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Carbon Dioxide Assimilation by Leaves, Isolated Chloroplasts, and Ribulose Bisphosphate Carboxylase from Spinach[1]

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ABSTRACT

The relationship between rate of photosynthesis and CO_2 concentration has been reinvestigated using isolated spinach (*Spinacia oleracea*) chloroplasts. The apparently low CO_2 concentration required for half-maximal photosynthesis is shown to result partly from a ceiling imposed by electron transport. In double reciprocal plots of rate against CO_2 concentration, this ceiling results in departures from linearity at high CO_2 concentrations. If these rate limitations are disregarded in extrapolation the "true" CO_2 concentration required for half maximal carboxylation by intact chloroplasts is approximately 46 μM (CO_2).

When assayed under comparable conditions, ribulose bisphosphate carboxylase from these chloroplasts also shows an apparent Km (CO_2) of approximately 46 μM, suggesting that its characteristics are not modified by extraction. An improved assay for ribulose bisphosphate carboxylase yielded rates of carboxylation considerably higher than those previously reported, the highest maximal velocities recorded approaching 1000 μmoles CO_2 fixed mg^{-1} chlorophyll hr^{-1} at 20 C. With such Km and V_{max}, values the carboxylase would be able to achieve, at concentrations of CO_2 less than atmospheric, rates of CO_2 fixation equal to those displayed by the parent tissue or by the average plant under favorable conditions in its natural environment.

Under optimal conditions of near saturating light, relatively high temperatures and CO_2 concentrations above atmospheric, land plants can assimilate carbon in the light at rates of up to 500 to 600 μmoles CO_2 mg^{-1} Chl hr^{-1} (47). In recent years, plants have been divided into two groups, C3 and C4, according to whether the first formed product of carboxylation is 3-P-glycerate, or oxaloacetate (6, 16, 17, 24). During photosynthesis, the C3 species (such as tobacco and spinach) release newly fixed CO_2 to the atmosphere, *i.e.* they photorespire, whereas C4 plants (such as maize and sugar cane) do not (6, 16, 17, 24, 47). Lack of photorespiration may be a consequence of the ability of C4 plants to concentrate CO_2 within an inner compartment of the leaf (15, 24), and it probably accounts for the faster rates of net photosynthesis usually achieved by C4 species under conditions of high light, high temperature, and low CO_2 (6, 16, 17, 47). Particularly as temperatures rise above 20 C, respiration and photorespiration increasingly widen the gap between "apparent" photosynthesis (net uptake of CO_2) and "true" photosynthesis (CO_2 actually fixed). For these reasons, it would be futile to attempt to derive an "average" rate of photosynthesis, embracing both groups, over a wide range of conditions. At temperatures up to 20 C, the differences in photosynthetic activity between C3 and C4 plants are less marked and the difference between "apparent" and "true" photosynthesis is comparatively small. As a first approximation, therefore, the figure of 20 mg CO_2 dm^{-2} leaf surface hr^{-1} proposed by Rabinowitch (31) for temperatures close to 20 C is not without merit.

Similar rates have subsequently been reported for a wide range of plants at temperatures near 20 C (5, 33, 44), although the maximum rates for a few species and varieties may be much higher (13). Rabinowitch's value applies to the plant in favorable conditions in its natural environment and is equivalent to 100 μmoles CO_2 mg^{-1} Chl hr^{-1} at a Chl concentration of 0.045 mg cm^{-2} (the Chl content of many species [18] lies within the range 0.03 to 0.06 mg cm^{-2}). In these particular circumstances, photosynthesis would be limited not by light but by CO_2. Except in urban areas, atmospheric CO_2 is usually near 0.033% (or about 8–12 μM in solution, depending on the temperature and presence of other solutes) and a 2- to 3-fold acceleration in photosynthesis occurs if the CO_2 is increased to between 0.10 and 0.15% (28). In some respects, the behavior of isolated spinach chloroplasts is not far removed from the "average" plant. Particularly if allowance is made for the presence of ruptured chloroplasts (which contribute to the Chl estimation but not significantly to the CO_2 fixation [39]), rates in excess of 100 μmoles mg^{-1} Chl hr^{-1} can be obtained routinely in saturating bicarbonate (Table I). Less commonly, rates as high as 400 μmoles mg^{-1} have been observed (U. Heber, unpublished). Half-maximal velocity for intact chloroplasts is attained at 0.3 to 0.6 mM bicarbonate (7, 12, 22), which at the temperature and pH of the assay mixtures, is approximately equivalent to air levels of CO_2. Half-maximal velocities at 0.03% CO_2 have also been recorded by Goldsworthy (14) for intact leaves of C3 and C4 species illuminated in N_2 under laboratory conditions.

