxylase activity increases with increased temperature. The increased O_2 sensitivity of the enzyme at a higher temperature is caused by a reduced affinity of the enzyme for CO_2 and a slightly increased affinity of the enzyme for O_2. Badger and Andrews (1974) have suggested that the temperature response of photorespiration is due to a substantially higher activation energy of RuBP oxygenase reaction relative to the carboxylase reaction. Thus the ratio of RuBP carboxylase activity of RuBP oxygenase activity is not necessarily a fixed value, but can vary under certain conditions with predictable subsequent alterations in the ratio between photosynthesis and photorespiration.

Ku and Edwards (1977 a,b), have demonstrated that the magnitude of percentage inhibition of photosynthesis by the atmospheric level of O_2 in C_3 species *Solanum tuberosum* L., *Medicago sativa* L., *Phaseolus vulgaries* L., *Glycine max* L., and *Triticum aestium* L., increases in a similar manner with an increase in the apparent solubility ratio of O_2/CO_2 in the leaf over a range of solubility ratio from 24–45. The solubility ratio of O_2/CO_2 can be increased by increasing leaf temperature, under constant atmospheric levels of O_2 and CO_2 (since the solubility of O_2 decreases less regularly than the solubility of CO_2 with increasing temperature); by increasing the relative levels of O_2/CO_2 in the atmosphere at a given leaf temperature, or by increased

stomatal resistance. The decreased solubility of CO_2 relative to O_2 may be partly responsible for the increased percentage inhibition of photosynthesis by O_2 under atmospheric conditions with increasing temperature. Based on their kinetic measurements of O_2 inhibition of the photosynthesis of wheat leaves, they conclude that the competitive component of O_2 inhibition is considered as a major component of O_2 inhibition of photosynthesis under atmospheric CO_2 levels and is relatively independent of temperature at a given O_2/CO_2 ratio if the allowance for the temperature effects in the solubilities of the two gases is made.

The nutrient supply to the plant systems also seems to alter RuBP oxygenase-carboxylase activity. Work of Kabaki et al. (1979) showed the effect of nitrogen phosphorus and potassium deficiencies on photosynthesis, transpirations, chlorophyll, soluble protein, and enzyme activities. The activity ratio of RuBP oxygenase/RuBP carboxylase is low in phosphorous deficiency whereas nitrogen and potassium deficiency do not seem to affect this ratio. Thus, it may be feasible to alter nutrient supply and control photorespiration under physiological conditions. It is however to note that phosphorus deficiency may lower photosynthetic activity by affecting RuBP and phosphorylation and may result in an increase in internal CO_2 concentration which will in turn possibly lower the oxygenase activity and photorespiration but the result of such a control is trivial as phosphorus deficiency will affect photosynthesis.

Attempts should also be made to modulate the enzyme by genetic means, since only this can provide a stable configuration of the enzyme, which may allow increased carboxylation or decreased oxygenation or both. Our knowledge of properties and constitution of this enzyme is at present meagre. Almost nothing is known about the composition of its catalytic or activator sites. Thus it is futile to try for chemical or genetic modification of the enzyme without the thorough understanding. Simply to extend our understanding research programs aiming at modifing RuBP carboxylase-oxygenase enzyme probably should focus on following areas: (i) characterization of the active sites of RuBP, carboxylase and oxygenase, (ii) characterization of activation sites which also involve, CO_2 and Mg^{2+}, (iii) determination of role and assembly of the small subunit of the enzyme, (iv) elucidation of molecular biogenesis of each subunit and assembly of the holoprotein, (v) study of behaviour of this enzyme towards varying concentrations of CO_2 and O_2 at different *p*H ranges in C_3, C_4 and intermediate plants, (vi) search of chemical effectors which can differentially regulate carboxylase and oxygenase functions, and (vii) study of manifestations of carboxylation and oxygenation reactions of the enzyme from plants grown at different environmental regimes like low or high temperature, water stress and hypoxic conditions as in high altitude.

