Biochimica et Biophysica Acta, 635 (1981) 393—404

BBA 48034

CORRELATION BETWEEN PHOTOSYNTHESIS AND THE TRANSTHYLAKOID PROTON GRADIENT

RUDOLF E. SLOVACEK * and GEOFFREY HIND **

Biology Department, Brookhaven National Laboratory, Upton, NY 11973 (U.S.A.)

(Received September 1st, 1980)
(Revised manuscript received December 22nd, 1980)

Key words: Photosynthesis; Oxygen evolution; Proton gradient; Quercetin; Antimycin; Thylakoid membrane

Summary

In isolated intact chloroplasts, maximal rates of photosynthetic O_2 evolution (in saturating HCO_3^-) are associated with a critical transthylakoid proton gradient as a result of the stoichiometric consumption of 2 mol NADPH and 3 mol ATP/mol CO_2 fixed. Studies with the fluorescent probe 9-aminoacridine reveal that in the illuminated steady state the critical ΔpH is 3.9.

CO_2-dependent O_2 evolution is inhibited by increases of 0.1—0.2 in ΔpH that occur when catalase is omitted from the medium, NO_2^- is included as an electron acceptor, or when chloroplasts are illuminated under low partial pressures of O_2. Low concentrations of antimycin (0.33 μM) or NH_4Cl (0.33 mM) decrease ΔpH and relieve this inhibition of electron flow. The energy transfer inhibitor quercetin lowers the high ATP/ADP ratio associated with these conditions, but does not lower ΔpH or relieve the inhibition.

A decrease of ΔpH below 3.9 by weaker illumination, millimolar levels of NH_4Cl or micromolar levels of antimycin, results in lower rates of photosynthesis owing to limitation by the phosphorylation rate.

These findings show that in absence of rate limitation by the carbon cycle, the extent of thylakoid energization is related to the ratio of ATP to NADPH production and in turn, the rate of CO_2 assimilation.

* Present address: Corning Glass Works, Sullivan Park FR-6, Corning, NY 14830, U.S.A.
** To whom correspondence should be addressed.
Abbreviations: Tricine, *N*-tris(hydroxymethyl)methylglycine; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

Introduction

Photosynthetic CO_2 fixation requires that coupled electron transport reactions produce reductant (NADPH) and establish a proton gradient across the thylakoid for driving phosphorylation. Earlier work with thylakoids showed that electron flow rates are rapid when the proton gradient is collapsed by uncouplers [1—3] or, to a lesser extent, by ADP and P_i [1—3]; conversely, high rates of phosphorylation depended upon a large proton gradient [2,4]. Maximal electron transport and phosphorylation rates can thus not be coincidental: one or other should limit the rate of CO_2 fixation in the absence of limitation imposed by the carbon reduction pathway.

In intact chloroplasts, decreased rates of 3-phosphoglycerate or CO_2 reduction observed upon lowering the light intensity from 70 to 15 W/m^2 were accompanied by a decline in the stromal ATP/NADPH ratio [5]. At high light intensity, the uncoupling of phosphorylation from electron flow with amines or carbonyl cyanide *m*-chlorophenylhydrazone also inhibited CO_2 fixation [5,6] and reduction of 3-phosphoglycerate [6]. The rate of photophosphorylation clearly limits the rate of CO_2 or 3-phosphoglycerate reduction in the above situations.

In contrast, several studies of light- and HCO_3^--saturated photosynthesis reported rate accelerations upon addition of NH_4Cl [7,8] or antimycin [9,10]. The mechanism for acceleration of photosynthesis by NH_4Cl was assumed to involve penetration of NH_3 into the stroma, where the uncharged base would be protonated and so raise the pH toward the optimum of 8.1 for CO_2 fixation [6]. The effect of antimycin was ascribed to an indirect increase in fructose-1,6-bisphosphatase activity [11] resulting from higher levels of reduced ferredoxin and operation of the ferredoxin-thioredoxin activator system [12]. Subsequent work showed that these rate increases occur only when photosynthesis is initially suppressed by suboptimal assay conditions resulting from, for example, H_2O_2 poisoning in the absence of added catalase [13,14], excessive levels of P_i [14,15], low partial pressure of O_2 [16] or the presence of electron acceptors such as nitrite [8,14]. Antimycin and NH_4Cl are equally effective in restoring electron flow under these inhibited conditions [14]. In instances where adenine nucleotide distribution [3,7—10] or the proton gradient [7,8, 14] was measured, the rate increases always correlated with a decline in the ATP/ADP ratio or in the proton gradient. Such finding supported the proposal [16] that in some instances photosynthesis is regulated by the inhibitory effect on electron transport of a large proton gradient.

