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Light and CO_2 limitation of photosynthesis and states of the reactions regenerating ribulose 1,5-bisphosphate or reducing 3-phosphoglycerate

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Spinach leaves were illuminated at various temperatures or CO_2 concentrations until steady-state photosynthesis could be measured. Subsequently, they were frozen rapidly in liquid nitrogen and freeze-dried. From the dry material, chloroplasts were isolated in a mixture of organic solvents in which polar metabolites are insoluble. Metabolite levels were determined in the chloroplast fraction. From measured levels of dihydroxyacetone phosphate, fructose 6-phosphate (Fru-6-P), ribulose 1,5-bisphosphate (Rbu-1,5-P_2), ATP and ADP, mass-action ratios of the reaction dihydroxyacetone phosphate + 2 glyceraldehyde 3-phosphate + 3 ATP + Fru-6-P → 3 Rbu-1,5-P_2 + 3 ADP + P_i were computed. They increased at constant light intensity with increasing CO_2 concentration or increasing temperature as photosynthetic flux increased. Surprisingly, however, mass action ratios decreased as flux increased with increasing light intensities. Moreover, mass-action ratios were linearly correlated to light-limitation coefficients which were obtained by computing the light limitation of photosynthesis from the slopes of light and CO_2 response curves and multiplying obtained values with that increment of photosynthesis which was measured on increasing the light intensity to saturation. The results are interpreted to indicate tight enzymic control of the formation of ribulose bisphosphate by light. As light intensities are increased, light-regulated enzymes are activated to an extent which permits a decrease in the mass action ratios instead of the increase expected to drive increased carbon flux. Since the reactions catalyzed by phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase and triosephosphate isomerase are close to thermodynamic equilibrium even when photosynthetic fluxes are large, ratios of dihydroxyacetone phosphate to 3-phosphoglycerate indicated the state of chloroplast phosphorylation potentials and the redox state of NADP which together form the assimilatory power $[ATP] \cdot [ADP]^{-1} \cdot [P_i]^{-1} \cdot [NADPH] \cdot [NADP^+]^{-1}$. Assimilatory power decreased as carbon flux increased with increasing light intensity and increasing CO_2 concentration, but increased as carbon flux increased with increasing temperature. Again this indicates a decrease in the flow resistance of the carbon cycle as light or CO_2 is increased. The decrease in the flow resistance is attributed to enzyme activation when light is increased, or to increased carboxylation when CO_2 is increased.

Introduction

In photosynthesis, the activities of three major pathways which drain carbon from the triosephosphate pool have to be adjusted to one another. The reactions involved in the regeneration of ribulose 1,5-bisphosphate, in starch synthe-

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Abbreviations: P_i, inorganic phosphate; GAP, glyceraldehyde 3-phosphate; PGA, 3-phosphoglycerate; DHAP, dihydroxyacetone phosphate; Rbu-1,5-P_2, ribulose 1,5-bisphosphate; Fru-6-P, fructose 6-phosphate.

sis and in assimilate export from the chloroplasts are subject to regulation. In a simplified model of the Calvin cycle Woodrow et al. [1] have shown that at least one kinetic restriction has to be placed upon each of these pathways. Flux of carbon in the Calvin cycle is mainly controlled, at the level of enzymes, by chloroplast fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase, ribulose-5-phosphate kinase [2] and ribulose-1,5-bisphosphate carboxylase oxygenase [3]. Control in the pathway to starch is exercised by ADP-glucose pyrophosphorylase [4] and in the pathway to sucrose by cytosolic fructose-1,6-bisphosphatase [5]. From results obtained with spinach chloroplasts which had been isolated by nonaqueous means from leaves photosynthesizing under defined conditions it had been concluded that the reactions catalyzed by fructose-1,6-bisphosphate aldolase, 3-phosphoglycerate kinase and glyceraldehyde 3-phosphate dehydrogenase are near equilibrium. This was found to be true even at maximum rates of photosynthesis under CO_2 and light saturation [6]. In contrast, a constant ratio of fructose 1,6-bisphosphate to fructose 6-phosphate at both low and high CO_2 concentrations indicated that the irreversible reaction fructose 1,6-bisphosphate → fructose 6-phosphate which is catalyzed by chloroplast fructose 1,6-bisphosphatase was tightly regulated and tuned to the rate of Calvin cycle turnover. Similarly, the activation state of the ribulose 1,5-bisphosphate carboxylase is regulated in response to the photon fluence rate [7,8]. This makes it difficult to assess the relative extent of control exercised by the carboxylation reaction and the reactions involved in the regeneration of ribulose 1,5-bisphosphate on flux in the Calvin cycle. The slow kinetics of the deactivation of the carboxylase upon a decrease in the photon fluence rate has been used by Mott et al. [9] to investigate the relationship between the CO_2 fixation rate and the pool size of ribulose 1,5-bisphosphate in *Xanthium strumarium*. Only a transient rate-limitation by the reactions leading to the regeneration of ribulose 1,5-bisphosphate was apparent after a decrease in the light intensity. Subsequently, the activation state of the carboxylase was readjusted so that in the steady state of photosynthesis before and after lowering the light intensity the chloroplast level of ribulose 1,5-bisphosphate was very similar. These results gave some insight into the regulation of the Calvin cycle in vivo [6,9].