By contrast, the enzymic carboxylation of RBPCase[3] (which is central to photosynthetic carbon assimilation in both C3 and C4 species) was at first reported to require 11 mM bicarbonate in order to attain half-maximal velocity (45). At neutral pH and 20 C, 11 mM bicarbonate is in equilibrium with about 6% CO_2 in the gas phase (37), so that there was apparently an

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[3] Abbreviation: RBPCase: ribulose-1,5-bisphosphate carboxylase.

enormous discrepancy between the CO_2 affinity of the intact plant (14) and that of the isolated enzyme. This puzzled biologists for many years. Some doubted the wisdom of attempting to apply classic kinetic concepts to chloroplasts containing massive quantities of RBPCase. Others suggested that the low affinity of the enzyme might be related to the fact that it evolved in primeval atmospheres, rich in CO_2 (34). Only recently has it become apparent that the problem might not be so large as had been first imagined. In 1969, Cooper *et al.* (10) showed that the carboxylase utilizes CO_2 rather than bicarbonate and, though this important observation did not, in itself, offer a solution, it allowed the value of 22 mM by then accepted as the K*m* (bicarbonate) to be recalculated as 1.8% CO_2 in the gas phase (41). In addition, Sugiyama *et al.* (36) extended the earlier work of Pon *et al.* (30) and found that the K*m* (bicarbonate) in higher Mg concentrations was 5.6 mM. Subsequently, large responses to increased Mg were observed during work on chloroplast extracts (21, 25, 40, 41) and there have been reports of increased activity in the presence of fructose-6-P (7) and of 6-P-gluconate (8). Multiple forms of RBPCase have also been proposed by Bahr and Jensen (3) including one with an apparent K*m* of 0.5 to 0.8 mM bicarbonate (11–18 μM CO_2) which is "comparable to the values exhibited by intact chloroplasts during photosynthesis." The problem is not entirely one of affinity; it is also a question of maximal velocity and quantity of enzyme present. Thus the "low K*m* form" of Bahr and Jensen (3), which gave a rate of 24 μmoles mg^{-1} Chl hr^{-1} in 9 μM CO_2, was slightly inferior in performance to an "intermediate K*m* form" (with lower CO_2 affinity but a higher V_{max}) which gave a rate of 27 μmoles mg^{-1} Chl hr^{-1} at the same CO_2 concentration. The quantity of enzyme present has been acknowledged since it became clear that fraction I protein, which often accounts for more than 50% of leaf protein, is largely, if not entirely, RBPCase (23). On the other hand, the over-all lack of activity has frequently been a matter for concern and has led to speculation about active transport of CO_2 (12, 43, 46).

This communication extends our own previous observations (25, 40, 41) and those of Jensen and Bahr (3, 21) and shows that if the carboxylase is assayed (26) under conditions which are probably nearer to those which obtain *in vivo*, both the K*m* and the V_{max} are more in accord with the behavior of the intact chloroplast and the parent tissue.

