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Assimilatory power as a driving force in photosynthesis

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Intact spinach chloroplasts were permitted to photoreduce added 3-phosphoglycerate until oxygen evolution was replaced by oxygen uptake. The chloroplast suspensions were then analyzed for dihydroxyacetone phosphate and residual 3-phosphoglycerate. Ratios of dihydroxyacetone phosphate to phosphoglycerate served to calculate assimilatory power ([ATP]/[ADP][P_i]) × ([NADPH]/[NADP]). Extrapolation yielded maximum values of assimilatory power P_A of about 4000 (M^{-1}) as long as the chloroplasts continued to oxidize dihydroxyacetone phosphate via ribulose bisphosphate oxygenase. When the oxygen concentration was reduced to 25 μM, maximum values of P_A approached 25000 (M^{-1}) at light saturation of phosphoglycerate reduction. Maximum P_A declined as chloroplasts aged. When the light intensity was reduced, P_A decreased and intact chloroplasts oxidized dihydroxyacetone phosphate which had previously been exported into the medium. This observation explains the transient inhibition of photosynthesis of leaves after a sudden reduction in light intensity. Maximum P_A calculated for broken chloroplasts by multiplication of NADPH/NADP ratios and maximum phosphorylation potentials [ATP]/[ADP][P_i] measured separately in thylakoid suspensions at light saturation was as high as $2.5 \cdot 10^6$ (M^{-1}). The discrepancy between P_A in the stroma of intact chloroplasts and the values calculated for thylakoid suspensions is explained by inefficient cyclic electron flow which is incapable of raising phosphorylation potentials to high levels when NADP is reduced in chloroplasts. In leaves, maximum P_A in chloroplasts was even lower than in isolated chloroplasts. Turnover of ATP and NADPH in situ prevents P_A from reaching high levels even when net assimilation is zero. P_A was higher in leaves at low light intensities when carbon reduction was slow than at high light intensities when it was fast. This can explain the Kok effect. The apparent paradox that photosynthetic flux is increased as the driving force P_A is decreased is explained by regulation of enzymes of the Calvin cycle. Maximum rates of electron flow and phosphorylation and therefore also of photosynthesis are possible only when levels of P_A are kept low. Rapid use of P_A requires high activities of the enzymes of the Calvin cycle and may explain the necessity of enzyme activation.

Introduction

In photosynthesis, CO_2 enters into the reductive carbon cycle through the reaction catalyzed by ribulose bisphosphate carboxylase. The prod-

Abbreviations: PGA, 3-phosphoglycerate; P_i, inorganic phosphate; RuBP, ribulose 1,5-bisphosphate; DHAP, dihydroxyacetone phosphate.

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uct of the reaction, 3-phosphoglycerate (PGA), is first phosphorylated by light-generated ATP and subsequently reduced to the carbohydrate level by the light-generated reductant NADPH. The enzymes involved in three reactions are localized in the chloroplast stroma and have been shown to operate close to thermodynamic equilibrium which is

$$\frac{[PGA^{3-}]}{[DHAP^{2-}]}\,\frac{[ATP^{4-}]}{[ADP^{3-}][P_i^{2-}]}\,\frac{[NADPH][H^+]}{[NADP^+]} = K = 9.8\cdot 10^{-6} \quad (1)$$

[1,2]. Dihydroxyacetone phosphate (DHAP) is the triosephosphate formed from the primary product of reduction, glyceraldehyde phosphate. The stroma concentration of H^+ is higher in the dark than in the light and decreases on illumination owing to the pumping of protons both from the stroma to the intrathylakoid space [3] and to the external medium [4,5]. Eqn. 1 shows that the ratio of the carboxylation product phosphoglycerate to the carbohydrate dihydroxyacetone phosphate is determined by the product of the phosphorylation potential $[ATP]/[ADP][P_i]$ and the redox ratio of NADPH to NADP which is termed assimilatory power P_A [6] *. Maximum values of phosphorylation potential of illuminated intact chloroplasts were calculated from measured stroma levels of ATP, ADP and phosphate [7,8] to be between 200 and 500 (M^{-1}) and redox ratios of NADPH to NADP were between 2 and 9 [1,9]. Thus, maximum values of assimilatory power should be between 400 and 4500 (M^{-1}).

Actually, values deduced for leaves from measurements of phosphoglycerate and dihydroxyacetone phosphate in nonaqueously isolated chloroplasts were as low as 120 to 150 (M^{-1}), and thus on the lower side of the maximum calculated from adenylate, phosphate and pyridine nucleotide measurements for isolated chloroplasts [10].

Whereas available data indicate that intact chloroplasts are unable to adjust phosphorylation potentials to high levels, thylakoids liberated during chloroplast rupture are known to support high phosphorylation potentials. Kraayenhof [11] has observed phosphorylation potentials as high as 40000 (M^{-1}) and Giersch et al. [7] as high as 80000 (M^{-1}). The large discrepancies between maximum phosphorylation potentials calculated for leaves [2,10] and intact chloroplasts [7] on the one side and for broken chloroplasts [7,11] on the other side require explanation. It appears possible that part of the substrate measured after extraction had been bound inside the cells or organelles to proteins or other cellular constituents. This part would be unavailable to drive a reaction. In the present work, attempts are made to measure assimilatory power of intact chloroplasts so as to avoid this source of error which could render calculations of phosphorylation potentials or assimilatory power meaningless. We have then compared data from leaves, isolated intact chloroplasts and thylakoids and attempted to rationalize observed differences.