In this communication regulatory principles governing flux in the Calvin cycle are further examined. It is shown that there is an unexpected relationship between light limitation of photosynthesis and the state of the reactions which regenerate ribulose 1,5-bisphosphate from triosephosphates and fructose 6-phosphate. Furthermore, assimilatory power as reflected by the states of the chloroplast phosphorylation potential and the chloroplast NADP system often does not increase as photosynthetic flux increases, but may actually decrease with increasing flux.

Material and Methods

Spinach plants were grown in a green house (10 h light and 14 h dark period). Excised leaves were gassed with air and illuminated as indicated in the legends to the figures. The temperature of the leaves was controlled with a thermocouple. After 20 min of steady-state photosynthesis the leaves were rapidly frozen by pouring liquid nitrogen into the cuvette containing the leaves. The illumination was not interrupted during freezing. Floating of the leaves was prevented by a nylon net which held the leaves at the bottom of the cuvette. In the experiments to Figs. 2 and 4 the petioles of the leaves were supplied with water. Frozen leaves were freeze-dried for one week at $-30°C$. After homogenization of the dry material in nonpolar organic solvents chloroplasts were isolated nonaqueously as described by Heber and Willenbrink [10].

Chlorophyll was determined according to the method of Arnon [11]. Ribulose 1,5-bisphosphate and 3-phosphoglycerate [12], dihydroxyacetone phosphate [13], fructose 6-phosphate [14], ATP [15], and ADP [16] were determined after 30 min extraction of the nonaqueously prepared chloroplasts in 6% $HClO_4$ on ice. After sedimentation, aliquots of the extracts were neutralized with 5 M K_2CO_3 and 250 mM triethanolamine to about pH 7.6.

The measurements were performed at least in duplicate with an American Instrument Company DW 2 spectrophotometer in the dual-wavelength mode at 340 nm (measuring beam) and 400 nm

(reference beam). The assay temperature was 30°C.

CO_2 gas exchange was measured with an IRGA system (Binos, Leybold Heraeus, Hanau, F.R.G.) as described before [17]. Enzymes were purchased from Boehringer. Ribulose-1,5-bisphosphate carboxylase was prepared as described in Ref. 6.

Results

Rates of photosynthesis and regeneration of Rbu-1,5-P_2

Spinach leaves were subjected to various light, CO_2 and temperature regimes so that rates of photosynthesis were varied over a wide range. After reaching the steady state of photosynthesis, they were rapidly frozen in liquid nitrogen. The nonaqueous chloroplast fractions prepared from the freeze-dried leaves are thought to preserve the metabolite status of the chloroplasts at the time of killing the leaves. Metabolites were measured by standard procedures.

Figs. 1–4 show rates of CO_2 fixation (A), ratios of ATP to ADP (B), and pool sizes of ribulose 1,5-bisphosphate, fructose 6-phosphate and dihydroxyacetone phosphate (C), limitation of photosynthesis by light vs. limitation by CO_2 (D) and two further parameters R' and $\Lambda(E)$. R' describes the state of the ribulose 1,5-bisphosphate regenerating reactions, and Λ the residual flux potential of the Calvin cycle which is not used due to limitation of the system by light. Detailed definitions are given below. Conditions of photosynthesis can be characterized as follows: light intensities were varied at limiting CO_2 concentration (Fig. 1); light was varied at saturating CO_2 (Fig. 2); CO_2 was varied under high intensity illumination (Fig. 3); temperature was varied in the presence of saturating CO_2 under high intensity illumination (Fig. 4). Measured levels of metabolites were converted into concentrations under the assumption that 1 mg chlorophyll corresponds to a chloroplast volume of 30 mm^3 [18]. Glyceraldehyde 3-phosphate was assumed to be close to equilibrium with dihydroxyacetone phosphate (i.e., its concentration is 4.55% of the concentration of dihydroxyacetone phosphate). This assumption is justified in view of the observation that the maximum activity of triosephosphate isomerase which catalyses the conversion of dihydroxyacetone phosphate into glyceraldehyde 3-phosphate exceeds the required activity by a factor of 20 [19].