MATERIALS AND METHODS

Spinach. *Spinacia oleracea* (True Hybrid 102, Arthur Yates & Co., Sydney, Australia [the same as American Hybrid 424]) was grown in water culture under natural light augmented by mercury and tungsten lamps. Illumination was restricted to 8 hr to suppress induction of flowering, and the day temperatures varied between 25 to 35 C.

Chloroplasts. These were isolated from freshly harvested leaves in sorbitol-pyrophosphate media using conventional techniques (9). The percentage of intact chloroplasts in each preparation was determined by its ability to evolve O_2 in the presence of ferricyanide before and after osmotic shock (19). Where necessary, chloroplasts were shocked in the reaction vessels by adding chloroplasts to dilute buffer prior to the other additives.

Chloroplast Extract. This extract was prepared by osmotic shock of intact chloroplasts (25) and was stored at 0 C in the presence of 5 mM dithiothreitol. The volume of chloroplast extract released from chloroplasts of measured Chl content allowed measured RBPCase activity to be expressed on a Chl basis.

Oxygen Evolution. Oxygen evolved by chloroplasts was measured polarographically using a twin-channel Clark-type electrode (11).

Illumination (11). Chloroplasts and leaf discs were illuminated by 150-w quartz iodine slide projectors. The light beam was passed through 15 cm of water and a Balzer Calflex C interference filter to give white light, mainly in the wavelength range of 400 to 750 nm at an irradiance of 830 w m^{-2}. Except where indicated in Table II, a red perspex filter (I.C.I.400) was also inserted to give light in the wavelength range 590 to 750 nm range at an irradiance of 300 w m^{-2}.

Carbon Dioxide Uptake. Circular discs of 10 cm^2 area were cut from spinach leaves with a sharp punch and illuminated in an open differential gas analysis system, type 225 (Analytical Development Co., Salisbury Road, Rye Park, Hoddesdon, Herts.). Air at 20 to 21 C containing 0.0305% CO_2 was passed over the discs at 1 liter/min and rates of apparent photosynthesis were calculated from the observed decrease in CO_2 concentration in the steady state.

RBPCase. An improved spectrophotometric assay (26) was used in which the reduction of newly formed 3-P-glycerate is coupled to NADH oxidation. Essential features (see "Reaction Mixtures" below) include the presence of an ATP regenerating system, preincubation with CO_2, and high concentrations of $MgCl_2$, and initiation of the reaction by ribose-5-P. Simultaneous measurements made with $^{14}CO_2$ have shown that the optical assay gives a true measure of carbon fixed and that ribose-5-P gives better rates than commercial ribulose-1,5-bisphosphate.

Chlorophyll. Chlorophyll was extracted from chloroplasts or leaf discs in 80% acetone and measured according to Arnon (1).

Reaction Mixtures. All reaction mixtures were prepared and used at 20 C and contained 0.33 M glucitol and 50 mM HEPES together with the further additions listed below. Mixtures B and C were prepared from bicarbonate free solutions and stored under N_2.

A: Ferricyanide-dependent O_2 Evolution. At pH 7.6 and in final volume of 2 ml, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 1.5 mM $K_3Fe(CN)_6$, 2.5 mM NH_4Cl, chloroplasts (100 μg of Chl), and 10 mM D,L-glyceraldehyde (to inhibit CO_2 assimilation, see ref. 35).

B: CO_2-dependent O_2 Evolution. At pH 7.6 and in a final volume of 2 ml, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 5 mM $Na_4P_2O_7$, and 0.5 mM Pi, chloroplasts (200 μg of Chl), and bicarbonate calculated from the Henderson-Hasselbach equation to give the CO_2 concentrations indicated in Figures 1 and 2. For Table I, 10 mM bicarbonate (equivalent to 584 μM CO_2 at pH 7.6) was used.