Material and Methods

Intact chloroplasts were isolated according to Jensen and Bassam [12] from spinach grown in a greenhouse under short-day conditions. They were illuminated with red light in an isotonic medium comprising 300 mM sorbitol/1 mM $MgCl_2$/1 mM $MnCl_2$/2 mM ethylenediaminetetraacetate/30 mM KCl/0.25 mM KH_2PO_4/50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate (pH 7.6, 7.9 or 8.3). 3-Phosphoglycerate was added to the suspension either before illumination (Fig. 4) or after residual CO_2/HCO_3^- of the medium had been reduced in the light (Figs. 1–3). Oxygen exchange was recorded by a Clark-type oxygen electrode [13]. Samples were withdrawn from the electrode vessel and injected into $HClO_4$ (final concentration, 0.35 M). After neutralization with K_2CO_3 and removal of $KClO_4$ and precipitated chloroplast material, 3-phoshpoglycerate and dihydroxyacetone phosphate were determined enzymatically in one assay by a combination of the

* Actually, Arnon et al. [6] used this term not as a ratio, but rather to describe formation of both ATP and NADPH by the electron transport chain. In Laisk's terminology, assimilatory power denotes the capacity of a leaf for carbon fixation [45]. Recently, Hill has drawn attention to different usages of the term power [46]. Strictly speaking, power is work per unit time. The ratio used by us should more properly be described as a force. For historical reasons, however, we prefer to retain the term coined by Arnon et al. [6].

procedures described by Michal and Beutler [14] and Czok [15]. Although the concentration of phosphoglycerate inside the chloroplasts was higher and that of dihydroxyacetone phosphate lower than the corresponding concentrations in the suspension medium [8], measured concentrations are practically identical with concentrations outside the chloroplasts, because chloroplast volumes were only about 0.5% of the volume of the medium. Light-dependent NADPH formation from 0.1 mM NADP by broken chloroplasts (30 μg chlorophyll/ml) was measured spectrophotometrically at 340 nm after addition of a small amount of ferredoxin. The medium was the same as that used for measuring phosphoglycerate and dihydroxyacetone phosphate, but contained also 10 mM $MgCl_2$/1.5 mM dithiothreitol/0.3 mM ATP/0.1 mM ADP/1 mM P_i (pH 7.9). Reduction of NADP was completed by adding 2 mM glucose 6-phosphate and 8 μg glucose-6-phosphate dehydrogenase/ml after the light-dependent reaction had come to a standstill.

Predarkened spinach leaves were illuminated in air containing 330 ppm CO_2. After different times of illumination with 200 $W \cdot m^{-2}$, or after having approached steady-state photosynthesis at different light intensities, they were rapidly frozen in liquid nitrogen, while illumination was continued. This minimized the danger of artifacts arising from changes in pool sizes of photosynthetic intermediates during freezing. After freezing, the leaves were freeze-dried. Chloroplasts were isolated non-aqueously from the dry material [2] and dihydroxyacetone phosphate and phosphoglycerate were determined in the chloroplasts. Biochemicals were obtained from Boehringer, Mannheim.

Results and Discussion

Phosphoglycerate-dependent oxygen evolution of intact chloroplasts

Fig. 1 shows oxygen evolution by intact spinach chloroplasts which were illuminated with rate-saturating red light in the presence of a low concentration of bicarbonate (about 0.2 mM). After CO_2-dependent oxygen evolution had declined because available CO_2 and bicarbonate had been largely exhausted, 0.6 mM phosphoglycerate was added. Reduction of phosphoglycerate according

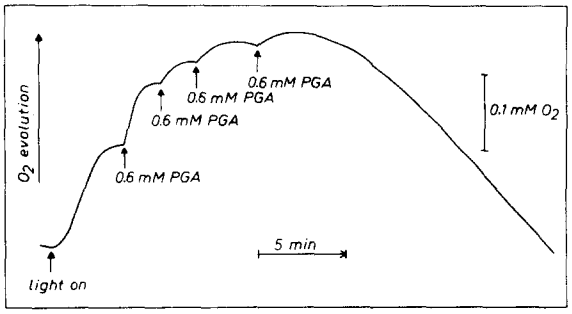

Fig. 1. Light-dependent oxygen exchange by intact spinach chloroplasts. When the decline of oxygen evolution indicated consumption of endogenous bicarbonate, 3-phosphoglycerate (PGA) was added in portions of 0.6 mM. Chlorophyll concentration, 150 μg/ml; pH, 7.6; light, 380 $W \cdot m^{-2}$

to

$$2\ PGA \rightarrow 2\ DHAP + O_2 \qquad (2)$$

restarted oxygen evolution. The rate of oxygen evolution declined before the stoichiometric amount of oxygen (0.3 mM) was released. Addition of another 0.6 mM phosphoglycerate again started oxygen release, but the amount of oxygen evolved was smaller after the second than after the first addition. Two further additions led to transient oxygen evolution which was followed by oxygen uptake which was light-dependent (not shown). As illumination was continued, more oxygen was consumed by the chloroplasts than had initially been evolved in the presence of substrate (not shown).

The observations are explained by the simultaneous occurrence of reactions involving oxygen production and oxygen consumption. Simplified versions of oxygen evolving reactions are given by

$$3\ CO_2 + 2\ H_2O + HPO_4^{2-} \rightarrow DHAP^{2-} + 3\ O_2 \qquad (3)$$

which describes the first phase of oxygen evolution in Fig. 1, and by Eqn. 2 which describes the second phase.

