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Rubisco: its role in photorespiration

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The release of CO_2 during photosynthesis that is due to the production and metabolism of glycollic acid is usually regarded as outward evidence for the wasteful process of photorespiration in plants. In the light, glycollic acid is produced almost entirely as a result of the oxygenase activity of ribulose bisphosphate carboxylase–oxygenase (Rubisco). Metabolism of the glycollic acid not only releases recently assimilated carbon back into the atmosphere but also uses a considerable amount of energy to recycle remaining carbon from the glycollate to intermediates of the photosynthetic carbon reduction cycle. Furthermore, nitrogen from amino acids is released as ammonia during the metabolism of glycollate; some further energy is needed for this ammonia to be reassimilated. The oxygenation of ribulose bisphosphate is competitive with carboxylation and it appears to be the relative concentrations of oxygen and carbon dioxide present in cells containing the enzyme that mainly determine the relative rates of the two reactions in leaves. Systems which concentrate carbon dioxide in photosynthetic cells decrease the extent of photorespiration in C_4 species, certain algae and cyanobacteria. However, carboxylases from different species also vary considerably in their relative capacities to catalyse carboxylation and oxygenation of ribulose bisphosphate under standard conditions. This variation allows some hope that photorespiration might be decreased without recourse to energy-dependent systems for increasing cellular CO_2 concentrations.

Introduction

The earliest studies of photosynthetic carbon assimilation, with radioactive carbon dioxide, showed that glycollic acid was a significant product (Benson & Calvin 1950). It was clearly not the first product of assimilation but was derived by subsequent metabolism of one of the early products. The uniformity of distribution of isotopes between the two carbon atoms of the glycollic acid and a related pattern in the carbon atoms of glycine and serine suggested that these amino acids were derived from glycollate (Schou *et al.* 1950). The proportion of radioactivity in glycollate after short periods of photosynthesis from $^{14}CO_2$ was increased in *Chlorella* by decreasing the CO_2 concentration or by increasing the oxygen concentration (Benson & Calvin 1950; Coombs & Whittingham 1966*a*, *b*). Synthesis of glycollate relative to other substances was also increased by increased light intensity (Coombs & Whittingham 1966*b*). These initial observations stimulated very extensive studies of glycollate metabolism described already in many review articles (Goldsworthy 1970; Jackson & Volk 1970; Tolbert 1971; Zelitch 1971). The enzymes involved and their intracellular locations were largely established by 1970. Glycollate in leaves is oxidized to glyoxylate, which is then aminated to glycine. Serine is formed from two molecules of glycine, with the release of one carbon as CO_2 and one amino group as ammonia, and is then involved in a transamination. The resulting hydroxypyruvic acid is converted to glyceric acid by reduction. Finally glyceric acid is phosphorylated to glycerate 3-phosphate. The various steps take place in either the chloroplasts, the peroxisomes or mitochondria of leaves, as shown in figure 1. Although much of the evidence