To summarize, there is extensive literature to show that carboxylase and oxygenase are the manifestations of a single bifunctional enzyme and that these are not two separate enzymes. The active sites for carboxylation and oxygenation by CO_2 and O_2 respectively are not so tightly coupled in this bifunctional enzyme that activities of RuBP carboxylase-oxygenase cannot be regulated differentially even when assayed under identical conditions. Either the catalytic sites for the two activater are not identical or the mechanisms by which these reactions are

catalysed are different. As the molecular configurations and charge distribution of O_2 and CO_2 are different, they (O_2 and CO_2) may be acting with different charged groups even in the same active site. Therefore, the possibility of preferential binding of CO_2 over O_2 in one active site by masking the binding site of the latter by chemical means cannot be precluded. Hence, continued attempts should be made for chemical modification of the said bifunctional enzyme either at the catalytic site or at the activator site so as to bring about preferential CO_2 binding to aid in the carboxylation rather than the oxygenation of RuBP.

Direct Oxygen Inhibition of Photosynthesis

As described eariier the very process of O_2 inhibition of photosynthesis consists of two components: (i) oxygen stimulated formation of glycolate by RuBP oxygenase action as discussed earlier, and (ii) a direct inhibition of photosynthesis by competition of oxygen for reductants generated during electron transport. Thus to block the oxygen inhibition of photosynthesis both the components should be checked rather than the former component alone.

According to modern concepts of electron transport during photosynthesis, the electrons from water are transferred to the physiological acceptor NADP through a number of intermediary carriers (figure 2). Unfortunately O_2 may be considered as a Hill oxidant, which can substitute for the physiological acceptor of electrons NADP. It is a familiar fact that NADPH is not auto-oxidizable i.e., the transfer of the electron from it to the moleculer oxygen cannot proceed, without intermediate electron carriers. The oxidation of NADPH may involve an auto-oxidizable flavin enzyme or cytochrome-b or polyphenol oxidase or many other systems. O_2 also can accept electron from ferredoxin or NADP reductase directly (Detechev et al. 1969).

Chlorophyll *a* (Chl *a*) fluorescence reflects redox-state of carriers and can be quenched by molecular oxygen. Vidaver et al. (1980, 1981) have described three phases of Chl*a* fluorescence quenching by O_2 in green algae, green plants and isolated chloroplasts: (i) a sensitive quenching site on the reducing site of PS-II, (ii) a site of intermediate sensitivity for quenching at the reaction centre of PS-II, and (iii) a least sensitive site for O_2 quenching of Chl fluorescense of excited PS-II antenna Chl. The work of Vidaver and his associates suggests that the most sensitive site of O_2 attack may be localized between PQ pool of PS-II and PS-I acceptor (see figure 2) It is suggested that plastocyanin (PC) (see figure 2) may transfer electrons to O_2 in addition to P700, the reaction centre of PS-I. It is now suggested that PC may function as a mobile link between the two photosystems. Thus besides the electron carriers located on the reducing side of PS-I, the carriers located on the oxidizing side of PS-I are also susceptible to O_2 attack.

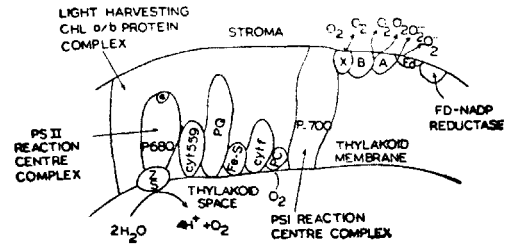

Figure 2 Schematic representation of the electron transport system in chloroplasts showing possible sites of interaction with oxygen (*see* Foeyr & Hall 1980)