The relationships between the transthylakoid proton gradient and the rates of electron flow, phosphorylation and CO_2 assimilation are further explored here. The proton gradient was experimentally manipulated by changing the illumination or assay conditions, and by the addition of antimycin or uncouplers.

Materials and Methods

Chloroplasts were isolated from spinach as previously described [16]; they were at least 80% intact and gave light- and HCO_3^--saturated photosynthesis

rates of 100—200 μmol/mg chlorophyll per h. Thylakoids were prepared by osmotic rupture of intact chloroplasts in 1.0 mM Tricine and 10 mM $MgCl_2$ at pH 8.1. Polarographic measurements of O_2 evolution with HCO_3^-, or of O_2 uptake with methyl viologen were performed at 19°C with chloroplasts or thylakoids, as indicated, suspended in 0.35 M sorbitol/10 mM NaCl/0.3 mM K_2HPO_4/50 mM Tricine adjusted to pH 8.1. Adenine nucleotide levels were determined by the luciferin-luciferase method after $HClO_4$ extraction [3]. Phosphorylation was measured in thylakoids by monitoring the pH change [17] in a suspension medium containing 0.35 M sorbitol, 10 mM NaCl, 5.0 mM $MgCl_2$, 1.0 mM K_2HPO_4, 0.5 mM Tricine, 0.5 mM Tris, 0.66 mM ADP, 20 μM *N*-methylphenazonium methosulfate and 10 μM 9-aminoacridine; the initial pH was adjusted to 8.0 except in the case of 9-aminoacridine fluorescence measurements where the pH was buffered at 8.1 with 10 mM additional Tricine.

Simultaneous measurements of 9-aminoacridine fluorescence and O_2 evolution or uptake were performed in a single cuvette in a fluorometer [18]. Samples contained 10 μM 9-aminoacridine and were illuminated with 235 W/m^2 of red actinic light (Corning 2-58 filter). The fraction of 9-aminoacridine uptake or fluorescence quenching was calculated as $Q = (F_{dark} - F_{light})/F_{dark}$. From the equation of Schuldiner et al. [19]: $\Delta pH = \log(Q/(V - VQ))$, where V, thylakoid volume per sample, was calculated assuming a thylakoid volume of 4.55 μl/mg chlorophyll. This value came from the relative thylakoid to stromal space ratio of 0.13 measured for illuminated chloroplasts [20] and from the average sorbitol-impermeable osmotic space of 35 μl/mg chlorophyll for intact chloroplasts [21]. The chlorophyll concentration was routinely 20—22 μg/ml. Deaerated samples (less than 20 μM O_2) were obtained by bubbling N_2 through reaction medium for 10 min prior to adding less than 25 μl of chloroplast suspension.

Results

Determination of ΔpH

The quenching of 9-aminoacridine fluorescence by illuminated thylakoid suspensions is due to redistribution of the probe in response to the proton gradient and binding of the probe to the membranes [7]. Correction of the fluorescence signal for membrane binding can be made by measuring light-induced quenching as a function of chlorophyll concentration. In Fig. 1, extrapolation to zero chlorophyll shows that light-induced binding introduces an error of approx. 0.65 pH unit at 22 μg chlorophyll/ml, which corresponds to 31% quenching [19] or a binding error of 1.4%/μg chlorophyll in agreement with previous estimates [7,14]. Determinations of ΔpH were corrected accordingly by use of $Q_{corr} = Q_{obs}/1.45$.