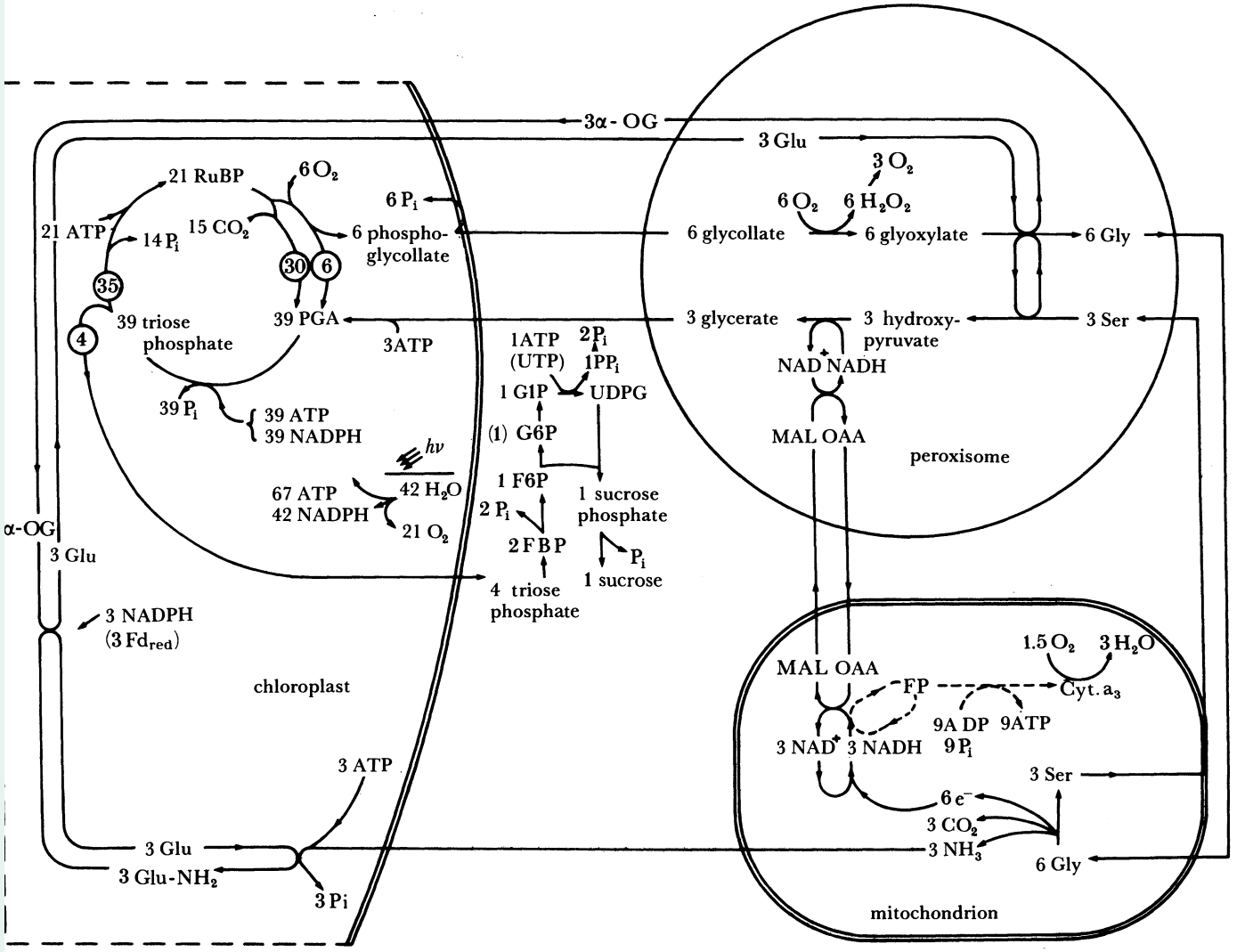

FIGURE 1. Photosynthetic carbon metabolism in a mature wheat leaf with sucrose as the sole product. The relative rate of carboxylation to oxygenation of ribulose bisphosphate is arranged at 2.5 by appropriate attention to the stoichiometry of both interacting cyclic pathways. Thus gross production of CO_2 in the photorespiratory pathway corresponds to 20% of gross CO_2 uptake by carboxylation. This situation is consistent with a carboxylase having a specificity factor of 80, where the internal CO_2 concentration in the leaf is 8.3 μM and the O_2 concentration 265 μM. These concentrations of CO_2 and O_2 can easily be justified for the natural situation, and the rates of photorespiration reported for wheat leaves are of the same order of magnitude as predicted in the figure. In the figure each mol of sucrose synthesized require 67 mol ATP and 42 mol NADPH; without photorespiration, the corresponding values would be only 37 and 24.

(Abbreviations used: Gly, glycine; Ser, serine; PGA, glycerate 3-phosphate; G6P, glucose 6-phosphate; F6P, fructose 6-phosphate; RuBP, ribulose bisphosphate; OAA, oxaloacetate; MAL, malate; FBP, fructose 1,6-bisphosphate; UDPG, uridine diphosphoglucose; α-OG, 2-oxoglutarate; Glu, glutamate; Fd_{red}, reduced ferredoxin; $Glu\text{-}NH_2$, glutamine; P_i, orthophosphate; PP_i, inorganic pyrophosphate; *hν*, light energy; Cyt a_3, cytochrome a_3.)

for production of glycollate came from studies with unicellular algae, the pathway for glycollate metabolism outlined above was elucidated mainly from studies with leaves; in at least some algae the pathway is different (Tolbert 1979) but not fully characterized.