Egneus et al. (1975) using labelled oxygen in isolated intact chloroplast of *Spinacea electacea* L. have claimed that during CO_2 reduction less ATP becomes available by the electron transport to NADP than it is needed to drive conversion of CO_2 into sugars if the ATP/NADPH ratio falls below 1.5. As the availability of ATP limits NADPH oxidation during reduction of CO_2, the NADPH pool is depleted and NADPH accumulates even under light intensity which is limiting for CO_2 reduction (hence there is no electron pressure to force reduction of oxygen because of this safety valve reaction). When NADP levels become low, electrons are diverted to oxygen. This results in ATP formation and relieves the ATP deficiency thus permitting NADPH oxidation. As the NADP level increases electrons are directed back to NADP. In this model the distribution of electrons between NADP and oxygen is regulated by the coupling efficiency of the thylakoid membranes (NADP for affinity reasons being the preferred electron acceptor in chloroplasts). However, most of the workers do not agree with this as they believe that the ratio of NADPH/ATP cannot be known acurately and even if there is not enough ATP, the cyclic photophosphorylation, which does not involve any molecular oxygen, can operate. Thus, the mechanism of pseudocyclic photophosphorylation needs further experimental evidences. Forti and Gerola (1977) have observed that inhibition of phosphoglycerate reduction (but not of CO_2 reduction) is abolished under conditions where ATP is available in excess of NADPH. This is taken as an indication that electron flow from PS-I is diverted to O_2 (Mehler reaction, which produces H_2O_2) when the unavailability of ATP is limiting the rate of reoxidation of NADPH. Thus the Mehler reaction is suggested as a physiological process, supplying ATP for photosynthesis. The work of Mukhin et al. (1977) on ATP: NADPH ratio for isolated chloroplasts of *Pisum sativum* showed that this ratio is influenced by environmental factors. The ratio of less than unity was observed when chloroplasts were exposed to light at a temperature above 25° C thus increasing NADP reduction and inhibiting photophosphorylation and the ratio exceeds unity if chloroplast reactions were carried out at low positive temperature or when plants are grown under low light intensity. This clearly suggests the possibility that there may be some mechanism by which ATP: NADP ratio is changed with the change in environmental conditions. Thus under given conditions the required ATP: NADPH ratio can be achieved which would lead to control of O_2 inhibition of photosynthesis.

Heber et al. (1978) using labelled O_2 have observed that as the light intensity is increased from 9 Wm^{-2} to 120 Wm^{-2} $^{18}O_2$ uptake by intact spinach chloroplasts does not increase with CO_2 dependent oxygen evolution, i.e., oxygen uptake during photosynthesis saturates at very low light intensity. This suggests that a high saturating light intensity for CO_2 fixation, electron transport to oxygen cannot contribute towards production of extra ATP needed for photosynthesis. Our contention is that Mehler's reaction, even though it may be to limited extent a physiological process, does not provide significant extra ATP for CO_2 fixation. Either it plays some other unknown physiological role or is simply a minor wasteful process as oxygen competes with NADP for the reductant generated at PS-I. The above mentioned investigators working upon the light scattering properties of intact leaves placed in varying atmospheres (i.e., from nitrogen to

21% O_2), have assigned a physiological role to oxygen alleging that it prevents over reduction of components of cyclic photophosphorylation and thus keeps cyclic ATP generation at an appreciable level. Thus the cyclic electron transport is considered to be under delicate redox control and inhibition of cyclic photophosphorylation due to excessive reduction can be checked by oxygen. However, their arguments though apparently reasonable, have not been well substantiated. In our opinion, as the oxygen uptake remains almost constant at low as well as high intensities, while CO_2 fixation and cyclic electron transport rates increase with increasing light intensity, oxygen probably has no physiological role to play in the photosynthesis of intact chloroplasts. Rather it selectively and wastefully competes, to a limited and fixed extent, with the NADP for the reductant generated at PS-I. It represents a futile pathway in which O_2 uptake wastes a little photosynthetically generated reducing power, a minor imperfection of the system, perhaps enhanced by air treatments.

There also remains a great deal of controversy as to the amount of H_2O_2 produced in the intact chloroplasts since H_2O_2 could be inhibitory to photosynthesis, depending on the mode in which H_2O_2, once formed, is removed from chloroplasts. The first possibility of H_2O_2 removal from chloroplasts may be that, since there is no catalase in the chloroplasts, a rapid H_2O_2 diffusion from the chloroplasts takes place down a steep concentration gradient to the peroxisomal catalase, where it is dismulated to H_2O and $\frac{1}{2}O_2$. A second possibility of H_2O_2 removal may be that reactive substrates (AH_2) such as reduced ascorbate and glutathione present in chloroplasts may convert H_2O_2 to water by the following reaction.