Figs. 1A–4A show rates of CO_2 fixation by spinach leaves under the conditions described above (lower curve). Rates increased when the light intensity was increased at low (Fig. 1A) or at high CO_2 (Fig. 2A), when the CO_2 concentration was increased (Fig. 3A), or when the temperature was increased (Fig. 4A). The maximum rate of CO_2 uptake which was measured upon increasing the light intensity to saturation under otherwise unchanged conditions is also shown in Figs. 1A–4A as the upper limitation of the hatched area which represents the photosynthetic potential not realized under the conditions of the substrate measurements. It can be seen that the increase in photosynthesis brought about by increasing the light intensity to saturation was small when CO_2 concentrations (Fig. 3A) or temperatures (Fig. 4A) were low. The changes in ribulose 1,5-bisphosphate concentration observed during changes in light intensity or CO_2 concentration were similar to those measured by Perchorowicz and Jensen [8], Badger et al. [21] and by Dietz and Heber [6]. With increasing temperature, the concentration of ribulose 1,5-bisphosphate decreased from 4.5 to 2.8 mM. This temperature change caused an increase in CO_2 fixation rate from 160 to 265 μmol per mg chlorophyll per h, and a considerable increase in the ATP to ADP ratio (Fig. 4B). The fructose 6-phosphate concentration decreased from 3.0 to 0.9 $mol \cdot m^{-3}$ and the dihydroxyacetone phosphate concentration from 0.91 to 0.70 $mol \cdot m^{-3}$ when the temperature was raised from 12° to 30°C.

A very simple approach was chosen to describe tentatively the status of the reactions regenerating ribulose 1,5-bisphosphate from dihydroxyacetone phosphate. The mass action ratio of the reaction

$$DHAP + 2\,GAP + 3\,ATP + Fru\text{-}6\text{-}P \rightarrow 3Rbu\text{-}1,5\text{-}P_2 + 3ADP + P_i \quad (1)$$

was calculated under omission of phosphate

$$R' = \frac{[DHAP][GAP]^2[ATP]^3[Fru\text{-}6\text{-}P]}{[Rbu\text{-}1,5\text{-}P_2]^3[ADP]^3} \quad (2)$$