Studies of the physiology of photosynthesis by plants provided many puzzling facts. Thus

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Warburg (1920) showed that photosynthesis in algae was inhibited by oxygen. Similar inhibition was later recognized in higher plants (Turner & Brittain 1962). Inhibition by oxygen was decreased in conditions where the CO_2 concentration was high (Wassink *et al.* 1938; Briggs & Whittingham 1952). Furthermore, many plants could not remove all the CO_2 present when photosynthesizing in a closed system. The concentration remaining was regarded as the result of a respiration process balancing exactly the rate of photosynthesis at a residual concentration of CO_2, which was called the compensation point. It was subsequently shown that the value of the CO_2 compensation point was increased by increases in temperature and oxygen concentration (Tregunna *et al.* 1964). Plants with a substantial CO_2 compensation point also showed high rates of respiratory CO_2 production in darkness immediately following a period of illumination before a steady rate of CO_2 evolution, related to dark respiration, began. Because of the rapid change from net assimilation to net release of CO_2 upon darkening, there appeared to be a pulse of CO_2 recorded, which was called the postillumination CO_2 burst (Decker 1955; Tregunna *et al.* 1961). This burst was proposed by Decker & Tio (1959) to be due to a respiratory process that they called photorespiration and which was seen as a continuation in darkness of a light-stimulated respiratory process. Maize was among species recognized at an early stage to have a CO_2 compensation point near to zero (Meidner 1962) and to show no significant postillumination CO_2 burst (Tregunna *et al.* 1964). Zelitch (1979) has described his experiences at this time when photorespiration and the oxygen inhibition of photosynthesis became accepted as causally related to glycollate production.

It was possibly the continued physiological studies of photosynthesis by Krotkov and his colleagues, exemplified in two papers published in 1966 (Forrester *et al.* 1966*a*, *b*), that did most to rationalize in the minds of scientists a relationship between glycollate metabolism, the oxygen inhibition of photosynthesis, the postillumination burst of CO_2 and CO_2 compensation points. They showed that photosynthesis by soybean was inhibited progressively by increased oxygen from 0–100% and much more strongly when the CO_2 present was decreased; this was the reverse of the response of glycollate production in algae to oxygen and CO_2. Oxygen increased the size of the postillumination burst of CO_2 in higher plants but did not stimulate dark respiration. Indeed extrapolation of the CO_2 compensation point to zero O_2 suggested that the normal processes responsible for dark respiration were suppressed in the light. Furthermore, maize had a CO_2 compensation point near to zero at all oxygen concentrations tested. Significantly also, O_2 increases from 0 to 21% in the atmosphere did not inhibit photosynthesis by maize, although further increases in O_2 above 21% were inhibitory.

Contemporary work by Hatch & Slack (1966) confirmed and extended four earlier reports from different laboratories that the path of carbon in photosynthesis in sugar cane and maize was not the same as that observed for other plants, namely the Calvin cycle with Rubisco as the main carboxylating enzyme. This finding was followed later by a specific hypothesis that sugar cane and maize used light energy to power a biochemical pathway involving fixation of CO_2 into C_4 acids and its subsequent release into cells containing the enzymes for the Calvin cycle mechanism of photosynthesis. In these cells the concentration of CO_2 was increased, thus decreasing the inhibitory effect of oxygen on photosynthetic assimilation (see Hatch & Slack 1970; Hatch 1976) and the associated photorespiration. Such plants are known now as C_4 plants.