C: Ribulose Bisphosphate Carboxylation (26). At pH 7.9 (20) and in a final volume of 0.5 ml, chloroplast extract equivalent to 2.5 μg Chl, 10 mM KCl, 15 mM $MgCl_2$, 1 mM EDTA, 1 mM NADH, 5 mM dithiothreitol, 5 mM ATP, 5 mM phosphocreatine, creatine phosphokinase (1 activity unit, Sigma), NAD-glyceraldehyde-3-P dehydrogenase and P-glycerate kinase (2.5 units each, Sigma), 1 mM ribose-5-P, and $NaHCO_3$ as specified in Figure 3. For Table I the bicarbonate in this assay was constant at 10 mM (equivalent to 302 μM CO_2 at pH 7.9).

RESULTS AND DISCUSSION

The photosynthetic activities of intact chloroplasts, and of RBPCase in extracts prepared from the same chloroplasts, were measured in parallel experiments and compared over a wide range of CO_2 concentrations (Fig. 1). The carboxylase was assayed at pH 7.9 because the pH of the stroma compart-

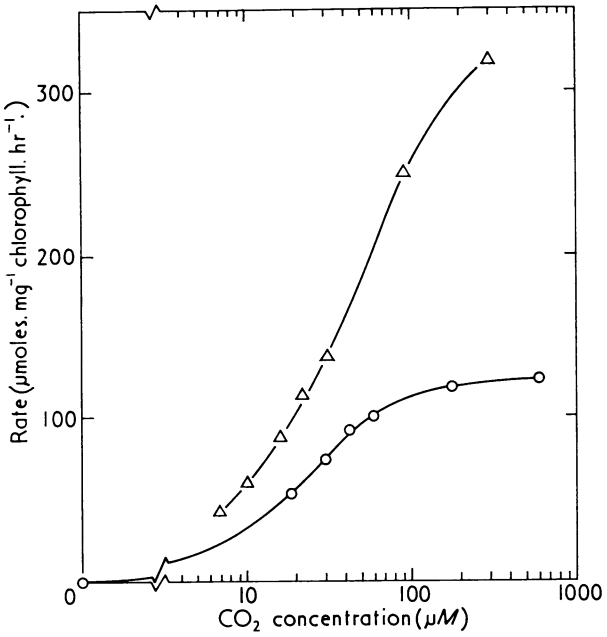

FIG. 1. Effect of CO_2 concentration on CO_2-dependent O_2 evolution by intact (68%) chloroplasts (○) and on RBPCase activity (△) in an extract from the same chloroplasts.

ment of intact chloroplasts is about this value when the chloroplasts are illuminated in pH 7.6 medium (20). The measured CO_2-dependent O_2 evolution by the intact chloroplasts is equivalent to CO_2 fixation under the conditions employed (42). The activity of RBPCase was considerably higher than the photosynthetic activity of the chloroplasts at each concentration of CO_2 used, including that approximating to the CO_2 concentration in solutions in equilibrium with air (11.5 μM in 0.33 M sugar at 750 mm Hg pressure and 20 C). Such values are usually calculated from the solubility coefficient of CO_2 in pure H_2O (0.878 ml CO_2/ml H_2O at 760 mm Hg pressure and 20 C) (37). The solubility coefficient is slightly affected by the large amounts of sugar present as osmoticum in chloroplast reaction mixtures, and for all calculations we have used the value 0.796 ml CO_2/ml 0.33 M sucrose (at 760 mm Hg pressure and 20 C), interpolated from the data of Usher (38). Equilibrium between dissolved CO_2 and bicarbonate in reaction mixtures containing chloroplast extracts can be assumed because of the very high activity of carbonic anhydrase in such extracts (3, 46).