Table I lists the 9-aminoacridine fluorescence quenching values and estimates ΔpH for various conditions. The quenching at pH 7.6 with HCO_3^- as terminal acceptor resembles earlier data [7] for intact chloroplasts incubated in medium at a pH lower than that of the illuminated chloroplast stroma (pH 8.1); it probably registers the net ΔpH between the medium and the thylakoid interior. A pH of 8.1 was accordingly selected for the reaction medium so that the 9-aminoacridine responses would reflect mainly the transthylakoid ΔpH

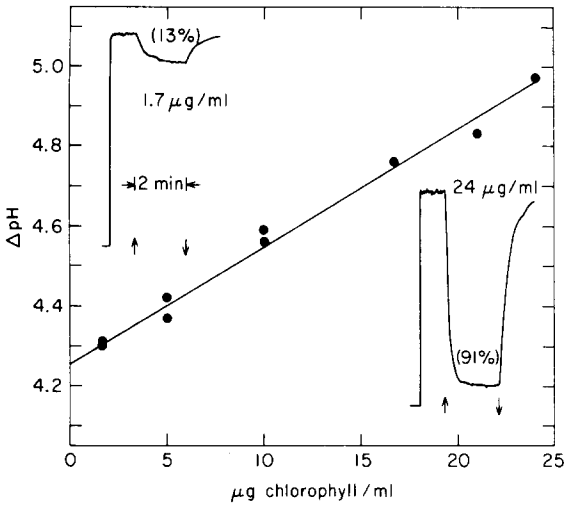

Fig. 1. The dependence of ΔpH calculations on chlorophyll concentration. Samples contained 1600 units/ml of catalase, 10 μM 9-aminoacridine, 25 μM methyl viologen and intact chloroplasts giving the indicated chlorophyll concentration. Insets: fluorescence traces at 1.7 and 24 μg chlorophyll/ml; actinic light on at upward arrow, off at downward arrow. Values in parentheses: fluorescence quenching (%).

(light-induced alkalization of the stroma is small at this pH [20]). More of the probe it taken up at pH 8.1 than in more acidic media, and with either methyl viologen or HCO_3^- present the estimated ΔpH in the light is 0.3 unit higher

TABLE I

9-AMINOACRIDINE FLUORESCENCE QUENCHING AND ESTIMATED ΔpH FOR VARIOUS REACTION CONDITIONS

Intact and broken chloroplasts were prepared as described in Materials and Methods. For reactions at pH 7.6, chloroplasts were suspended in 50 mM Hepes buffer instead of Tricine. All samples contained 1600 units/ml of catalase. 10 mM $NaHCO_3$, 25 μM methyl viologen (MV) and 20 μM *N*-methylphenazonium methosulfate (PMS) were added as indicated.

Conditions	9-Aminoacridine fluorescence quenched (%)	ΔpH
pH 7.6; intact		
+ HCO_3^-	42	3.63
+ MV	61	3.89
pH 8.1; intact		
+ HCO_3^-	62	3.90
+ MV	84	4.18
+ MV, + 5 μM monensin	23	3.30
+ PMS	85	4.19
pH 8.1; broken		
+ MV	86	4.21
+ 1.3 mM ADP	74	4.05
+ 13 μM monensin	11	2.93
pH 8.1; broken		
+ PMS	87	4.22
+ 0.7 mM ADP	81	4.15
+ 13 μM monensin	10	2.89

TABLE II

THE EFFECTS OF PHOSPHORYLATION INHIBITORS ON INTACT CHLOROPLAST PHOTOSYNTHESIS

Samples contained 10 mM $NaHCO_3$, 1600 units/ml of catalase and 20 μg/ml of chlorophyll in reaction medium. Numbers indicate steady-state values after 4 min of illumination. Illumination was 324 W/m^2 of blue (Corning CS 4-96) light for O_2 and adenine nucleotide determinations, and 235 W/m^2 of red (Corning CS 2-58) light for 9-aminoacridine fluorescence measurements used in calculations of ΔpH.

Conditions	O_2 evolution (μmol/mg chlorophyll per h)	ATP/ADP	ΔpH
Control	121	0.88	3.89
+ 1.0 μM antimycin	63	0.52	3.78
+ 2.0 mM NH_4Cl	56	0.46	3.25
+ 60 μM quercetin	51	0.46	3.91

(Table I). The quenching values with methyl viologen present are the same for intact and freshly broken chloroplasts, in support of the view that fluorescence of 9-aminoacridine is registering the ΔpH across the thylakoid. However, at low values of estimated ΔpH (possibly less than 3.0 [20]) light-induced alkalization may be inadequate to bring the stroma into pH equilibrium with the medium (at pH 8.1); in these cases the measured ΔpH will be an overestimate.

Addition of ADP to shocked chloroplasts (under phosphorylating conditions) decreases ΔpH only slightly, whereas the ionophore monensin is an effective inhibitor.