At this stage there was no convincing theory concerning the mechanism of glycollate production in leaves. Reviews of photorespiration appearing in 1970 (Goldsworthy 1970;

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Jackson & Volk 1970) scarcely mentioned ribulose bisphosphate carboxylase but by then Ogren & Bowes (1971; Bowes & Ogren 1972) were considering the reason for oxygen inhibition of carboxylation catalysed by ribulose bisphosphate carboxylase. It became evident that the enzyme catalysed an oxygenation of RuBP in addition to carboxylation. Phosphoglycollate was one of the products (Bowes *et al.* 1971; Andrews *et al.* 1973). A phosphoglycollate phosphatase in chloroplasts was already known (Richardson & Tolbert 1961) so that a hypothesis that photorespiration derived from glycollate produced from ribulose bisphosphate by way of phosphoglycollate was available to be tested. Laing *et al.* (1974) showed that the rates of oxygenation of ribulose bisphosphate catalysed by the carboxylase responded to temperature in a way reminiscent of the situation with photorespiration in leaves, and also that the responses of net photosynthesis to temperature, oxygen and CO_2 were qualitatively those predicted by the properties of Rubisco. It appeared that oxygen and CO_2 were alternative competing substrates for reaction with ribulose bisphosphate at a common catalytic site. There have been reports which are not consistent with this conclusion, but none has withstood the test of time (see reviews: Chollet & Ogren 1975; Andrews & Lorimer 1978; Lorimer & Andrews 1981; Lorimer 1981; Ogren 1984) and now most models of single leaf photosynthesis contain parameters of the kinetic equations governing catalysis of reactions of alternative competing substrates (Dixon & Webb 1979) to give rates of photosynthesis and photorespiration. Except for a few of the fine details, the interactions of photorespiratory and photosynthetic metabolism outlined in figure 1 have been confirmed by selection and study of mutants (Ogren 1984).

The remainder of this review is concerned with properties of ribulose bisphosphate carboxylase *in vitro*, the likely concentrations of its substrates *in vivo* and the extent to which these two aspects can be used to account for the known relationship between photosynthesis and photorespiration in various photosynthetic species.

Properties of Rubisco relevant to rates of oxygenation and photorespiration

Theoretically a knowledge of the kinetic constants K_m(RuBP), $K_m(CO_2)$, $K_i(CO_2)$, $K_m(O_2)$, $K_i(O_2)$, V_{max} (carboxylase) and V_{max} (oxygenase) for the enzyme-catalysed carboxylation and oxygenation might sufficiently define the rates of oxygenation and carboxylation at given O_2, CO_2, RuBP and enzyme concentrations and at the temperature to which the values for these parameters refer. However, this is not the situation, because the catalytic activity (V_{max}) of the enzyme is modulated by the concentrations of its cofactors, CO_2 and Mg^{2+}, and by many other substances present in cells. Thus the enzyme loses both catalytic activities if it is incubated in a CO_2-free medium. At equilibrium, in buffer containing sub-saturating CO_2 or Mg^{2+} the appropriate V_{max} values to be used in calculations of velocities of carboxylation or oxygenation are much lower than the maximum specific activities that can be attained. The problem of maintaining the state of activation of the enzyme must also be taken into account when the K_m value for CO_2 or the oxygenase activity are determined; initial rates must be measured on addition of activated enzyme to an otherwise complete reaction mixture (Lorimer *et al.* 1976, 1977). Otherwise, at low CO_2 concentrations the enzyme becomes deactivated progressively with time so that low oxygenase activities and high $K_m(CO_2)$ values are recorded. In the activation by CO_2, there is a synergistic effect of a divalent metal ion. The divalent metal most frequently used *in vitro* is Mg^{2+} because this is probably the ion present with the enzyme *in vivo*.

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Activating CO_2 reacts with a lysyl group in the enzyme to form a carbamate, which is stabilized by Mg^{2+} (Lorimer 1981; Miziorko & Lorimer 1983). The problem of defining appropriate V_{max} values for operation of the enzymes in cells is further complicated by other substances present that affect the activity of the enzyme. Thus, if CO_2 and Mg^{2+} are regarded as cofactors then NADPH, 6-phospho-D-gluconic acid, 3-phospho-D-glyceric acid, orthophosphate and many other substances are effectors of the enzyme (Badger & Lorimer 1981; McCurry *et al.* 1981; Gutteridge *et al.* 1982; Parry *et al.* 1985). There are two essential features of the mode of operation of these substances that need to be recognized. First, they decrease the concentration of CO_2 necessary to achieve near to maximum activation of the enzyme, and secondly, they competitively alter the binding of RuBP by the enzyme (the K_m(RuBP)). There is at least one further factor to be considered; in certain species of higher plant there is a powerful inhibitor of Rubisco that is formed during the night and which disappears slowly during the day (Vu *et al.* 1983; Servaites 1985).