$$2H_2O_2+2AH_2\rightarrow 4H_2O+2A.$$

H_2O_2 may oxidise sugar phosphate components of Calvin cycle. Indeed H_2O_2 oxidation of dihydroxyethylthiamine pyrophosphate (addition complex to transketolase) produces glycolate (Gibbs 1969). Since ribulose bisphosphate is also a relatively easily oxidizable molecule, H_2O_2 may also oxidize it to phosphoglycolate which can be further hydrolyzed by chloroplastic phosphoglycolate phosphatase, as has been recently investigated in detail (Christeller & Tolbert 1978). Further oxidation of glycolate to CO_2 occurs outside the chloroplast.

Kaiser (1976) has claimed that low concentrations of added H_2O_2 to intact chloroplasts strongly inhibit CO_2 fixation. According to his account addition of catalase to a suspension of intact chloroplasts stimulates CO_2 fixation by 2–6 fold indicating that this process is partially inhibited by endogenous H_2O_2 formed in Mehler reaction.

In intact chloroplasts capable of CO_2 fixation, the catalase activity is considerrable. Since catalase is not a chloroplastic enzyme but is localized in the peroxisome, its presence in intact chloroplast preparations must presumably result from contamination of chloroplast suspension by peroxisomes. Removal of contamining catalase by extensive washing of chloroplasts is undesirable, if high photosynthetic activity is to be maintained. As H_2O_2 itself is inhibitory to CO_2 fixation, and if O_2 reduction occurs in intact chloroplasts, inhibition of catalase in this system should also lead to inhibition of photosynthesis itself. Allen and Whatley (1978) have compared the effect of cyanide, azide and aminotriazole on photosynthetic CO_2 fixation and catalase activities. Cyanide and azide inhibit catalase as well as CO_2 fixation to a greater extent,

whereas aminotriazole at different concentrations can differentially inhibit the above processes. Thus a 50% inhibition of catalase occurs at an aminotriazole concentration with which photosynthesis is enhanced by only 20%. From this observation, they suggest that the relatively small effect of aminotriazole on photosynthesis makes it likely that any Mehler reaction in intact chloroplasts is not rapid enough to produce inhibitory concentrations of H_2O_2 under conditions optimal for CO_2 fixation. Further investigation is needed to elucidate whether H_2O_2 is produced in vivo to a concentration which is appreciably inhibitory to photosynthesis. However, as discussed earlier, it is also possible that even if H_2O_2 is produced in the photosynthetic organisms by the Mehler reaction to an extent potentially inhibitory to photosynthetic CO_2 fixation, it may be dismutated to H_2O and O_2. Inspite of the fact that H_2O_2 produced by Mehler reaction is inhibitory to subsequent photosynthetic CO_2 fixation or not, one potential role of Mehler reaction is apparent that it competes with physiological acceptor NADP for the low potential reductant generated at PS-I. Thus occurrence of Mehler reaction by itself is detrimental for photosynthesis.

Hydrogen peroxide production during photosynthesis has also been demonstrated in the intact photosynthetic organism *Anacystis nidulans* (Patterson & Myers 1973). Based upon the sensitive assay using the fluorescence of scopoletin, which allows continuous recording of H_2O_2 production in illuminated intact cells of *Anacystis nidulans*, they observed that after the onset of illumination there was 5 to 10 seconds lag, a burst of very rapid production of H_2O_2 continuing up to 5 minutes and finally a slow and steady rate of H_2O_2 production. Size of the H_2O_2 burst is decreased by diuron (DCMU), by low O_2 concentrations and, by certain Calvin cycle intermediates. It is increased by highlight intensity, CO_2 depletion and Calvin cycle inhibitors like iodoacetamide. They explain the H_2O_2 production by the hypothesis that a low potential reductant is produced more rapidly than it can be used in the normal pathway of CO_2 reduction and this reacts with oxygen.

Glidewell and Raven (1975), using labeled oxygen have demonstrated that intact photosynthetic alga *Hydrodictyon africanum* reduces oxygen to an appreciable extent, in a reaction which is different from O_2 uptake by the RuBP carboxylase oxygenase reaction.