The averaged data of Table I show that the activity of RBPCase in chloroplast extracts at high CO_2 concentrations exceeded the rate of ferricyanide-dependent O_2-evolution (Hill reaction) exhibited by the chloroplasts after rupture by osmotic shock and in the presence of uncoupling concentrations of NH_4Cl. The uncoupled rate of electron transport from H_2O to ferricyanide exhibited by envelope-free chloroplasts is somewhat faster than the coupled rate of transport from H_2O to NADP in intact chloroplasts and therefore constitutes a measure of the upper limit imposed on CO_2 fixation by the primary photochemical events. Even when allowance was made for the proportion of ruptured chloroplasts in the preparations, the average rate of CO_2-dependent O_2 evolution by the intact chloroplasts at saturating CO_2 concentration was substantially lower than the upper limit set by electron transport to ferricyanide. Conversely, RBPCase activity was always much higher than the rate of electron transport (Table I). In short, RBPCase *in situ* cannot manifest its full potential for carboxylation at high CO_2 concentrations, because reduction of the carboxylation product, and hence regeneration of the CO_2 acceptor, is appreciably slower than the maximum rate of CO_2 fixation which would be theoretically possible if these constraints were lifted. If the true maximum is not attained, the CO_2 concentration required to support a rate of half the apparent maximum velocity will be correspondingly smaller. For the same reason, reciprocal plots of the photosynthetic activity of intact chloroplasts against CO_2 concentration (Fig. 2) are nonlinear at high CO_2 concentrations. (Hatch and

Table I. *Comparison of Ribulose Bisphosphate Carboxylase Activity with Chloroplast Photosynthetic Activity*

	Mean ± SEM μmoles CO_2 or O_2 mg^{-1} Chl hr^{-1} for 14 Preparations	Mean Corrected to 100% Intact Chloroplasts
Ribulose bisphosphate carboxylase activity in chloroplast extracts	426 ± 22	573[1]
Whole chloroplasts, rate of CO_2-dependent O_2 evolution	134 ± 4	180
Chloroplasts ruptured by osmotic shock and uncoupled, rate of ferricyanide-dependent O_2 evolution	266 ± 11[2]	

[1] All of the results listed relate to measurements at 20 C. The carboxylase has a normal Q_{10} of about 2. The corresponding value for the spinach carboxylase obtained in 1961 by Peterkofsky and Racker (29) was adjusted by them, assuming a Q_{10} of 2, to 156 at 20 C. More recent values (4) for six C4 and six C3 species fall in the range of 60 to 252 if adjusted to 20 C on the same basis.

[2] These rates are substantially higher than many in the literature (*e.g.* 32) and that the ceiling which they impose (see text) is therefore also relatively high.

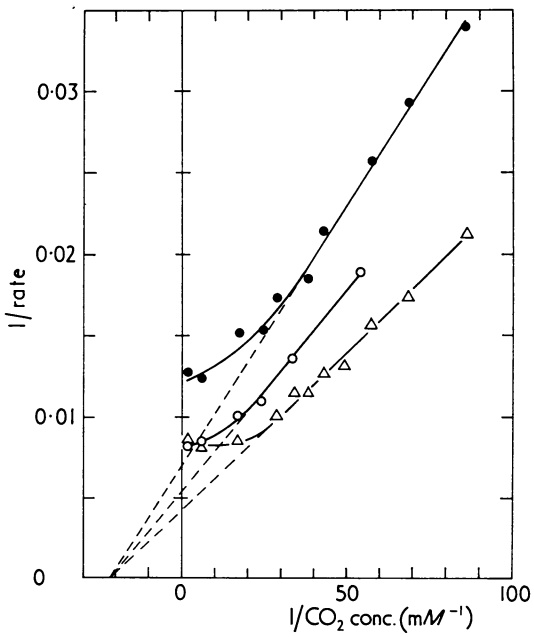

FIG. 2. Reciprocal plots of rates of CO_2-dependent O_2 evolution (μmoles of O_2 mg^{-1} of Chl hr^{-1}) against CO_2 concentration, for three chloroplast preparations containing 62% (●), 69% (○), and 66% (△) intact chloroplasts. A linear rate of O_2 evolution was recorded when an appropriate amount of $NaHCO_3$ was added to the reaction mixture following 3-min preillumination.