CO_2 fixation limited by phosphorylation

Table II compares the effects on HCO_3^--supported O_2 evolution of several reagents which decrease photophosphorylation. Antimycin (an inhibitor of cyclic electron flow [22]), NH_4Cl (an uncoupler [1]) and quercetin (an energy transfer inhibitor [23]) decrease O_2 evolution by 50% and reduce the ATP/ADP ratio by approx. 40% at the concentrations indicated. Quercetin differs from the other reagents in not decreasing ΔpH concomitantly, hence its effect on photosynthesis results from inhibition of the supply of ATP per se.

TABLE III

LIGHT INTENSITY DEPENDENCE FOR THE INHIBITORY EFFECT OF LOW ANTIMYCIN CONCENTRATIONS

Samples contained 10 mM $NaHCO_3$, 1600 units/ml of catalase and 23 μg/ml of chlorophyll. Blue illumination (Corning CS 4-96) was provided at the intensities listed. Antimycin A additions were 0.5 μM.

W/m^2	Additions	Oxygen evolution (μmol/mg chlorophyll per h)	Control (%)
680	—	131	100
	+ antimycin	121	93
260	—	73	100
	+ antimycin	57	78
45	—	61	100
	+ antimycin	33	54

TABLE IV

THE EFFECTS OF REACTION CONDITIONS ON CHLOROPLAST ADENINE NUCLEOTIDE RATIOS

Samples containing 8 mM $NaHCO_3$, 21 μg chlorophyll/ml and 1600 units of catalase/ml (except where noted) were incubated for 4 min in blue light (Corning CS 4-96) at 1000 W/m^2 before termination of the reaction with 5% $HClO_4$.

Reaction conditions	nmol/mg chlorophyll		
	ATP	ADP	ATP/ADP
Aerobic control	9.4	13.8	0.68
— Catalase	12.4	13.4	0.93
+ 2.0 mM P_i	13.9	11.3	1.23
+ 1.6 mM NO_2^-	10.3	11.2	0.92
Deaerated	12.5	12.6	0.99

At low light intensity, assimilation of CO_2 is even more sensitive to inhibitors of phosphorylation as shown in Table III, where 0.5 μM antimycin inhibits by 46% at low light intensity yet is almost ineffectual at 680 W/m^2. A similar sensitivity pattern has been observed using 0.33 mM NH_4Cl [14].

CO_2 fixation limited by electron flow

Increased CO_2 fixation rates resulting from addition of NH_4Cl [18] or antimycin [9,10] suggest that photophosphorylation is not always rate limiting. Table IV presents measurements of adenine nucleotide levels during photosynthesis under optimal conditions or under inhibition by excessive P_i [15], omission of catalase [13], addition of nitrite [8] or by suboptimal O_2 tension [16]. Higher ATP/ADP ratios accompany all these inhibitions, in contrast to the situations discussed above, and phosphorylation rates presumably do not limit photosynthesis.

Fig. 2. compares the effects of NH_4Cl, antimycin and quercetin on photosynthesis in control chloroplasts and those inhibited by omission of catalase. The initially high control rates of CO_2-dependent O_2 evolution are decreased by all

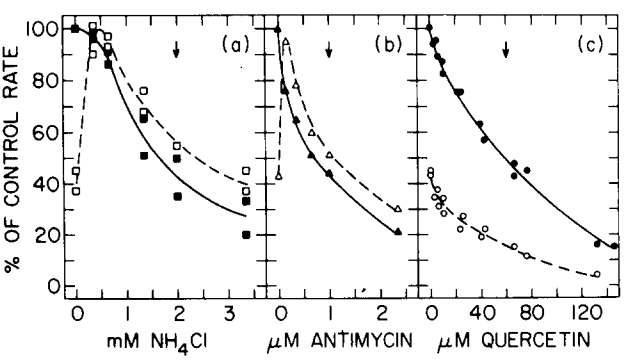

Fig. 2. The effects of varying NH_4Cl, antimycin and quercetin concentrations on chloroplast O_2 evolution with and without catalase. Samples containing 10 mM $NaHCO_3$ and 21—32 μg/ml of chlorophyll were illuminated with 1000 W/m^2 of blue (Corning CS 4-96) light. ■,▲,●, samples with 1600 units/ml of catalase and □,△,○, without catalase. Control rates in μmol O_2/mg chlorophyll per h were (a) 122, 144; (b) 152, and (c) 90, 121.