Oxygen uptake is catalyzed by RuBP carboxylase/oxygenase. The oxygenase reaction results in the oxidation of the dihydroxyacetone phosphate (and of other sugar phosphates) which had been formed by the reactions (2) and (3). Oxidation may be summarized as:

$$2\ DHAP + 1\tfrac{1}{2}O_2 \rightarrow 3\ \text{glycolate} + 2\ P_i \qquad (4)$$

It has been shown previously that sugar phosphates formed during photosynthesis are completely oxidized by isolated chloroplasts to glycolate and phosphate when the CO_2 supply is exhausted and oxygen is available [16]. Whereas only 1 mol oxygen is formed during reduction of two mol phosphoglycerate, $1\frac{1}{2}$ mol is consumed as the resulting dihydroxyacetone phosphate is oxidized to glycolate. This explains the observation that more oxygen is consumed than evolved in the experiment of Fig. 1. When oxygen is evolved, phosphate and phosphoglycerate enter the chloroplasts in exchange against dihydroxyacetone phosphate. During oxygen uptake, dihydroxyacetone phosphate is taken up in exchange for phosphate which is exported. Exchange is mediated by the phosphate translocator of the chloroplast envelope which is known to transport only divalent anions [17,18].

Relationship between maximum extrachloroplast ratios of dihydroxyacetone phosphate to phosphoglycerate and assimilatory power

When the phosphoglycerate reducing reaction reaches equilibrium which is described in Eqn. 1, the intrachloroplast ratio of dihydroxyacetone phosphate to the trivalent anion of phosphoglycerate is determined by available assimilatory power:

$$\frac{[DHAP^{2-}]\cdot 9.8\cdot 10^{-6}}{[PGA^{3-}][H^+]} = \frac{[ATP^{4-}]}{[ADP^{3-}][P_i^{2-}]}\,\frac{[NADPH]}{[NADP^+]} \qquad (5)$$

At equilibrium, substrate flux across the chloroplast envelope ceases. Flux equilibrium demands that the intrachloroplast ratio of dihydroxyacetone phosphate to divalent phosphoglycerate is identical with the extrachloroplast ratio of the same anionic species. Since

$$PGA^{3+} + H^+ \rightleftharpoons PGA^{2-} \qquad (6)$$

the Henderson–Hasselbach equation permits calculation of the relationship between the divalent and the trivalent phosphoglycerate anions. The pK of reaction (6) is 7.1. Calculation requires knowledge of pH. However, it is not necessary to know the pH inside the chloroplasts. It is sufficient to calculate the concentrations of the divalent and trivalent phosphoglycerate anions from measured total concentrations and the pH outside the chloroplasts. The product $[PGA^{3-}][H^+]$ is identical both inside and outside the chloroplasts, because the light-dependent decrease in the stroma concentration of H^+ is exactly balanced by an increase in the concentration of the trivalent phosphoglycerate anion.

Assimilatory power produced by intact isolated chloroplasts

Table I shows concentrations of phosphoglycerate and dihydroxyacetone phosphate measured in a chloroplast suspension which was illuminated with rate-saturating light in the presence of phosphoglycerate. Samples were taken some time after oxygen uptake had replaced oxygen evolution (see Fig. 1). From measured values, ratios of dihydroxyacetone phosphate to the trivalent phosphoglycerate anion were calculated.

Actually, determinations of the Michaelis constants of phosphoglycerate kinase for phosphoglycerate at different pH values gave no indication that the chloroplast enzyme used only the trivalent anion of phosphoglyceric acid as substrate. K_m values for total phosphoglycerate (PGA^{2-} plus PGA^{3-}) were 0.95 mM at pH 6.5 and 0.57 mM at pH 8 (data not shown). Still, as Eqn. 5 served as the basis for calculating assimilatory power, it was considered appropriate to use for the calculations PGA^{3-} instead of total phosphoglycerate. Since most measurements were performed at pH 7.9 or 8.3, the final result would not have been much different, if total phosphoglycerate had been inserted into equation (5) instead of PGA^{3-}.

Fig. 2 shows values of assimilatory power calculated from ratios of dihydroxyacetone phosphate to phosphoglycerate as measured after different times of illuminating the chloroplasts with 380 $W \cdot m^{-2}$ red light. The pH was 7.6 or 8.3. As in Fig. 1, the chloroplasts were first allowed to reduce available CO_2 for a few minutes before phosphoglycerate was added. At pH 8.3 phosphoglycerate was more easily measured than at 7.6 because its concentration did not decline as much as at pH 7.6. This is a consequence of the pH-dependence of the reaction catalyzed by glyceraldehydephosphate dehydrogenase (see Eqn. 1).

TABLE I

DIHYDROXYACETONE PHOSPHATE (DHAP) AND 3-PHOSPHOGLYCERATE (PGA) IN CHLOROPLAST SUSPENSIONS, WHICH HAD BEEN ILLUMINATED IN THE PRESENCE OF 3-PHOSPHOGLYCERATE

Measurements were taken after light-dependent O_2 uptake had replaced O_2 evolution. The volume of the external medium was large compared with the volume of the stroma so that chloroplast metabolites did not significantly influence the measurements. Data are from three different experiments.

Minutes after onset of illumination	DHAP (μmol/ml)	$PGA^{3-} + PGA^{2+}$ (μmol/ml)	$\frac{[DHAP]}{[PGA^{2-} + PGA^{3-}]}$	$\frac{[DHAP]}{[PGA^{3-}]}$
(A) Chlorophyll concn., 150 μg/ml; pH = 7.6; [PGA], initially 3.6 mM.				
38	0.59	0.17	3.47	4.57
41	0.695	0.165	4.21	5.54
44	0.61	0.115	5.3	6.97
48	0.60	0.10	6.0	7.89
53	0.52	0.07	6.94	9.13
(B) Chlorophyll concn., 200 μg/ml; pH = 7.6; [PGA], initially 1.2 mM.				
14	0.31	0.181	–	2.26
17	0.27	0.125	–	2.86
20	0.26	0.084	–	4.02
24	0.14	0.034	–	5.38
29	0.14	0.027	–	6.80
(C) Chlorophyll concn., 150 μg/ml; pH = 8.3; [PGA], initially 1.8 mM.				
27	0.239	0.377	–	0.67
30	0.217	0.282	–	0.81
33	0.245	0.283	–	0.91
37	0.239	0.215	–	1.17
42	0.232	0.181	–	1.36