More convincing arguments come from investigation of Radmer and Kok (1976) who by using ^{18}O mass spectrometer studies in algal suspensions of *Scendesmus* and *Anacystis*, have concluded that O_2 and CO_2 are in direct competition for photosynthetically generated reducing power of PS-I, with O_2 being the main electron acceptor during the initial induction period and under other conditions in which CO_2 reduction cannot keep pace with O_2 evolution. In their experiments, the high rate of oxygen uptake reaction observed in the presence of Calvin cycle inhibitors or photophosphorylation uncouplers suggest that a special high capacity oxidase distinct from RuBP oxygenase exists in whole cells leading to direct oxygen inhibition of light reaction of photosynthesis. The oxygen uptake rate by whole cells reported far exceeds the rates observed in broken chloroplasts. This implies that the above special high capacity oxidase involved is only loosely bound to the photochemical electron transport apparatus and is eliminated in the isolation and washing of the chloroplasts.

Later on Radmer et al. (1978) have also determined the O_2 affinity of the oxidase involved in this O_2 cycle and kinetics of O_2 uptake under low CO_2 conditions. They have used a mass spectrometer with a teflon membrane inlet to monitor light dependent O_2 evolution, O_2 uptake and CO_2 uptake in suspensions of the green alga *Scendesmus*. They have observed that the rate of O_2 uptake, which in presence of iodoacetamide replaces the uptake of CO_2, shows a distinct plateau (Vmax) beyond 30% O_2, and is half maximal at 8% O_2. Thus they conclude that light driven O_2 uptake process (which does not involve carbon compunds) is saturated at lower O_2 concentration than all photorespiration and glycolate formation. During the course of CO_2 depletion from the algal suspension medium electron flow to CO_2 is replaced by an equivalent flow to O_2.

Sorensen et al. (1978) observed an increase in photosynthesis and reduction in glycolate excretion in blue green alga *Anacystis nidulans*, when the system was excited at 614nm (excitation of PS-I and PS-II) and at 446nm (mainly excitation of PS-I), it resulted in enhancement of photosynthesis by 18% and reduction in glycolate excretion by 31%. This showed that a preferential excitation of the two systems by irradiating with blue as well as yellow light significantly reduced glycolate excretion to the medium, thus this could be a controlling factor of glycolate metabolism. Their work supported the hypothesis that some glycolate may be consumed in an oxidation process associated with photosystem I, when PS-II is poorly excited and the supply of electrons from the water splitting process of photosynthesis is low (figure 3).

In *Sinapis alba*, photorespiration like photosynthesis, was shown to be

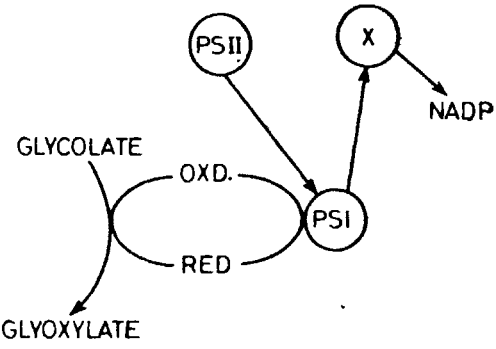

Figure 3 A model drawing of how glycolate dehydrogenase is oxidized to glyoxylate simultaneously as electrons are supplied to PS-I *(after Sorrensen et al. 1978)*

wavelength dependent (Nilson Stein et al. 1979). These workers reported that light is involved not only in the production of glycolate, but also in its further metabolism and confirmed that PS-I is a key factor in the regulation of glycolate metabolism and that the product formed from the consumption of glycolate via PS-I does not seem to be metabolized by the normal glycolate pathway.

Thus, in the light of above experiments, the following points may be raised:

(i) Egneus et al. (1975) and Krause and Heber (1976) have hypothesized that the extra requirement of ATP for CO_2 reduction diverts the electrons to oxygen in spinach chloroplasts. The C_4 plants have a higher requirement of ATP for CO_2 fixation than the C_3 plants. Assuming the hypothesis of above workers to be correct, one ought to ask if it is a fact that the leaves of C_4 plants posses a higher rate of Mehler's reaction than the leaves of C_3 plants or is the extra ATP formed by cyclic photophosphorylation?