TABLE V

DETERMINATIONS OF ΔpH UNDER OPTIMAL AND SUBOPTIMAL CONDITIONS FOR CO_2 FIXATION

Simultaneous measurements of O_2 evolution and 9-aminoacridine fluorescence were performed with samples containing 10 mM $NaHCO_3$ as described in Materials and Methods. 1600 units/ml catalase, 1.0 mM $NaNO_2$, 0.25 μM antimycin, 0.25 mM NH_4Cl, and 10 μM quercetin were present as indicated. Control rates of O_2 evolution ranged from 82 to 104 μmol/mg chlorophyll per h when catalase was present.

Conditions	O_2 evolution rate (%)	Fluorescence quenched (%)	ΔpH
+ Catalase	100	63	3.92
— Catalase	40	77	4.09
+ Antimycin	70	66	3.96
+ NH_4Cl	80	63	3.91
+ Quercetin	30	76	4.08
+ Catalase, + NO_2^-	45	76	4.08
+ Antimycin	90	68	3.98
+ NH_4Cl	95	61	3.89
+ Quercetin	—	75	4.07

three reagents tested (the concentrations required to half inhibit the control rates, as used in Table II, are indicated by arrows). The initially low rates obtained by omission of catalase are, however, accelerated by low concentrations of NH_4Cl or antimycin; inhibition follows at higher concentrations but the inhibition profiles are displaced to the right relative to the control (+catalase). Since low levels of quercetin do not accelerate the rate, the stimulatory effect of NH_4Cl or antimycin cannot be due to a lowering of the ATP/ADP ratio per se. Measurements of ΔpH were consequently performed to examine the role of the proton gradient in these effects.

Omission of catalase (Table V) increased the ΔpH by approx. 0.17 unit and NH_4Cl or antimycin, but not quercetin, overcame this effect. Comparable inhibitions then restorations of O_2 evolution rates were observed; again, quercetin was ineffectual. ΔpH also increased by approx. 0.16 unit when CO_2 fixation was inhibited by nitrite. The effect of nitrite on photosynthesis rate seen in Table V is unlikely to result from inhibition of carbon cycle enzymes by acidification of the stroma since this is negligible [8] if the external pH is close to the light-driven stromal pH [6]. Competition by nitrite for reduced ferredoxin may restrict operation of the enzyme activator system [12] with consequent inhibition of photosynthesis, but the effects of nitrite at pH 7.9 on the photosynthesis rate and pool sizes of carbon cycle intermediates [8] suggest this could not account for the extensive inhibition seen in Table V. As in the case of catalase omission, NH_4Cl or antimycin decreased ΔpH and so reversed the inhibition of photosynthesis by nitrite. A low concentration of quercetin had no such effect. Similar interactions were observed upon inhibition of photosynthesis by 2.0 mM P_i (data not shown, but see Ref. 14).

The data in Table V suggest that in the absence of catalase or presence of nitrite, an excessive proton gradient exerts inhibitory back-pressure on noncyclic electron flow.

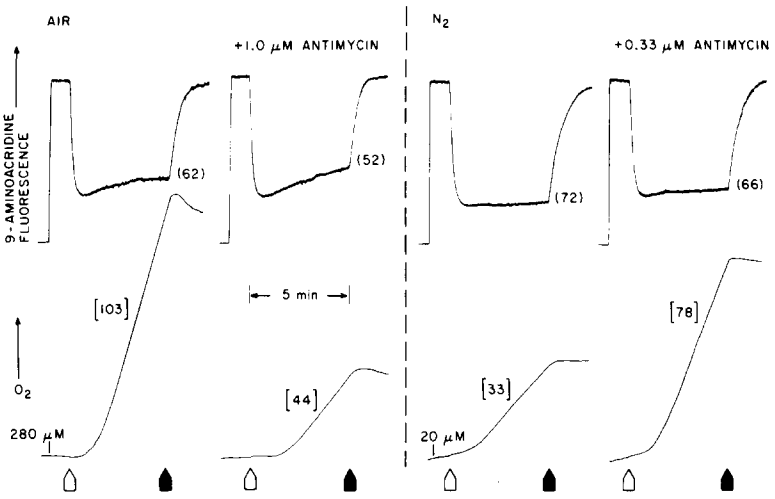

Fig. 3. Traces of 9-aminoacridine fluorescence quenching and O_2 evolution under aerobic (280 μM O_2) and deaerated (less than 20 μM O_2) conditions for CO_2 reduction. Samples contained 10 mM $NaHCO_3$, 10 μM 9-aminoacridine, 1600 units/ml of catalase and 22 μg/ml of chlorophyll in reaction buffer. Measurements were performed as in Materials and Methods with illumination by 860 W/m^2 of red (Corning CS 2-60) light.