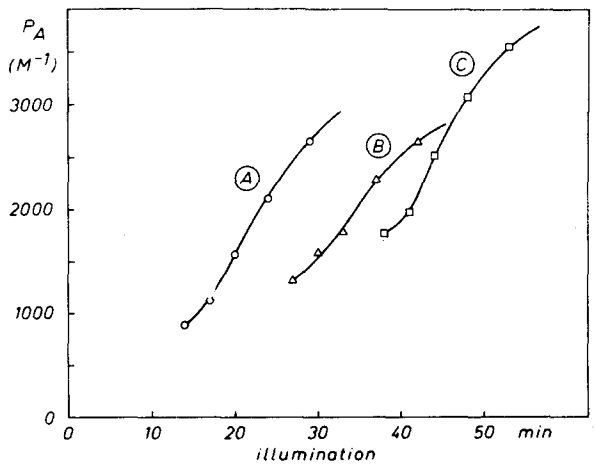

Fig. 2. Assimilatory power as calculated from ratios of dihydroxyacetone phosphate to the trivalent phosphoglycerate anion in suspensions of chloroplasts illuminated in the presence of different concentrations of phosphoglycerate at pH 7.6 or 8.3. The oxygen concentration was above 300 μM. Maximum values of assimilatory power may be obtained by extrapolation. They appear to be not far above 4000 (M^{-1}). (A) pH 7.6; (B) pH 8.3; (C) pH 7.6.

It should be noted that values of assimilatory power obtained by extrapolation of the data of Fig. 2 (about 4000 (M^{-1})) cannot be considered as maximum values. The latter can only be measured at light saturation and at reaction and flux equilibrium. No such equilibrium was reached. Light-dependent oxygen uptake indicating oxygenation of ribulose bisphosphate (see Fig. 1) is accompanied by ATP and NADPH turnover which will lower assimilatory power. Attempts were therefore made to inhibit oxygenation of ribulose bisphosphate by inhibiting formation of ribulose bisphosphate. Glyceraldehyde is known to block regeneration of ribulose bisphosphate in the Calvin cycle [19]. In its presence, the oxygen consumption seen after phosphoglycerate-dependent oxygen evolution had reached its peak (see Fig. 1) was indeed abolished indicating effective inhibition of ribulose bisphosphate oxygenation (data not shown). However, the amount of oxygen evolved from 1 mM phosphoglycerate decreased as the

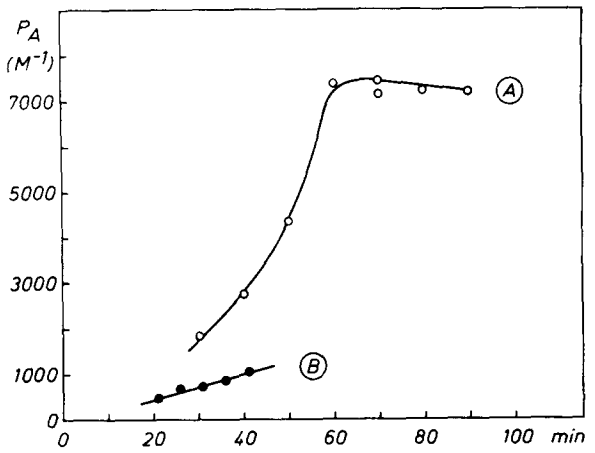

Fig. 3. Assimilatory power as calculated from ratios of dihydroxyacetone phosphate to the trivalent anion of phosphoglycerate in a suspension of chloroplasts illuminated with 380 $W \cdot m^{-2}$ red light at pH 7.9. Oxygen was close to 25 μM (curve A), or above 400 μM, with 5 mM D,L-glyceraldehyde (GA) also present (curve B).

level of glyceraldehyde was increased from 5 to 20 mM. In accordance with this observation, maximum ratios of dihydroxyacetone phosphate to phosphoglycerate were lower in the presence than in the absence of glyceraldehyde, and calculated assimilatory power was accordingly low (Fig. 3). Apparently, glyceraldehyde exerted effects on chloroplasts unrelated to its reported effects on the regeneration of ribulose bisphosphate. It may interfere with the translocation of dihydroxyacetone phosphate and phosphoglycerate by the phosphate translocator of the chloroplast envelope (Li and Gibbs, personal communication).