These various effects on the activity of the enzyme affect the carboxylase and oxygenase activities proportionally. Prediction of the velocity of the enzyme-catalysed oxygenation (and hence photorespiration) therefore requires knowledge of the concentrations of many constituents of the medium surrounding the enzyme in addition to the amounts of substrates and the amount of enzyme protein present.

Fortunately, there is a relationship that is derived from the Michaelis–Menten theory (Laing *et al.* 1974; Jordan & Ogren 1981) relating the oxygenase activity to the carboxylase activity. This relation applies apparently irrespectively of the state of activation of the enzyme or the presence of many effectors (Boyle & Keys 1984) and is also independent of the concentration of RuBP (Jordan & Ogren 1984). It shows that there is a constant, called the specificity factor (V_cK_o/V_oK_c; where V_c and V_o are the V_{max} values for carboxylation and oxygenation, and K_c and K_o are the Michaelis constants for CO_2 and O_2 respectively) which, when multiplied by the ratio of the concentrations of CO_2 to O_2 in solution gives the ratio of the rate of carboxylation of RuBP to the rate of its oxygenation. The main assumptions made are that $K_i(CO_2) = K_m(CO_2)$, $K_i(O_2) = K_m(O_2)$, (here $K_i(O_2)$ and $K_i(CO_2)$ refer to inhibition constants for, respectively, O_2 on carboxylation and CO_2 on oxygenation) and that the CO_2 concentration is small. For individual carboxylases, the only known way to change the specificity factor is to replace Mg^{2+} as the divalent metal ion cofactor (Wildner & Henkel 1978, 1979; Christeller & Laing 1979). The effect of Mn^{2+} is to cause a considerable decrease in the specificity factor, but replacement of Mg^{2+} with Ca^{2+} does not alter the specificity factor (Parry *et al.* 1983).

Table 1 shows that agreement between estimates of V_c, V_o, K_c and K_o is poor even for the higher plant enzymes that have been subject to much study and that complete sets of data from one source are not available. The V_{max} values refer to the maximum rates that can be achieved with the particular sample of enzyme under the defined conditions of assay; agreement on V_o is especially poor. The specific activity of the freshly extracted, fully activated enzyme is of some considerable interest in relation to recent reports that it is different between species of *Triticum* with different cytoplasmic genomes (Evans & Seeman 1984).

Table 2 shows a selection of reported specificity factors for various species. There are very clear differences in the enzyme in organisms of diverse habitat but within a group of C_3 terrestrial higher plants the variation is quite small. There appears for example to be a difference in specificity factor between the carboxylases from wheat and tobacco. A comparison in Table 3

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TABLE 1. KINETIC CONSTANTS FOR RUBISCO FROM SPINACH, SOYBEAN AND MAIZE WITH Mg^{2+} AS COFACTOR

species	temperature °C	K_m(RuBP) μM	$K_c(K_{ic})$ μM	$K_o(K_{io})$ μM	V_c μmol min^{-1} mg protein	V_o μmol min^{-1} mg protein	reference
spinach	25	—	11	500	1.5	0.77	Jordan & Ogren (1984)**
	30	—	14	600	1.9	1.10	Jordan & Ogren (1984)**
	25	—	14	480	—	—	Jordan & Ogren (1983)**
	25	17	17	—	—	—	Yeoh *et al.* (1981)*
	25	—	17	—	—	—	Andrews *et al.* (1975)*
	25	—	17.5 (19.5)	196 (354)	—	—	Badger & Andrews (1974)*
	25	—	10.2	(368)	0.37	—	Bird *et al.* (1982)*
soybean	30	—	21 (52)	690 (780)	0.94	0.21	Jordan & Ogren (1981)*
	30	180	34 (38)	390 (370)	—	—	Laing *et al.* (1974)*
	30	—	36	(544)	1.7	0.61	Christeller & Laing (1979)*
maize	25	—	34	810	—	—	Jordan & Ogren (1983)**
	25	—	56	—	—	—	Yeoh *et al.* (1980)*
	25	—	27	—(526)	0.80	—	Bird *et al.* (1982)**

* calculated from pK_a for H_2CO_3 at infinite dilution (no correction for ionic strength).
**calculated from corrected data for CO_2 or O_2.