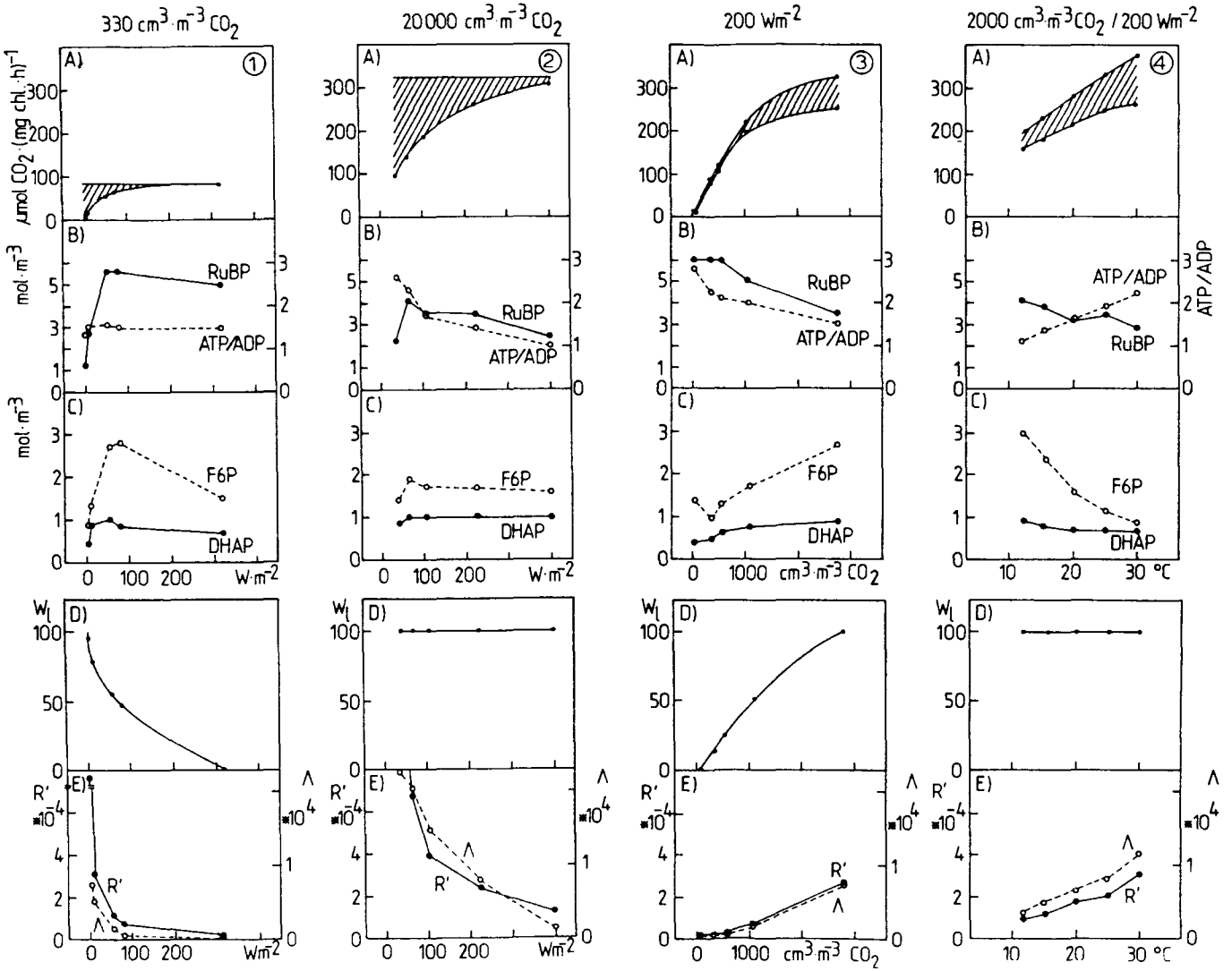

Fig. 1. Photosynthesis of spinach leaves in air as a function of light intensity. (A) Carbon reduction (lower curve). Upper line indicates maximum photosynthesis as observed when the light intensity was increased to saturation. (B) ATP/ADP ratios. (C) Substrate concentrations in the chloroplast stroma. (D) The sensitivity of photosynthesis to a change in light intensity W_l; for details see text. (E) Light limitation coefficient of photosynthesis Λ (in μmol CO_2 per mg Chl per h) and mass-action ratios of the reaction DHAP + 2 GAP + Fru-6-P + 3 ATP → 3 RBU-1,5-P_2 + 3 ADP + P_i; for details see text. R' is expressed in mM.

Fig. 2. Photosynthesis of spinach leaves at saturating CO_2 concentration as a function of light intensity. (A)–(E) as in Fig. 1.

Fig. 3. Photosynthesis of spinach leaves at constant light and temperature (200 W·m^{-2}; 25°C) as a function of CO_2 concentration. (A)–(E) as in Fig. 1.

Fig. 4. Photosynthesis of spinach leaves at constant light (200 W·m^{-2}) and saturating CO_2 as a function of leaf temperature. (A)–(E) as in Fig. 1.

Phosphate was omitted to avoid ambiguity. Concentrations of P_i did not decrease much with increasing photosynthetic flux in preparations of nonaqueous chloroplasts [6]. They were somewhat below 40 mM in the chloroplast stroma at 50 ppm CO_2 and 200 $W \cdot m^{-2}$ (net flux close to zero) and still 20 mM at 2750 ppm CO_2 (maximum flux). However, there is some uncertainty as to which extent contamination by vacuolar phosphate in the nonaqueous preparations leads to overestimation of chloroplast phosphate. In well-fertilized spinach, the main part of cellular phosphate resides in the vacuole. Maximal phosphate concentrations reported for isolated chloroplasts were well below those measured in nonaqueous chloroplast preparations (about 20 mM [20]). Even with phosphate omitted, and although two irreversible reactions (catalyzed by sedoheptulose-1,7-bisphosphatase and ribulose-5-phosphate kinase) are involved in the reaction sequence leading from dihydroxyacetone phosphate and fructose 6-phosphate to ribulose 1,5-bisphosphate, changes in R' were thought to give information on changes in the state of the reactions which generate ribulose 1,5-bisphosphate. An increase in the thermodynamic driving force of photosynthesis will result in increased carbon flux as long as the photosynthetic apparatus does not work at maximum capacity. At first sight, increased flux should be accompanied by an increased mass action ratio R'. Interestingly, Fig. 1E and Fig. 2E show that R' decreased when photosynthetic flux increased in response to increased light intensity. In contrast, R' increased when photosynthetic flux increased with increasing CO_2 at constant photon flux density (Fig. 3E) or in response to increasing temperature (Fig. 4E).

Figs. 1D–4D show the sensitivity of photosynthesis to changes in light intensity as compared to the sensitivity to changes in CO_2 concentration. The term W_l is used to describe sensitivity to light and relates the response of photosynthesis after a change in light intensity to the sum of the sensitivities to light and CO_2. It is defined by

$$W_l = \frac{100\, k \frac{dA}{dF_l}}{k\frac{dA}{dF_l} + \frac{dA}{dF_c}} \qquad (3)$$

where dA is an infinitesimal change in the rate of photosynthesis produced by either an infinitesimal change in light intensity dF_l or in CO_2 concentration dF_c, and k is a factor of correction in order to make photosynthetic responses to light and CO_2 comparable (for determination of W_l, see Ref. 17).