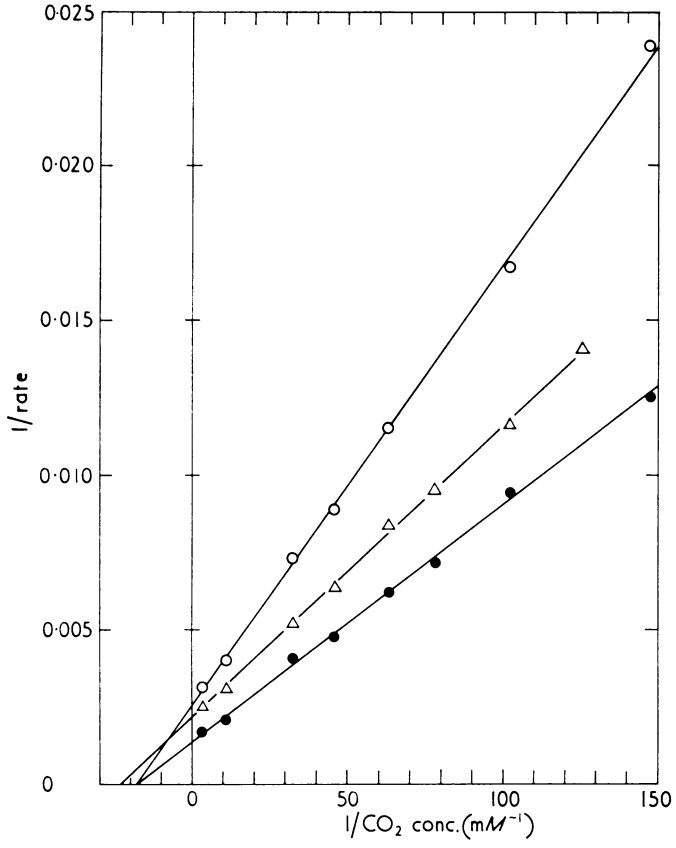

FIG. 3. Reciprocal plots of rates of CO_2 fixation (μmoles of CO_2 mg^{-1} of Chl hr^{-1}) by RBPCase in chloroplast extracts against CO_2 concentration. Data for enzyme activity in extracts from 3 chloroplast preparations containing 81% (●), 69% (○), and 76% (△) intact chloroplasts. From the reciprocals of the extrapolated intercepts, the following values were obtained: V_{max} (μmoles CO_2 fixed mg^{-1} Chl hr^{-1}): 758 (●), 392 (○), 463 (△). Apparent K*m* (CO_2): 58.5 μM (●), 56.5 μM (○), 43.5 μM (△). Equivalent values for K*m* (bicarbonate) at pH 7.9: 1.88 mM (●), 1.82 mM (○), 1.40 mM (△). The V_{max} values become 947, 671, and 517 if allowance is made for the envelope-free chloroplasts present in the original extract.

Slack [17] have already drawn attention to the fact that the relationship between bicarbonate concentration and activity of intact chloroplasts is not typical of enzyme kinetics.) However, the linear portions of each plot can reasonably be extrapolated to a common intercept corresponding to an "apparent K*m* (CO_2)" of 46.5 μM (about four times the CO_2 concentration in air-saturated assay medium and equivalent to 0.75 mM HCO_3^- at pH 7.6). This value lies well within the range of 43.5 to 58.5 μM for the apparent K*m* (CO_2) of the soluble enzyme, which gave a linear relationship in a double reciprocal plot of rate against substrate concentration (Fig. 3). It strongly suggests that there is no real difference between the affinity of the chloroplast for CO_2 and the affinity of the soluble enzyme for CO_2, whereas previous reports have stated or implied that the intact organelle has a much higher affinity than the carboxylase (3, 7, 22). In short, the enzyme in the functioning chloroplast seems to display characteristics (Fig. 2) which are very similar to those exhibited by the isolated enzyme (Fig. 3). Figure 2 also underlines the undesirability of ever applying the term "K*m*" to the CO_2 concentration at which a chloroplast suspension or leaf assimilates CO_2 in the light at half maximal velocity. It is obviously important that the concentration which gives half maximum velocity should be determined but if it is described as a "K*m*" (or an "affinity" in the sense of 1/K*m*) it inevitably carries the implication that the maximum rate of carboxylation by the leaf or organelle is itself governed by some intrinsic characteristic of the carboxylase. As Table I shows, this is most unlikely because the rates attained by the carboxylase in high CO_2 are so much faster than electron transport.