TABLE 2. SPECIFICITY FACTORS FOR RUBISCO FROM VARIOUS SPECIES

species		specificity factor (V_cK_o/V_oK_c)	species	specificity factor (V_cK_o/V_oK_c)
C_3 plants				
Glycine max		82	*Helianthus maximus*	77
Spinacea oleracea		80 (74*)	*Lycopersicon esculentum*	82
Lolium perenne		80	*Medicago sativa*	77
Nicotiana tabacum		77	*Petroselinum crispum*	77
C_4 plants				
Amaranthus hybridus		82	*Portulaca oleracea*	78
Zea mays		78	*Setaria italica*	58
Echinochloa crus-galli		83	*Sorghum bicolor*	20
green algae				
Scenedesmus obliquus		63	*Euglena gracilis*	54
Chlamydomonas reinhardii		61	*Chlorella pyrenoidosa*	(31*)
Cyanobacteria				
Aphanizomenon flos-aquae		48	*Plectonema boryanum*	54 (32*)
Cocochloris periocystis		47	*Aphanocapsa alpicola*	48
photosynthetic bacteria				
Rhodospirillum rubrum		15(8*)		
Rhodopseudomonas sphaeroides	L_2 type	9		
	L_8S_8 type	62		

Data from Jordan & Ogren (1981, 1983), except *from Kent & Tomany (1984).

(unpublished work) of the results of three different experiments supports this view but the difference is so small that yet more measurements, or improved methods, are needed to confirm the conclusion. Within a genus, differences in specificity factor between species are so small as to be indistinguishable by present methods (Holbrook *et al.* 1984). Measurements of K_c also show differences between carboxylases from higher C_3 plants (Yeoh *et al.* 1980, 1981) and differences in amino acid sequences are now well established (Lorimer 1981; Miziorko &

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TABLE 3. SPECIFICITY FACTORS FOR RUBISCO FROM WHEAT AND TOBACCO

experiment	species	specificity factor	s.d.	replicates
1	wheat	104	7.9	5
	tobacco	94	7.1	5
2	wheat	92	10.2	5
	tobacco	77	8.3	5
3	wheat 1	96	7.4	8
	wheat 2	99	3.6	8
	tobacco 1	86	3.1	8
	tobacco 2	91	7.8	8

Data from M. A. J. Parry, C. N. G. Schmidt, M. J. Cornelius, B. N. Millard, S. Burton, S. Gutteridge, T. A. Dyer & A. J. Keys, unpublished.

Lorimer 1983). Whether the differences in specificity factors for carboxylases from C_3 plants are sufficient to be detected as differences in photosynthetic characteristics is an interesting question. The differences in K_c are, however, associated with photosynthetic mechanism. Thus Yeoh *et al.* (1981) found K_c values for C_3 plants ranging from 12 to 25 μM, for C_4 plants from 28 to 34 μM, and for aquatic species from 30 to 70 μM. Associated with the higher K_c values is an increased specific activity (Seeman *et al.* 1984) and a trend towards a decreased specificity factor (Jordan & Ogren 1983).

The conclusion from our present knowledge of RuBP carboxylases is that, given the concentrations for CO_2 and O_2 in photosynthetic cells and a rate of gross photosynthesis, it should be possible to calculate a corresponding rate of RuBP oxygenation and thus of photorespiration. Alternatively, given the *in situ* potential activity of Rubisco, together with the concentrations of CO_2, O_2 and RuBP, one might calculate maximum rates of gross photosynthesis and photorespiration. However, we do not yet know enough about the effects of local environment on the operation of Rubisco at its site within cells to allow prediction of rates *in vivo* from the amount of carboxylase protein.