Reducing the oxygen concentration of chloroplast suspensions by gassing with N_2 is another means of minimizing oxygenation of ribulose bisphosphate. Fig. 3 shows values of assimilatory power as calculated from DHAP/PGA ratios in a chloroplast suspension whose oxygen content was kept at 25 μM. Complete anaerobiosis had to be avoided as it is known that in the absence of oxygen the electron-transport chain becomes overreduced [20]. Overreduction decreases the transhylakoid proton gradient and the phosphorylation potential. During illumination with 380 $W \cdot m^{2-}$ red light in the presence of low levels of oxygen, ratios of dihydroxyacetone phosphate to phosphoglycerate increased as should be expected. They reached a maximum which corresponded to a value of assimilatory power of 7000 (M^{-1}) (Fig. 3). At the chlorophyll concentration used for this experiment, the rate of phosphoglycerate-dependent oxygen evolution observed immediately after onset of illumination was about 80% of the rate of light-saturated oxygen evolution. When the light intensity was increased beyond light saturation, higher levels of assimilatory power were observed. Different chloroplast preparations yielded somewhat different results (compare Fig. 2A with 2C). The highest values of assimilatory power calculated from maximum DHAP/PGA ratios in suspensions of intact chloroplasts were between 20000 and 25000 (M^{-1}) (data not shown). Maximum assimilatory power observed at 20°C decreased as isolated chloroplasts aged during storage at 0°C. Under illumination saturating for phosphoglycerate-dependent oxygen evolution, it was 8000 (M^{-1}) 4 h after chloroplast isolation (exp. A in Fig. 4) and 5000 (M^{-1}) after 7 h (expt. B in Fig. 4). When the light intensity was decreased after maximum phosphorylation potentials had been established in rate-saturating light (illumination with two beams of 380 $W \cdot m^{-2}$ from two sides of a cuvette containing chloroplasts with 200 μg chlorophyll/ml, light-path 1 cm), ratios of dihydroxyacetone phosphate to phosphoglycerate decreased. After reducing the light intensity to 40 $W \cdot m^{-2}$ red light which supported 60% of a light-saturated rate of phosphoglycerate-dependent oxygen evolution immediately after the light was turned on, the maximum assimilatory power which is associated with minimum substrate turnover decreased from the 8000 (M^{-1}) observed under light saturation to about 1000 (M^{-1}) at low light (Fig. 4). Somewhat older chloroplasts (7 h storage in an ice bath) reduced assimilatory power from 5000 (M^{-1}) to 3000 (M^{-1}) when the light intensity was reduced so as to yield 80% of a light-saturated rate of phosphoglycerate-dependent oxygen evolution in a chloroplast suspension immediately after turning on the light.

It should be emphasized that assimilatory power as calculated in Figs. 3 and 4 represents maxima under conditions when metabolic turnover is very slow. Consumption of assimilatory power during fast turnover of photosynthetic substrates will decrease levels of assimilatory power even though

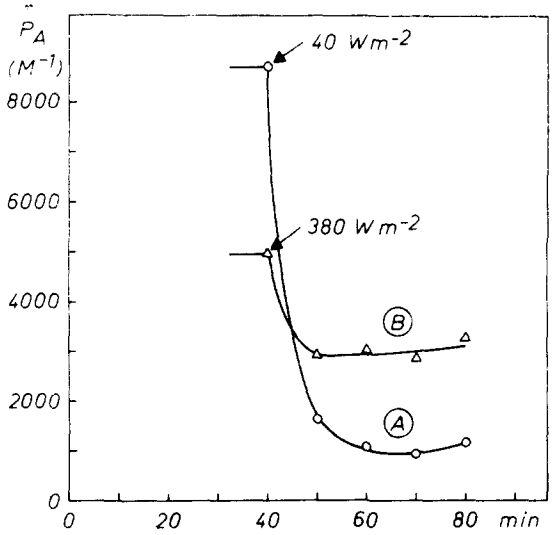

Fig. 4. Assimilatory power P_A as calculated from ratios of dihydroxyacetone phosphate to the trivalent anion of phosphoglycerate in suspensions of chloroplasts illuminated from two sides with beams of 380 $W \cdot m^{-2}$ red light at pH 7.9 and oxygen concentration of 25 μM. The chlorophyll concentration was 200 μg/ml, and the light-path 1 cm. When maximum P_A was reached after 40 min illumination, one beam was switched off and the other either reduced to 40 $W \cdot m^{-1}$ (curve A), or kept at an intensity of 380 $W \cdot m^{-2}$ (curve B). Experiments (A) and (B) were performed with the same batch of chloroplasts, but (B) was measured 3 h after (A). The difference in P_A between (A) and (B) after 40 min illumination is caused by chloroplast ageing.

the decreased levels are still fully competent to drive fast reduction in photosynthesis [10].

Oxidation of extrachloroplast dihydroxyacetone phosphate by intact chloroplasts during the transition from high-intensity to low-intensity illumination

As the light intensity was reduced in the experiments shown in Fig. 4, the direction of substrate flux across the chloroplast envelope was reversed. Dihydroxyacetone phosphate which had been exported from the chloroplasts during reduction of phosphoglycerate under high intensity illumination was taken up by the chloroplasts and oxidized inside to phosphoglycerate which was exported. This is illustrated in Table II which lists results of substrate measurements. Since not all dihydroxyacetone phosphate which disappeared from the chloroplast suspension gave rise to the export of phosphoglycerate, side reactions must also have occurred. Oxidation of dihydroxyacetone phosphate inside the chloroplasts results in the formation of NADPH and ATP. The pools of NADP and NADPH inside the chloroplasts (200 μg chlorophyll/ml) were less than 5 nanomol and of adenylates about 10 nanomol. The oxidation of about 100 nanomol dihydroxyacetone phosphate indicates turnover of NADPH and ATP. It is proposed that NADPH was oxidized by oxygen via reduced ferredoxin. The rate of this oxidation deduced from the decline of assimilatory power shown in Fig. 4 was about 3 μmol/mg chlorophyll per h or faster. The most likely fate of the ATP generated during oxidation of dihydroxyacetone phosphate was hydrolysis.

It should be noted that the adjustment of substrate levels shown in Table II can explain the transient inhibition of photosynthesis which is observed in leaves when the light intensity is suddenly decreased [21]. Obviously, as long as dihydroxyacetone phosphate is oxidized in response to a sudden lowering of assimilatory power instead of being produced by reduction, photosynthesis cannot continue.