Fig. 3 displays simultaneous traces of O_2 evolution and 9-aminoacridine fluorescence in aerobic (280 μM O_2) and deaerated (less than 20 μM O_2) samples. Addition of 1.0 μM antimycin to the aerobic sample inhibits O_2 evolution by almost 60% and decreases the uptake of 9-aminoacridine. The inhibition of photosynthesis can clearly be attributed to a diminished proton gradient leading to a lower ATP/ADP ratio (cf. Table II). When the O_2 tension is initially low, however, the rate of photosynthesis is depressed by a high H^+ gradient; 0.33 μM antimycin decreases this gradient toward the aerobic control level and the electron flow rate rises markedly.

It is evident from the above that ΔpH can become excessive under certain conditions. The associated decrease in the rate of photosynthesis does not result from a high ATP/ADP ratio, since partial inhibition of ATP synthesis by quercetin does not overcome the inhibition. Reagents that decrease ΔpH, on the other hand, do relieve the inhibition, which shows that photosynthesis is limited by the back-pressure of the proton gradient on electron flow.

Correlation between ΔpH and photosynthesis rate

The data in Tables II and V indicate that electron flow and phosphorylation may vary markedly over a small range of ΔpH. The relation of ΔpH to the rates of electron flow and phosphorylation, catalyzed by methyl viologen and *N*-methylphenazonium methosulfate, respectively, was studied as a function of uncoupler concentration. The results (Fig. 4) show that electron flow is sensitive to inhibition by ΔpH values above 3.9, whereas phosphorylation rates increase rapidly above a ΔpH of 3.5. A similar dependence of photophosphorylation [2,4] and electron flow [2] on ΔpH has been noted previously in broken chloroplasts. Maximum phosphorylation and electron flow rates clearly cannot

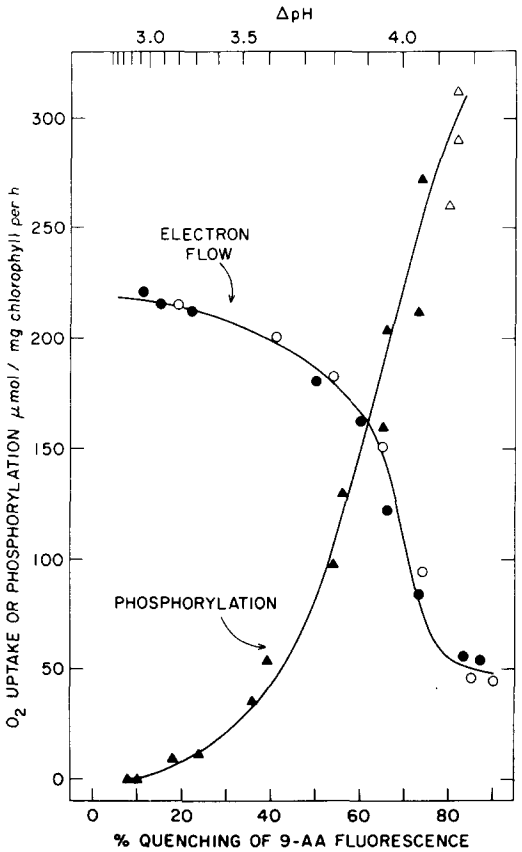

Fig. 4. Phosphorylation and electron flow rates as a function of ΔpH. Methyl viologen catalyzed O_2 uptake by intact chloroplasts (with 1.0 mM KCN present) and *N*-methylphenazonium methosulfate (PMS) catalyzed ATP production in thylakoid suspensions were measured as described in Materials and Methods. All samples contained 10 μM 9-aminoacridine. Electron flow was varied by adding up to 2.5 mM NH_4Cl (○) or up to 13 μM monensin (●); phosphorylation (△) was varied with up to 13 μM monensin (▲).

coexist; how the chloroplast makes the necessary compromise, and what ΔpH value is maintained during steady-state photosynthesis will now be discussed.