CONCENTRATIONS OF CO_2 AND O_2 IN PHOTOSYNTHESIZING CELLS

(*a*) *C_3 plants*

Currently favoured mathematical models for photosynthetic rates (Laing *et al.* 1974; Peisker 1974, Farquhar 1979; Farquhar *et al.* 1980; von Caemmerer & Farquhar 1981) in single leaves contain sub-models for the concentration of CO_2 at the site of operation of Rubisco, for the RuBP concentration, and for dark respiration (Krebs cycle respiration). In C_3 plants the oxygen concentration is thought to be close to that expected in an aqueous solution in equilibrium with the natural atmosphere (Raven & Glidewell 1981). The net amount of oxygen evolved in photosynthesis is small compared to the amount in the air spaces in the leaf, and the diffusion path from the site of release is short and through a large surface between the mesophyll cells and the intercellular air spaces in the leaf. The O_2 concentration in the air spaces is not significantly different from that in the outside air. On the other hand, consumption of CO_2 in the leaf during photosynthesis is high compared to the amount in the intercellular spaces in the leaf and relatively steep concentration gradients exist to the outside air. Again the diffusion path from the intercellular air spaces to the mesophyll chloroplasts is short and through

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a large surface area so that the concentration of CO_2 in the intercellular spaces may be in near-equilibrium with the concentration in the chloroplast (Bauwe *et al.* 1980; Lawlor & Pearlman 1981). The stomata control the diffusion path into the air spaces in the leaf from the ambient air; the gradient established depends mainly on stomatal aperture and the rates of CO_2 assimilation and release within the leaf. In bright light, under natural conditions, with 340 µl l^{-1} CO_2 in the atmosphere and with no restriction of water available to the plant, the air spaces may contain between 200 and 250 µl l^{-1} CO_2 (Wong *et al.* 1985). If one assumes the chloroplast stroma is pure water, the free CO_2 in solution at 25 °C will be between 6.8 µM and 8.5 µM, based on the solubility coefficient for CO_2 at 25 °C of 0.759. The concentrations of bicarbonate and carbonate in solution will depend on the pH. At pH 8.0, a probable value in the chloroplast stroma in bright light, bicarbonate will be the main inorganic form of carbon in solution, at concentrations of 290 µM–363 µM assuming a pk_a of 6.37 at 25 °C. However it is CO_2 in solution that is the substrate for Rubisco, and the ratio $[CO_2]:[O_2]$ will be between 0.026 and 0.032. For a specificity factor of 80 (Table 2) this suggests relative rates of carboxylation to oxygenation in a C_3 leaf at 25 °C of 2.1–2.6 and thus of gross photosynthesis to photorespiration of 4.2–5.2 (Net photosynthesis $= v_c - 0.5\,v_o - R_D$, where v_c = rate of carboxylation, v_o = rate of oxygenation and R_D = rate of dark respiration). Thus the stoichiometric representation of photorespiration shown in the composite diagram (figure 1) giving gross photosynthesis/photorespiration = 5 is not inconsistent with the known properties of purified carboxylase and with many estimates in the literature (Zelitch 1975).

There is much evidence that the rate of photorespiration relative to that of photosynthesis declines with decreasing temperature. This is consistent with a change in the relative rates of carboxylation to oxygenation (Laing *et al.* 1974; Ku & Edwards 1977) although whether it is the kinetic constants of the enzyme-catalysed reaction or the change with temperature in the relative solubilities of carbon dioxide and oxygen that are mainly responsible is a matter of dispute (Brooks & Farquhar 1985; Hall & Keys 1983; Jordan & Ogren 1984; Ku & Edwards 1977). It may not be profitable to pursue this question unless new evidence is produced for a significant alternative source of glycollate than the oxygenation of RuBP and hydrolysis of phosphoglycollate. At that stage the fact that the chloroplast stroma is not water, but a colloidal solution containing ionic substances other than bicarbonate, must be considered carefully, and the effect of such solutes on the solubilities of CO_2 (Harned & Davis 1943) and O_2 (Reynaforge *et al.* 1985) and on the kinetic parameters associated with the enzyme reactions catalysed by Rubisco needs to be evaluated in relation to any measurements made *in vitro* under often very different conditions of ionic strength (Yokota & Kitaoka 1985) and certainly at much lower enzyme concentrations. Another complication may be that a CO_2 concentrating system may be present even in C_3 plants (Espie & Coleman 1982; Machler *et al.* 1985; Volkita *et al.* 1981).