Redox state of chloroplast NADP system and chloroplast phosphorylation potentials

Assimilatory power has the two components phosphorylation potential and redox ratio NADPH/NADP. Maximum values of assimila-

TABLE II

CONCENTRATIONS OF DIHYDROXYACETONE PHOSPHATE (DHAP) ANBD 3-PHOSPHOGLYCERATE (PGA) IN A CHLOROPLAST SUSPENSION (200 μg CHLOROPHYLL/ml) WHICH HAD BEEN ILLUMINATED WITH 1.0 mM PHOSPHOGLYCERATE IN THE PRESENCE OF 25 μM OXYGEN

After maximum reduction of phosphoglycerate had been reached under high intesnity illumination (2×380 $W \cdot m^{-2}$, t_0); the light intensity was reduced to 40 $W \cdot m^{-2}$. Concentration values are in μM.

Minutes after high-intensity illumination	DHAP	PGA	DHAP + PGA
0	182	19	201
10	126	69	195
20	91	76	167
30	81	79	160
40	94	73	167

tory power close to 20000 (M^{-1}) would indicate phosphorylation potentials close to 7000 (M^{-1}), if the redox ratio were close to 3. If 95% of the NADP were reduced, phosphorylation potentials would be close to 1000 (M^{-1}), and if reduction approached 98%, phosphorylation potentials would be 500 (M^{-1}), i.e., close to the values calculated from direct measurements of adenylates and phosphate. Measurements of NADPH and $NADP^+$ in the chloroplast stroma usually indicate maximum reduction at an NADPH/NADP ratio of 2 or 3, very rarely of 9. However, since pyridine nucleotides are known to bind to proteins [22], the significance of such ratios is in doubt. We have therefore measured the extent of light-dependent NDAP reduction by ruptured chloroplasts in the presence of ferredoxin. Reduction was monitored at 340 nm. When after illumination reduction appeared to be complete, glucose 6-phosphate and glucose-6-phosphate dehydrogenase were added. The further increase in 340 nm absorption was 2–4% of the increase brought about by light (Fig. 5). It is concluded that more than 96% of the available NADP was subject to reduction by light. Thus, maximum NADPH/NADP ratios are at least 25, not 2–3 as usually measured in intact chloroplasts. The discrepancy suggests that direct measurements of NADP and NADPH in intact chloroplasts do not give quantitative information on intrachloroplast redox ratios. Furthermore, binding of pyridine nucleotide to chloroplast constituents can now explain the observation that measured redox ratios of the chloroplast NAD system and of the chloroplast NADP system differ [9], although redox potentials are similar. Chloroplasts contain both NAD- and NADP-dependent glyceraldehydephosphate dehydrogenase which should be expected to equalize redox states of the NAD and the NADP systems.

If NADP reduction similar to that observed in the presence of thylakoids also takes place in intact chloroplasts, the data on assimilatory power obtained by the determination of dihydroxyacetone phosphate and phosphoglycerate outside illuminated intact chloroplasts indicate phosphorylation potentials inside the chloroplasts which are higher by a factor of 2 or 3 than those measured by adenylate and phosphate analysis in the chloroplast stroma. Differences between measured and calculated potentials are explained by substrate binding. Since adenylates of intact chloroplasts have been observed to be close to adenylate kinase equilibrium at very different states of the chloroplast adenylate system, adenylate binding is unlikely to be responsible for the differences [23]. A more likely candidate for binding is inorganic phosphate which may be less available inside intact chloroplasts than indicated after extraction with acid.

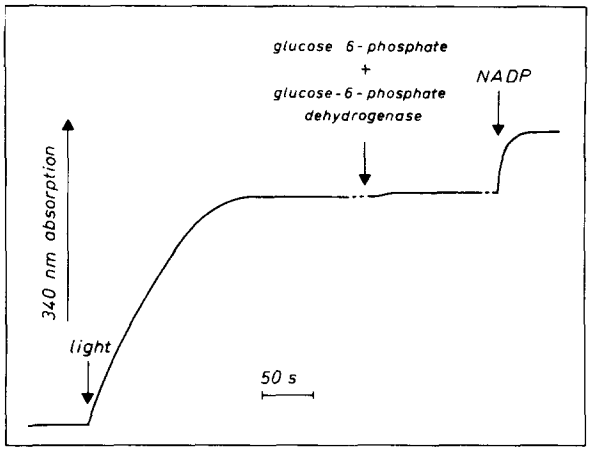

Fig. 5. NADP reduction by broken chloroplasts supplied with ferredoxin as indicated by 340 nm absorption of the chloroplast suspension. Light reduced added NADP almost completely. Little further reduction was brought about by glucose 6-phosphate and glucose-6-phosphate dehydrogenase.

Even the highest phosphorylation potentials indicated for intact chloroplasts by the ratios of dihydroxyacetone phosphate to phosphoglycerate are far below the maximum phosphorylation potentials measured in thylakoid suspensions by Kraayenhof [11] and Giersch et al. [7], who used phenazine methosulfate as a catalyst of cyclic photophosphorylation and ferricyanide or NADP as electron acceptors during noncyclic photophosphorylation. In recent unpublished work with methyl viologen, we have even measured phosphorylation potentials in excess of 100000 (M^{-1}). When maximum values are multiplied with an NADPH/NADP ratio of 25, assimilatory power becomes higher than $2.5 \cdot 10^6$ (M^{-1}). A comparison with the assimilatory power generated by intact chloroplast (about 20000 (M^{-1})) clearly shows

that electron transport and photophosphorylation are less effective in intact chloroplasts than in broken chloroplasts. There are two main possibilities to explain the large differences. One is that intact chloroplasts may contain a factor which controls electron flow or phosphorylation preventing dramatic increases of assimilatory power. On rupture, this factor would be released from the chloroplasts and diluted out. So far, attempts to demonstrate the existence of such a factor have been unsuccessful.