Mohanty and Boyer (1976) have compared the quantum yield of leaves of

sunflower, a C_3 plant, at 21% and 2% of O_2. They have observed that quantum yield in terms of moles CO_2 absorbed per einstein is higher at 2% O_2 than that at 21% O_2. Bjorkman (1976) and Ehleringer and Bjorkman (1977) have also measured the quantum yields of a C_3 plant *Atriplex alsbrtuscula* and C_4 plant *Atriplex argentea*. The quantum yield for the C_3 plant leaf is 0.051 mole CO_2 per absorbed einstein in the presence of normal air. When the oxygen concentration is decreased to 2%, the quantum yield increases to 0.073 mole CO_2 per einstein regardless of the CO_2 concentration used so the effect is unlikely to be due to photorespiration. Under low O_2 concentration, the quantum yields of C_3 plants are usually about one third higher than in C_4 plants. This lower quantum yield of C_4 plants as well as saturation at high light intensities is consistent with the notion that C_4 plants have smaller photosynthetic unit than C_3 plants and that C_4-photosynthesis requires 2 more ATP per CO_2 fixed than does C_3 photosynthesis. However, because of the presence of an oxygen inhibition of photosynthesis and the consequent lowering of the quantum yield in C_3 plants, but not in C_4 plants, their quantum yields in normal air are practically identical.

The effect of temperature on the quantum yield and its relation to the O_2/CO_2 solubility ratio was studied by Ku and Edwards (1978) in C_4 species, *Zea Mays* and in C_3 species, *Triticum aestivum* L. Whereas the quantum yield for C_4 species was more or less constant over a range of temperature of 16–35°C irrespective of O_2 atmosphere (1.5% or 21% O_2), the quantum yield for C_3 species decreased from under 1.5% O_2 to 21% O_2 for a range from 15°C to to 35°C temperatures. The possible cause for this decrease is attributed to a temperature dependent change in the solubility ratio of O_2/CO_2. However, *Zea Mays* is a warm weather plant and *T. aestivum* is adjusted to cold climates, so the difference is not necessarily due to the C_4-C_3 phenomenon.

It is certainly remarkable that the benefit of the abolishing of O_2 inhibition conferred by C_4 photosynthesis, is almost exactly offset by the higher intrinsic energy cost of this pathway. Since the Mehler's reaction is saturated at very low O_2 concentration, i.e., half saturation is achieved at 0.1% O_2 (Heber 1969), can the above results be extrapolated to indicate that low quantum yield of the C_4-plants in 2% O_2 is due to a higher rate of Mehler's reaction, (pseudocyclic electron transfer) to generate extra ATP in these plants?

(2) On the other hand, it has been argued, as discussed above that Mehler reaction is detrimental to photosynthetic CO_2 fixation, as O_2 competes with the low potential reductant generated by PS-I acting in series with PS-II. The magnitude and intensity dependence of the O_2 exchange reactions and their sensitivity to diuron (DCMU) suggests that O_2 uptake requires the cooperation of both the photosystems (Radmer & Kok 1976). Hence the following questions can be asked: are the C_4 plants protected against Mehler's reaction due to lack (or low activity of) PS-II in the bundle sheath chloroplasts preventing thereby generation of light generated auto-oxidizable reducing power and do these plants have higher rate of cyclic electron flow than C_3 plants to cope with increased ATP requirement for CO_2 fixation? However, one has to wonder why the mesophyll cells of C_4 plants with abundance in activity for PS-II, would not show a higher extent of Mehler reaction than C_3.

Shelp, Barry and Canvin (1980) demontrated that in *Chlorella pyrenoidosa* in which photorespiration does not occur, a CO_2 concentrating mechanism suppresses the oxygen inhibition of photosynthesis. Thus O_2 inhibition is discussed in terms of the oxygenase reaction and a Mehler reaction supporting pseudocyclic electron flow.