(*b*) *Leaves of C_4 plants and CAM plants*

In leaves of C_4 plants, Rubisco is present in the bundle sheath cells, where the CO_2 concentration is raised by a pumping mechanism in which light energy absorbed in the mesophyll provides energy to drive the pump. The concentration of CO_2 in the chloroplasts of bundle sheath cells is difficult to estimate but some calculations can be made based on volumes of the cell components and estimates of the pool of inorganic carbon present (Hatch 1971). The values thus calculated suggest $[CO_2]$ in excess of 80 µM (Hatch 1976). Under such a régime, where the oxygen concentration is near to equilibrium with that of the outside air, the oxygenase

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reaction would be less than one fifth that in C_3 plants. A more precise estimate would need to take into account the slightly different properties of carboxylases in C_4 compared to C_3 species already mentioned. Claims that the oxygen concentration may be less than an equilibrium value arise from the discovery that in certain C_4 plants of the maize type the bundle sheath chloroplasts are deficient in photosystem II activity. Thus the above may be an overestimate of oxygenation.

Plants that involve crassulacean acid metabolism (CAM) in their photosynthetic mechanism assimilate CO_2 into C_4 acids in the night. During the day, any stomata present close to restrict water loss and the concentration of CO_2 can rise to as high as 2% by volume in the intracellular air spaces in the middle of the photoperiod as the C_4 acids are decarboxylated (Cockburn *et al.* 1979). Although some increase in oxygen concentration in the intracellular air spaces occurs (Spalding *et al.* 1979) the assimilation of CO_2 by Rubisco takes place under favourable conditions for carboxylation and photorespiration is slow.

(*c*) *Algae and Cyanobacteria*

Aquatic photosynthetic species frequently seem to possess constitutive or inducible CO_2 concentrating mechanisms that are not yet fully defined (Raven *et al.* 1985). Thus despite the less favourable specificity factors associated with increased K_c values for the carboxylases in these species, their photosynthesis is less sensitive to oxygen than that of C_3 species and it can be concluded that glycollate production and metabolism is slower relative to carboxylation (Kerby & Raven 1985).

(*d*) *Photosynthetic bacteria*

Some carboxylases of photosynthetic bacteria have a poor specificity for carboxylation (see table 2). However these organisms are anoxygenic and often thrive only under relatively anaerobic conditions where the relatively high capacity for oxygenation of RuBP is not realised.

Concluding remarks

The extent of photorespiration relative to photosynthesis can be largely explained in terms of the properties of Rubisco. However, the absolute rates of photosynthesis and photorespiration cannot be related directly to the amount of Rubisco protein present in cells. This is because the enzyme is subject to regulating mechanisms depending on the concentrations of CO_2, Mg^{2+} and various metabolites and inhibitors present in the vicinity of the enzyme. However some measurement of the immediate activity of Rubisco in leaves can be made by rapid extraction and assay. The information then obtained for the response of photosynthesis and photorespiration to CO_2 concentration is consistent with a model based on the carboxylase and oxygenase properties if it is also assumed that at high CO_2, when light becomes rate-limiting, the regeneration of RuBP becomes inadequate to support photosynthesis (Farquhar *et al.* 1980).

The intense interest in Rubisco is only partly because it initiates photorespiration. This aspect is, however, of special interest in agriculture because many crop plants have the C_3 mechanism of photosynthesis in which photorespiration is rapid. In such species potential productivity is decreased compared with C_4 species where photorespiration is slow (Monteith 1978; Forrester *et al.* 1966*a*) and compared with C_3 species growing in conditions where photorespiration is decreased artificially (Bjorkman *et al.* 1968; Hardy & Havelka 1977).

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