Another explanation is that as NADP is reduced, electron flow becomes so slow that loss of protons by passive leakage from the thylakoids competes successfully with coupled proton flux through CF_1, which results in ATP formation. As a consequence, high phosphorylation potentials can no longer be established. If this explanation is correct, it implies that cyclic electron flow is ineffective in intact isolated chloroplasts.

Usually, it is though that as NADPH rises in response to a shortage of ATP in photosynthesis (assimilation requires more ATP than NADPH), electrons are diverted into the cyclic electron-transport pathway, thereby raising ATP levels by cyclic photophosphorylation. Oxygen must be present to prevent over-reduction of the electron-transport chain which would tend to block electron flow [20]. If cyclic electron flow were effective in intact chloroplasts, it should raise phosphorylation potentials to values similar to those observed during cyclic photophosphorylation of thylakoids. As only low levels of assimilatory power are established in intact chloroplasts, it must be concluded that cyclic electron flow cannot be fast. The question then arises as to how sufficient ATP for photosynthetic reactions is provided. Intact chloroplasts are known to be capable of photoreducing glycerate. Reduction requires an ATP/2e ratio of somewhat more than 2. Carbon reduction in the Calvin cycle requires an ATP/2e ratio of 1.5 or somewhat more. In contrast the ATP/2e ratio of ATP production by coupled linear electron flow is usually considered to be 1.3 [24]. If cyclic electron flow and electron flow to oxygen are ineffective in intact chloroplasts, Q cycle mechanisms must increase the efficiency of ATP production in coupled linear electron flow to NADP [25,26].

Assimilatory power in chloroplasts in situ

Fig. 6 shows assimilatory power in the chloroplasts of predarkened leaves as a function of illumination time. On illumination, assimilatory power increased rapidly to a maximum of about 200 (M^{-1}). As CO_2 uptake accelerated within the first minute of illumination, assimilatory power decreased to about 100 (M^{-1}). A lag phase in photosynthesis is a common feature of photosynthesis induction both in isolated chloroplasts and in leaves [27,28]. A slow decrease in assimilatory power followed after the initial rapid decrease. It was related to the increase in photosynthesis brought about by slow stomatal opening. The data of Fig. 6 were calculated from stromal levels of dihydroxyacetone phosphate and phosphoglycerate under the assumption that the stroma pH was 7.8. Obtained values can be directly compared with data on assimilatory power which were communicated in a previous publication where the same assumption had been made [10]. If the stromal pH is not 7.8 but 8.1 in leaves exposed to high intensity illumination as recent measurements indicate [29], the values shown in Fig. 6

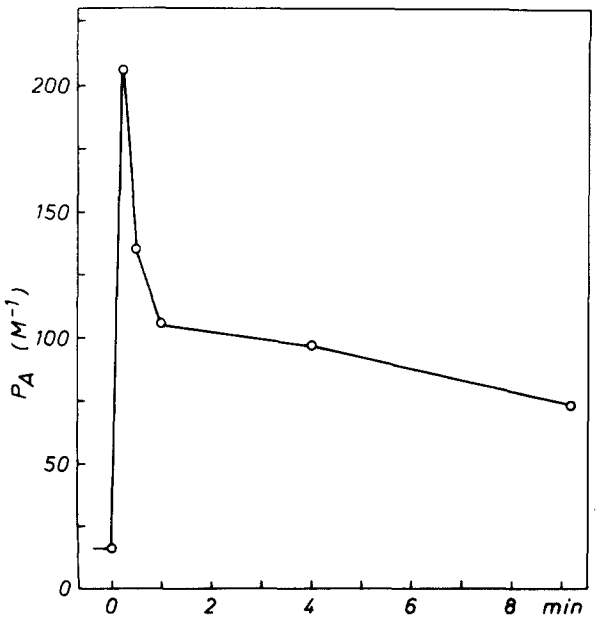

Fig. 6. Assimilatory power in chloroplasts of leaves as a function of illumination time. The leaves had been predarkened for 1 hour and then illuminated with 200 $W \cdot m^{-1}$ in air containing 330 ppm CO_2. Temperature was 26°C.

must be doubled. Even then they are much lower than maximum values measured in isolated chloroplasts when turnover of ATP and NADPH was very slow. Obviously, the low values observed in vivo indicate fast turnover which did not permit assimilatory power to reach high values. Indeed, as photosynthesis increased, assimilatory power decreased rapidly.

At the CO_2 compensation point (50 ppm CO_2), when net assimilation of leaves is close to zero, assimilatory power was about 120 (M^{-1}) [10]. This low level is a consequence of ATP and NADPH turnover in photorespiratory reactions. When photorespiration is suppressed in leaves by decreasing the oxygen concentration from 21 to 2%, thylakoid energization increased indicating an increase in the phosphorylation potential [30,31].

The large differences between maximum assimilatory power in chloroplasts in situ and in intact isolated chloroplasts oxidizing carbohydrate (Fig. 1 and 2) show that photorespiratory energy consumption is faster in situ than in isolated chloroplasts, which in contrast to leaves cannot metabolize glycolate. Reduction of the phosphoglycerate derived from glycolate, and of the CO_2 evolved during photorespiration, consumes assimilatory power and lowers its steady-state level. Turnover of assimilatory power appears to be required for protection of the photosynthetic apparatus against photodamage [32].