It is immediately apparent that light is the only limiting factor of photosynthesis in air at very low light intensities ($W_l = 100$). Above 300 $W \cdot m^{-2}$, photosynthesis no longer responds to light at air levels of CO_2 and W_l decreased therefore from 100 at low light intensities to zero at 300 $W \cdot m^{-2}$ (Fig. 1D). In the presence of saturating CO_2, i.e. $dA/dF_c = 0$, W_l has a constant value of 100 at various light intensities as can easily be seen from Eqn. 3 (Fig. 2D). Fig. 3D shows that at constant light intensity W_l increases with increasing CO_2 towards 100. In Fig. 4D, CO_2 is saturating, and therefore W_l is 100 at various temperatures. No relation between W_l and R' is apparent from a comparison of Figs. 1D–4D with Figs. 1E–4E. However, W_l only relates the light sensitivity of photosynthesis to the CO_2 sensitivity. It does not incorporate information on how far from maximum turnover the photosynthetic apparatus operates under various conditions. When W_l is multiplied with ΔA which is the increase in photosynthesis brought about by increasing the light intensity to saturation as shown in Figs. 1A–4A by the upper line limiting the hatched area, we can define a light-limitation coefficient

$$\Lambda = W_l \cdot \Delta A \qquad (4)$$

which describes how much of the capacity of the photosynthetic apparatus can still be realized when the light intensity is increased to saturation. When R' and Λ are compared, an interesting correlation becomes apparent. Only at very low light intensities (5 $W \cdot m^{-2}$, 330 ppm CO_2; 35 $W \cdot m^{-2}$, 20000 ppm CO_2), when ribulose 1,5-bisphosphate is far below the binding site concentration of ribulose 1,5-bisphosphate carboxylase, is R' much larger than Λ. Fig. 5 shows that the relationship between R' and Λ is linear. The regression coefficient is 0.95. The data reveal the surprising fact that as the gap is increased between the used capacity and the potential capacity for photosynthesis to respond to light, the ratio of substrates to products in the reaction sequence leading from triosephosphate

and hexosemonophosphate to ribulose 1,5-bisphosphate increases correspondingly.

Rates of photosynthesis and states of the chloroplast phosphorylation potential and the redox ratio of NADP

It has previously been shown that the enzymic capacity of the chloroplast triosephosphate oxidation system which catalyzes reduction of 3-phosphoglycerate in the light is so high that this reaction is not far displaced from equilibrium even when photosynthetic fluxes are large [6]. This permits calculation of the product between the chloroplast phosphorylation potential $[ATP]\cdot[ADP]^{-1}\cdot[P_i]^{-1}$ and the chloroplast NADPH/NADP ratio from measured concentrations of dihydroxyacetone phosphate and 3-phosphoglycerate. This product is termed assimilatory power P_A, a designation which has been introduced, in a somewhat different context, by Arnon et al. [22]. Table I defines P_A and compares calculated P_A values with rates of photosynthesis. It should be noted that calculated values are minimum values. Depending on how far the reaction

$$PGA + ATP + NADPH + H^+ \leftrightarrow DHAP + ADP + P_i + NADP^+ \quad (5)$$

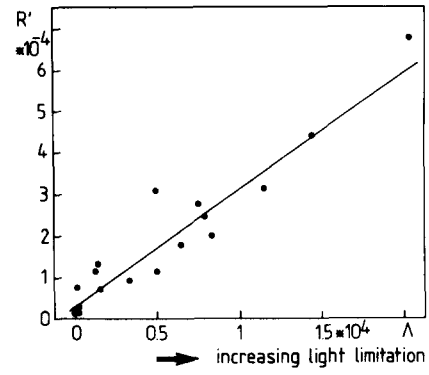

Fig. 5. Relationship between light limitation of photosynthesis as described by Λ and the state of the ribulose 1,5-bisphosphate regenerating reactions as described by the mass action ratio R'. Λ and R' data were taken from the experiments shown in Figs. 1–4. Regression analysis reveals $R' = 0.28\ \Lambda + 400$ ($r = 0.95$).