The V_{max} values, derived from the vertical intercept in Figure 3, were in the range 392 to 758 μmoles CO_2 fixed mg^{-1} Chl hr^{-1} or 516 to 947 when allowance was made for the presence of ruptured chloroplasts (which contribute to the Chl estimate but do not release significant quantities of carboxylase on osmotic shock). The enzymic activity in chloroplast extracts appeared to be stable and did not exhibit significant change in activity or affinity for CO_2 during storage for up to 4 hr at 0 C (*cf.* ref. 30).

Bahr and Jensen (3) have reported that an unstable kinetic form of RBPCase with apparent K*m* (CO_2) of 11–18 μM could be detected if the enzymic activity was assayed immediately after osmotic shock of intact chloroplasts. This "low K*m*" form of the enzyme was stabilized by including ribose-5-P, ATP, and $MgCl_2$ in the hypotonic medium in which the chloroplasts were ruptured, and also exhibited 3.5- to 4-fold lower maximum velocity than an "intermediate K*m*" form of the enzyme which was observed when untreated chloroplast extract was preincubated in $MgCl_2$ and bicarbonate. Measurements by the assay used here (26) of RBPCase activity in similar chloroplast extracts (prepared by osmotic shock in medium containing 2.5 mM ribose-5-P, 3 mM ATP, and 25 mM $MgCl_2$), confirmed a decrease in activity at all CO_2 concentrations, due to inactivation (30) by the RBP formed from these additives. However, the resulting kinetics, unlike those of the untreated enzyme, were complex, and their interpretation in terms of enzyme function does not seem prudent at this time (26). The "intermediate K*m*" form of the enzyme described by Bahr and Jensen (3) appears to have similar kinetic properties to those described here.

Comparisons between the performance of intact leaves and isolated enzymes are notoriously difficult to make and any conclusions drawn must therefore be treated with great caution. Nevertheless, at a time when the K*m* of the carboxylase was put at 22 mM bicarbonate and the fastest measured rates at 20 C (with saturating CO_2) were believed to be in the region of 150 μmoles mg^{-1} Chl hr^{-1}, it seemed inescapable that the parent tissue must operate some form of active or facilitated transport of CO_2 (12, 43). Our present results do not in any way rule out this possibility, but they do bear on the question of whether or not there is a theoretical necessity to invoke such a mechanism in order to account for the rates of photosynthesis displayed by intact tissues or organelles. Unfortunately, the accurate measurement of true photosynthesis in leaves is no easier than the measurement of the isolated carboxylase. Allowances have to be made for photorespiration and "dark" respiration and interpretation of published values may be complicated by the increases in leaf temperature which sometimes follow irradiation with saturating light. Our own experience leads us to believe that, although spinach can reach high rates of apparent photosynthesis at high temperatures and in high CO_2 concentrations (22), its behavior in air at 20 C is not far removed from the average value of 100 μmoles mg^{-1} Chl hr^{-1} already mentioned. For example, when large discs were taken from the same tissue as those used for the preparation of active chloroplasts (*cf.* refs. 2, 27), they gave rates of CO_2 uptake of about 80 μmoles mg^{-1} Chl hr^{-1} when illuminated with saturating white light in air at 20 C (Table II), and under these conditions the correction for respiration and photorespiration would not be

expected to be large. Similar, or slightly lower, values for spinach have been previously recorded for temperatures in the 20 to 25 C range (33, 47) (see also refs. 2 and 27 concerning earlier comparisons between chloroplasts and their parent tissue).