When assimilatory power was measured as a function of light intensity, the results shown in Fig. 7 were obtained. Even low light intensities, which can support only low rates of photosynthesis, produced considerable assimilatory power as calculated under the assumption of constant stromal pH. Increased light intensities which increased photosynthetic flux did not increase assimilatory power further, but actually decreased it. This is a very surprising observation. Flux in any system should increase, when the driving force is increased, not when it is decreased. At first sight, the observations raise doubts whether the assumption of constant pH is justified. If the stromal pH were significantly lower at low light intensity than at high intensities, then values of assimilatory power calculated according to Eqn. 5 would be lower at low light than indicated in Fig. 7. However, it has been shown that owing to the buffer-

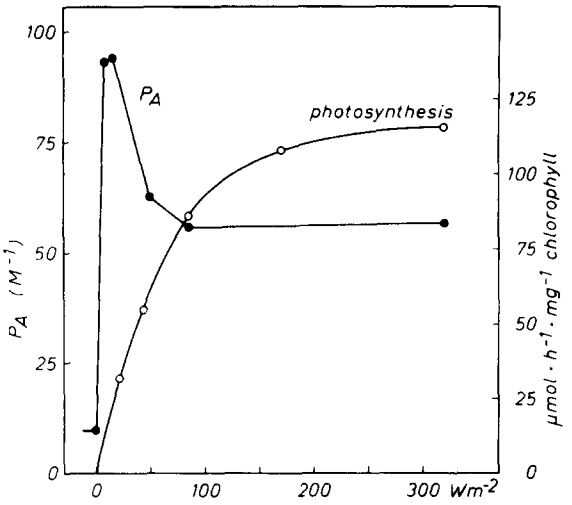

Fig. 7. Assimilatory power in chloroplasts of leaves as a function of light intensity. The leaves were illuminated in air containing 330 ppm CO_2 until steady state photosynthesis was established. Temperature was 26°C.

ing characteristics of chloroplast stroma and thylakoids the alkalization of the stroma is largely light-saturated at light intensities as low as 10 $W \cdot m^{-2}$ [33], so that the assumption of constant pH satisfies the requirements of the calculations even if it is not entirely correct. Therefore, the dilemma remains that increased flux which should be driven by an increased driving force is actually accompanied by a decrease in the driving force. It is accenuated by the observation that light scattering and energy-dependent fluorescence quenching, which are indicators of the transthylakoid proton gradient [34–37], and reduction of the electron acceptor Q_A [38] are low under low intensity illumination. They increase with light intensity in a sigmoid fashion. Obviously, the relationship between the reduction state of Q_A, the magnitude of the proton gradient and the state of assimilatory power need to be explored.

However, the postulate of proportionality between flux and driving force as it is derived from Fick's law or Ohm's law is of limited applicability to enzyme-catalyzed systems. Moreover, it presupposes that resistance to flux remains constant. Apparently, this is not the case. Light activation of enzymes [39] will decrease resistance to flux in the Calvin cycle. Decreased resistance to flux permis increased photosynthesis even though the

driving force does not increase, but actually decreases.

It should be noted that the observation of high P_A values at low light intensities is important in relation to the explanation of the Kok effect [40] which has been proposed to reflect inhibition of mitochondrial oxidation in photosynthetic cells at very low light intensities. Inhibition has been thought to be caused by a light-dependent increase in the phosphorylation potential of the cytosol which is mediated by shuttle transfer of transport metabolites between chloroplasts and cytosol [17]. It would be difficult to explain when photosynthesis at low light intensities were associated with low levels of P_A. The high levels recorded for low light intensities in Fig. 7 lend additional support the the assumption that an increased cytoplasmic ratio of dihydroxyacetone phosphate to phosphoglycerate controls mitochondrial oxidation via the cytosolic phosphorylation potential. This assumption has been challenged recently when increased cytosolic phosphorylation potentials were not indicated by measured ATP/ADP ratios after aqueous fractionation of illuminated mesophyll protoplasts [41–43]. However, we believe that it is difficult to maintain in vivo levels of the phosphorylation potentials during aqueous fractionation. Moreover, phosphate as a component of the phosphorylation potential which decreases in the light has not been measured. Even when the cytosolic ATP/ADP ratio is held constant in the dark and in the light, a decrease in phosphate concentration, which is known to occur in the light, leads to an increase of the phosphorylation potential.

At constant light intensity, photosynthetic flux is increased with CO_2 until photosynthesis is saturated in respect to CO_2. As shown in Fig. 7 for the relationship between photosynthesis and light intensity, the increase in photosynthesis was accompanied by a decrease in assimilatory power [10]. In this case, increased carboxylation of ribulose bisphosphate decreased resistance to carbon flux. Maximum rates of photosynthesis in the presence of rate-saturating CO_2 have been observed at values of assimilatory power close to 60 (M^{-1}). Similar values are recorded for photosynthesis in air in the experiment of Fig. 7. In the latter experiment, the full capacity for photosynthesis was not realized, because CO_2 was subsaturating. Increasing the CO_2 concentration would have stimulated photosynthesis and decreased assimilatory power further, below the ceiling shown in Fig. 7. The data show that production of assimilatory power cannot be considered a main limiting factor of photosynthesis even when both light and CO_2 are saturating. Similar conclusions have been drawn on the basis of other evidence [37,44]. Of comparable significance as production of assimilatory power is its consumption. A decrease in flow resistance in the Calvin cycle by increased enzyme catalysis will aid production of assimilatory power. Our observations illustrate the interdependence of catalysis in the Calvin cycle on one side and the efficiency of electron transfer and phosphorylation by the thylakoid system on the other side. The electron-transport chain operates efficiently only when NADP, ADP and phosphate are in good supply, i.e. when values of assimilatory power are low. Our data clearly show that both phosphorylation and electron flow become inefficient when assimilatory power increases to high levels. Thus, high rates of photosynthesis are possible only in the presence of low levels of assimilatory power. Levels are kept low only when catalysis of the reactions of the Calvin cycle is efficient, i.e., when sufficient amounts of enzymes are present or light-modulated enzymes are activated.

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