As discussed earlier, Chollet (1976a) has reported that glycidate stimulates the rate of glycolate biosynthesis in isolated chloroplasts in contrast to reported leaf disc experiments of Zelitch (1974). It appears probable that in the above experiments of Chollet, glycidate might have checked direct O_2 inhibition effect of photosynthesis by somehow inhibiting the above high capacity oxidase or facilitating *dismutation* of H_2O_2 produced during Mehler reaction to H_2O and O_2.

Once the basic mechanism of direct oxidation of light generated reducing power at PS-I by O_2 is established, it may be possible to control the above process by chemical or genetic means. Nevertheless it remains uncertain how large is this direct oxidation of reductant by O_2, what its nature is, and whether or not O_2 competes with CO_2 reduction, or is it possible that O_2 also directly quenches the excited state? All these questions suggest that the study of direct effect of O_2 on photoelectron transport will be a fruitful area of endeavour particularly under conditions of temperature, water stress and senescence which affect photosynthesis.

Conclusion

From the foregoing discussion, it is apparent that a fundamental controversy exists as to the specific site at which the glycolate pathway which can be blocked to achieve reduced rate of photorespiration. The debate involves the following possibilities: (1) the glycolate pathway may be controlled at any specific step even after glycolate synthesis, and (2) glycolate pathway should be checked only at the level of glycolate synthesis, since a block at any other site of the pathway may lead to accumulation of metabolites and depletion of Calvin cycle intermediates (such as RuBP) resulting in the reduced rate of CO_2 fixation. We favour the latter position believing that glycolate pathway should be blocked at the level of glycolate synthesis. Alternatively, attempts should be made to convert glycolate to any useful Calvin cycle intermediate. This might be achieved through by-passing the site of glycine conversion to serine and CO_2. But this may not be a useful solution as glycine has to be reshuffled into Calvin cycle. A reduced rate of glycolate synthesis is possible if we can genetically modulate the enzyme RuBP carboxylase oxygenase so as to bring about an increased rate of carboxylation relative to oxygenation. Our contention is that since the electronic configuration of O_2 and CO_2 are drastically different, these competing substances may be binding to different charged groups even in the same active site region of the bifunctional enzyme. However, there is no experimental evidence to support this argument (possibly the enzyme can be changed utterly by obliterating the site of O_2 binding). However, this task of genetic modulation of the enzyme cannot be accomplished until the active sites and activator sites of the enzyme are thoroughly characterised and their interrelationships are established. Even then the task might remain formidable since evolution has yet to accomplish it.

In our opinion, direct inhibition of photosynthesis by O_2 is brought about by

two interlinked steps. Firstly competition of O_2 with NADP for the reductant generated at photosystem-I or some other sites PQ or PC which depletes the level of the reducing component necessary for CO_2 fixation, and thus photosynthesis is inhibited. How large this effect is remains, uncertain since we do not know how competitive the reaction really is. It is doubtful that in intact plants oxygen beneficially acts as terminal acceptor for pseudo cyclic electron transport merely to supply extra ATP needed for CO_2 fixation. The extra ATP needed for CO_2 fixation more probably comes from cyclic electron transport. Secondly the H_2O_2 produced by above Mehler reaction may also contribute to a part of glycolate synthesized by peroxidation of a Calvin cycle intermediate. If so, attempts to completely check the glycolate synthesis cannot be accomplished by genetic modulation of RuBP carboxylase-oxygenase enzyme alone. Hence, interaction of oxygen with the reductant generated at PS-I should also be checked to completely control oxygen inhibition of photosynthesis. Attempts to block direct O_2 inhibition of photosythesis must await a thorough understanding of mechanism of O_2 action at the reducing side of PS-I and PS-II in intact green plants. Also, if any CO_2 concentrating mechanism exists in a system, it may be possible to suppress the oxygen inhibition of photosynthesis. This is the essence of the C_4-pathway. The other control measure that can be taken with regard to photorespiration and oxygen inhibition of photosynthesis is by specific inhibition of oxygenase activity of RuBP carboxylase by certain chemicals. Whether or not this is possible remains to be seen.

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