It is relevant to ask what concentration of CO_2 would be required at the carboxylation site in order to maintain such rates, if the enzyme *in situ* behaves in the same way as it does in the assay medium. For a rate of fixation of 100 μmoles mg^{-1} Chl hr^{-1}, the concentration required by the carboxylase preparations used in Figure 3 ranged between 7 and 11.8 μM CO_2 (when the rates were corrected for the proportion of broken chloroplasts in each preparation). This CO_2 concentration range, equivalent to 0.020 to 0.034% CO_2 in the gas phase, would therefore be required in the stromal compartment of the chloroplast if the leaf is to achieve a rate of true photosynthesis of 100 μmoles CO_2 fixed mg^{-1} Chl hr^{-1}. This may be compared to the concentrations of about 0.03% CO_2 in the air outside the leaf and of approximately 0.010 to 0.018% CO_2 in the intercellular spaces within the leaf under similar conditions (18, 28). Even though the present apparent K*m* (Fig. 3) is still four to five times the concentration of CO_2 in air, this would be sufficient, when combined with maximal velocities of the order of 1000 μmoles mg^{-1} Chl hr^{-1}, to account for the observed rates of photosynthesis by the parent tissue. Although the best of our measured rates (*e.g.* V_{max} of 942 μmoles mg^{-1} Chl hr^{-1} in Fig. 3) approached this value, there is no doubt that photosynthesis would be favored either by facilitated diffusion of CO_2 along a pathway of very low resistance from the exterior or by active transport from the intercellular spaces to the chloroplast such that the relative CO_2 concentration in the stroma was increased by a factor of 2 to 3.

Alternatively, it could be argued that, while our procedures undoubtedly permit the carboxylase to exhibit rates considerably faster than those previously reported, it is unlikely that an optimal assay has yet been devised or that separation of the enzyme from the leaf has been accomplished without loss. It seems increasingly likely that our estimates of the number of intact chloroplasts in a preparation may be too high because of rupture and self-sealing following the loss of stromal protein. If, for these reasons, the real performance of the enzyme *in situ* is only moderately superior to that reported here, any theoretical requirement for active transport or facilitated diffusion would be extremely difficult to sustain.

In air, some C3 species such as sunflower (see *e.g.* 13, 47) can photosynthesize much more rapidly than spinach or its hypothetical equal, the average plant. Whether they do so because they have more RBPCase or simply because they have leaves which offer a lower diffusive resistance to CO_2 remains to be established. With K*m* and V_{max} values similar to those reported here (or even with values substantially less favorable) RBPCase would be more than equal to its task in C4 photosynthesis, provided that all of the species concerned were able to maintain CO_2 in the bundle sheath at concentrations approaching those which have been reported for maize and *Amaranthus* (15).

CONCLUSIONS

New measurements indicate that for half-maximal photosynthesis the CO_2 requirement of the intact chloroplast is somewhat higher than previously supposed and that the affinity of RBPCase for CO_2 is not changed when it is released from the chloroplast. Improved assay procedures yield values of the apparent K*m* (CO_2) and V_{max} which would make the carboxylase in C3 plants potentially equal to its task *in vivo* at concentrations of CO_2 below those in the external atmosphere.

Table II. *Rates of Apparent Photosynthesis by Leaf Discs*

Disc	Fresh Wt	Chl	Rate of CO_2 Uptake			
			White[1]	Red[2]	White	Red
	g	*mg*	*mg dm^{-2} hr^{-1}*		*μmoles mg^{-1} Chl hr^{-1}*	
1	0.574	0.56	20.5	17.8	83.4	72.2
2	0.497	0.62	21.6	17.1	79.2	62.8
3	0.460	0.55	18.5	16.4	76.4	67.7

[1] White light in wavelength range 400 to 750 nm at an irradiance of 830 wm^{-2}.

[2] Red light in range 590 to 750 nm at 300 wm^{-2}.

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