TABLE I

CONCENTRATIONS OF 3-PHOSPHOGLYCERATE (PGA) AND DIHYDROXYACETONEPHOSPHATE (DHAP), RATIOS OF PGA TO DHAP AND OF ATP TO ADP, AND VALUES DESCRIBING THE ASSIMILATORY POWER (P_A) UNDER VARIOUS CONDITIONS OF PHOTOSYNTHESIS

P_A was calculated under the assumption that the reactions involved in reduction of 3-phosphoglycerate to dihydroxyacetonephosphate are near thermodynamic equilibrium [6]. P_A of darkened chloroplasts is below 10 M^{-1}. For further explanations, see text.

$$P_A = \frac{[ATP][NADPH]}{[ADP][P_i][NADP^+]} = \frac{[DHAP]\cdot 9.8\cdot 10^{-6}}{[PGA]\cdot 1.585\cdot 10^{-8}}\ (M^{-1})$$

Conditions for photosynthesis			Rate of CO_2 uptake (μmol per mg Chl per h)	[PGA] (mM)	[DHAP] (mM)	[DHAP]/[PGA]	[ATP]/[ADP]	P_A (M^{-1})
Light intensity ($W\cdot m^{-2}$)	CO_2 concn. (ppm)	Temperature (°C)						
200	2000	12	160	9.88	0.91	0.092	1.13	57
200	2000	20	216	4.74	0.72	0.152	1.63	94
200	2000	30	265	2.82	0.70	0.248	2.25	153
35	20000	25	95	3.53	0.83	0.235	2.6	145
105	20000	25	180	4.43	1.0	0.226	1.7	140
400	20000	25	310	7.2	1.0	0.139	1.6	86
200	50	25	8	1.96	0.37	0.189	2.8	117
200	350	25	80	2.4	0.48	0.2	2.25	123
200	550	25	105	3.6	0.60	0.167	2.15	103
200	1100	25	200	6.8	0.68	0.10	2.0	62
200	2750	25	250	9.46	0.86	0.091	1.5	56

is displaced from equilibrium, real values will be higher than calculated values. Not surprisingly, assimilatory power increased as rates of photosynthesis increased with increasing temperature. However, it decreased with increasing light intensity or increasing CO_2 concentration, although rates of photosynthesis increased under these conditions. It has been observed before that the transthylakoid proton gradient, which is a driving force of ATP synthesis, decreased when rates of photosynthesis increased at increased CO_2 concentrations [34]. The highest values of P_A calculated from measured ratios of dihydroxyacetone phosphate to phosphoglycerate were about 150 (M^{-1}). This corresponds to a phosphorylation potential of 50 (M^{-1}), if the NADP system is 75% reduced, of 150 (M^{-1}), if it is 50% reduced, or of 450 (M^{-1}), if it is 25% reduced. Maximum phosphorylation potentials measured in intact chloroplasts were about 200–300 (M^{-1}) [23,24] and in broken chloroplasts 30000–80000 (M^{-1}) [24,25]. The large discrepancy between these figures is unexplained.

Discussion

Carbon flux from triosephosphates and fructose 6-phosphate to ribulose 1,5-bisphosphate

As long as turnover limitations do not enforce a ceiling, flux F in a biochemical reaction sequence is governed by the general flux equation

$$F = \Delta G / R \qquad (6)$$

where ΔG is the driving force and R the resistance to flux. R incorporates information on state and amount of enzymes. Changes in ΔG will predict changes in flux only when the resistance R is constant. However, enzyme modulation modifies R. Figs. 1 and 2 show that in low and high CO_2 concentrations carbon flux is increased with increasing light intensity, whereas the substrate ratio R' is decreased. This ratio may be considered to reflect ΔG. The conclusion is inevitable that with increasing light intensities the resistances within the reaction sequence are reduced to an extent that a reduction in the substrate ratio results even though flux is increased. Enzymes known to be regulated in the path to ribulose 1,5-bisphosphate are fructose 1,6-bisphosphatase, sedoheptulose 1,7-bisphosphatase and ribulose 5-phosphate kinase. Indeed, activation of these enzymes is reported to depend on the incident light intensity (for a review, see Refs. 2 and 26). Whereas R' decreases with increasing light intensity, it increases with increasing CO_2 concentration and with increasing temperature (Figs. 3 and 4). The increase in carbon flux observed under these conditions may be explained by an increased driving force ΔG as revealed by R'. Enzymes whose activity is modulated via a regulation of substrate affinity may show increased turnover although the substrate concentration is lowered. Sedoheptulose 1,7-bisphosphate is regulated in such a manner [27]. Ribulose 5-phosphate kinase is regulated by changes in the maximum activity at constant substrate affinity [35]. In the following we consider the role of sedoheptulose-1,7-bisphosphatase in the reaction sequence which regenerates ribulose 1,5-bisphosphate from triosephosphates and fructose 6-phosphate.

The influence of the ratio of sedoheptulose 1,7-bisphosphate to sedoheptulose 7-phosphate on the ribulose 5-phosphate concentration

If the reversible reactions involved in the regeneration of ribulose 1,5-bisphosphate from triosephosphates and fructose 6-phosphate were close to thermodynamic equilibrium the concentration of ribulose 5-phosphate can be calculated as a function of the ratio of sedoheptulose 1,7-bisphosphate to sedoheptulose 7-phosphate. Hydrolysis of sedoheptulose 1,7-bisphosphate is the only irreversible reaction in this reaction sequence. The sedoheptulose 1,7-bisphosphate to sedoheptulose 7-phosphate ratio is adjusted by sedoheptulose-1,7-bisphosphatase which is subject to light regulation. Fig. 6 shows ribulose 5-phosphate concentrations calculated for different ratios of sedoheptulose 7-phosphate to sedoheptulose 1,7-bisphosphate from the concentrations of dihydroxyacetone phosphate and fructose 6-phosphate which were measured in the chloroplast fractions prepared from leaves photosynthesizing in saturating CO_2, at 200 $W \cdot m^{-2}$ and 30°C. It can be seen that a change in the ratio of sedoheptulose 7-phosphate to sedoheptulose 1,7-bisphosphate by a factor of 10 causes a change in ribulose 5-phosphate concentration by a factor of only about 2. This suggests that fine regulation of sedoheptulose 1,7-bis-

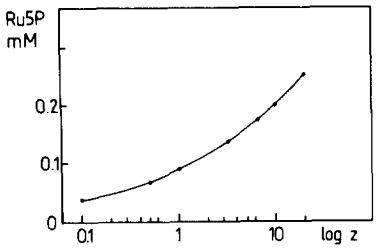

Fig. 6. Dependence of the ribulose 5-phosphate concentration on the ratio z of sedoheptulose-7-phosphate (S7P) to sedoheptulose-1,7-bisphosphate (SBP) at constant dihydroxyacetone phosphate (DHAP), glyceraldehyde-3-phosphate (GAP) and fructose 6-phosphate (Fru-6-P) concentrations. The calculation of the ribulose 5-phosphate concentration was based on the following assumptions: all reversible reactions in the reaction sequence are in thermodynamic equilibrium; $[E4P]\cdot[Xy5P]\cdot[F6P]^{-1}\cdot[GAP]^{-1} = 0.84$, $[E4P]\cdot[DHAP]\cdot[SBP]^{-1} = 8.1$ mM (in analogy to fructose-1,6-bisphosphate aldolase), $[S7P]/[SBP] = z$, $[Xy5P]\cdot[R5P]\cdot[S7P]^{-1}\cdot[GAP]^{-1} = 0.95$, $[Ru5P]/[R5P] = 0.3$, $[Xy5P]/[Ru5P] = 1.5$ (Barman, T.E., 1969) [33]; from these data [Ru5P] is calculated as $0.6667\sqrt[3]{4.433z\,[GAP]^2[DHAP][Fru\text{-}6\text{-}P]}$. Calculations are based on metabolite concentrations measured at 30°C, 2000 ppm CO_2 and 200 $W\cdot m^{-2}$, i.e., $[DHAP] = 0.68$ mM, $[Fru\text{-}6\text{-}P] = 1.5$ mM; $[GAP] = 0.0455\,[DHAP]$. E4P, erythrose-4-phosphate; Xy5P, xylolose-5-phosphate; Ru5P, ribose-5-phosphate.

phosphate is less critical in determining flux to ribulose 1,5-bisphosphate than regulation by ribulose-5-phosphate kinase. A sudden change in the rate of CO_2 fixation which is unaccompanied by an immediate change in the activation state of the sedoheptulose-1,7-bisphosphatase will cause drastic changes in the substrate ratio of sedoheptulose 7-phosphate to sedoheptulose 1,7-bisphosphate, but much smaller changes in ribulose 5-phosphate concentration.

Light-limited photosynthesis and the regeneration of ribulose 1,5-bisphosphate

By definition, limitation of photosynthesis by light is limitation by ATP and/or NADPH. ATP is consumed in the reactions catalyzed by 3-phosphoglycerate kinase and ribulose-5-phosphate kinase. The former is a reversible reaction. The same is true for the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase which consumes NADPH. Since reaction (5) is usually not far from the thermodynamic equilibrium [6]:

$$\frac{[PGA]}{[DHAP]}\,\frac{[ATP][NADPH][H^+]}{[ADP][P_i][NADP^+]} = \text{constant} = 9.8\cdot10^{-6} \quad (7)$$

changes in the ratio of 3-phosphoglycerate to dihydroxyacetone phosphate indicate changes in the state of both the chloroplast phosphorylation potential $[ATP]\cdot[ADP]^{-1}\cdot[P_i]^{-1}$ and the chloroplast NADP-system:

$$\frac{[DHAP]}{[PGA]}\,\frac{9.8\cdot10^{-6}}{[H^+]} \approx \frac{[ATP]}{[ADP][P_i]}\,\frac{[NADPH]}{[NADP^+]} = P_A \quad (8)$$

The components of assimilatory power P_A, phosphorylation potential $[ATP]/[ADP]\cdot[P_i]$ and redox ratio $[NADPH]/[NADP^+]$, can be measured separately [23,28]. The phosphorylation potential of isolated intact chloroplasts is about 40–60 (M^{-1}) in the dark and 200–300 (M^{-1}) in saturating light in the absence of ATP-consuming reactions [24]. The redox state of the chloroplast NADP system is very variable both in the dark and in the light [28,29]. Moreover, direct measurements after acidic or alkaline extraction include bound pyridine nucleotides, whereas the redox ratio of Eqns. 7 and 8 is the ratio of free and thermodynamically active pyridine nucleotides. It is therefore more appropriate to describe assimilatory power by the measured ratio of dihydroxyacetone phosphate to 3-phosphoglycerate as shown by Eqn. 8 than by adenylate and pyridine nucleotide measurements. Although it would be more correct thermodynamically to include the proton concentration of the chloroplast stroma in the redox ratio (i.e., $[NADPH]\cdot[H^+]/[NADP]$), for reasons of simplicity of expression it has been preferred in Eqn. 8 to consider (H^+) to be ocnstant under the conditions of our measurements and to combine it with the equilibrium constant of the triosephosphate oxidation reaction $9.8\cdot10^{-6}$. For light intensities between about 10 $W\cdot m^{-2}$ and light saturation this is justified in view of the large buffering capacity of the chloroplast stroma (about 30 $\mu eq.H^+/ml$ stroma per pH unit close to pH 8; see Ref. 30 and of the light-intensity characteristics of the transthylakoid proton gradient [31]. The pH of the stroma has been assumed to be 7.8 in the light ($[H^+] = 1.585\cdot10^{-8}$ (M^{-1})).

It is generally agreed that ATP and NADPH drive photosynthesis. Accordingly, assimilatory power should be expected to increase as rates of

photosynthesis increase. However, the experimental observations documented in Table I show that assimilatory power may, depending on photosynthetic conditions, either increase as photosynthetic flux increases or actually decrease with increasing flux. The former is observed when photosynthesis increases with increasing temperature, the latter, when photosynthesis increases with increasing light intensity or with increasing CO_2 concentration. Assimilatory power is produced by light, and there is no doubt that light drives photosynthesis. If assimilatory power declines as shown in Table I, although rates of photosynthesis increase when either the light intensity is increased at constant CO_2, or CO_2 is increased at constant light, validity of the flux equation (Eqn. 6) demands a decrease in the photosynthetic flux resistance R. As discussed above, a decrease in R is achieved by enzyme activation, when the light intensity is increased. When flux is increased with increasing CO_2 concentration and assimilatory power declines, the decrease in R is likely to occur mainly at the level of ribulose-1,5-bisphosphate carboxylase. An increase in CO_2 increases the carboxylation efficiency of this enzyme.

In terms of Eqn. 7, 3-phosphoglycerate assumes a role as a driving force of photosynthesis, when assimilatory power declines with increasing photosynthetic flux. This is also documented in Table I. Fast formation and accumulation of phosphoglycerate which originates from the irreversible carboxylation of ribulose 1,5-bisphosphate depends on fast regeneration of ribulose bisphosphate which is under the control of phosphoribulokinase and other regulated enzymes. These relations further illustrate the importance of enzyme regulation in photosynthesis. Depending on the state of enzyme activation, photosynthesis may be considered as a reaction driven by light or as a reaction consuming light. As Table I shows, assimilatory power drives carbon reduction, when an increase in temperature leads to increased rates of photosynthesis, but it is consumed by carbon reduction, when rates are increased by increasing light or CO_2.

In 1905, Blackman [32] applied the concept of limiting factors to the process of photosynthetic carbon assimilation. His axiom was that a process which is controlled by a number of separate factors will be limited only by the pace of the slowest one. He considered CO_2, water, light intensity, chlorophyll content and temperature as factors controlling photosynthesis. In this communication CO_2 concentration, light intensity and temperature are taken into account. The presented results give some insight into the complex regulatory mechanisms which are at work to ensure optimal functioning of the photosynthetic apparatus. These regulatory mechanisms explain why the concept of Blackman "minimum factor = limiting factor" fails over a wide range of factor concentrations in photosynthesis.

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