Fig. 5 displays simultaneous ΔpH and O_2 evolution measurements for chloroplasts assimilating CO_2 under various light intensities and with additions of antimycin or NH_4Cl. At low light or when antimycin is present, ΔpH values fall below the optimum and assimilation is apparently governed by a low phosphorylation rate (the decline on this side of the peak, as ΔpH approaches 3.0, may be less steep than shown owing to overestimation of ΔpH for reasons given earlier). Values of ΔpH above 3.9 attained by intense illumination at low O_2 tensions, by omission of catalase, or by addition of nitrite, are also associated with suboptimal assimilation. ATP production rates are highest in this region of ΔpH (Fig. 4) therefore electron flow is more likely to be rate limiting. Support for this view is provided by the restoration of high rates upon decreasing ΔpH and phosphorylation with addition of antimycin or NH_4Cl.

The sharp ΔpH optimum in Fig. 5 presumably results from the rigid stoi-

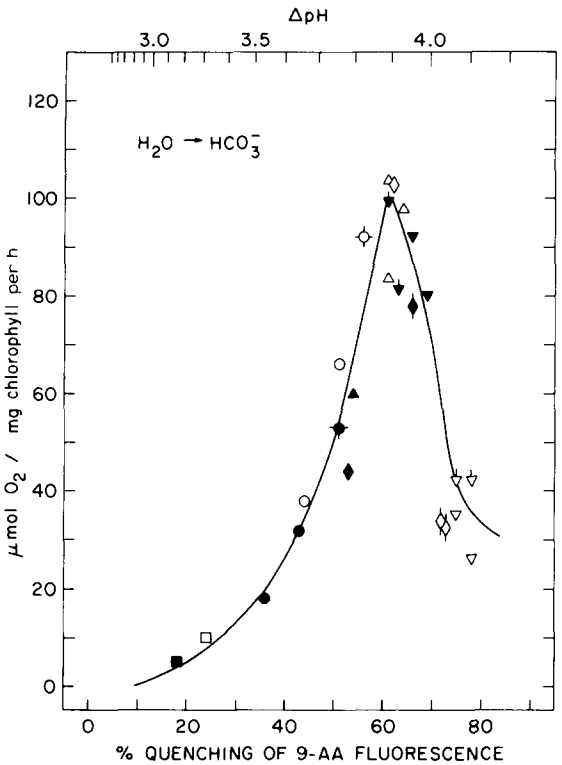

Fig. 5. CO_2-dependent O_2 evolution as a function of ΔpH. All samples contained 10 mM $NaHCO_3$ and 10 μM 9-aminoacridine. Illumination was with red (Corning CS 2-58 or 2-60) light. Assay conditions were varied as described in the text and noted as follows: ♦,◇, 1000 W/m^2, +/—1.0 μM antimycin; ♦,◇, 860 W/m^2, +/—1.0 μM antimycin; ♦,◇, 860 W/m^2 deaerated, +/—0.33 μM antimycin; ▲,△, 235 W/m^2, +/—1.0 μM antimycin; ▼,▽, 235 W/m^2 minus catalase, +/—0.25 μM antimycin or 0.25 mM NH_4Cl, ♦,◇, 235 W/m^2 plus 1.6 mM KNO_2, +/—0.25 μM antimycin or 0.25 mM NH_4Cl; ●, ○, 110 W/m^2, +/—1.0 μM antimycin; ■,□, 25 W/m^2, +/—1.0 μM antimycin.

chiometric requirement for 3 ATP and 2 NADPH mol/mol CO_2 reduced in turnover of the Calvin cycle [24]. At least within the range of ΔpH studied, the thylakoid membrane is apparently unable to vary coupling efficiency (ATP produced/H^+ transferred) to match the energy demand of photosynthesis. Flexible coupling is known in other systems and was most recently documented for the plasma membrane proton pump of *Neurospora* [25].

Conclusions

Photoassimilation of CO_2 is shown in Fig. 5 to be critically dependent on ΔpH; increases or decreases of 0.2 unit are sufficient to inhibit by 50—75%, hence minor perturbations in ATP production and consumption strongly influence the assimilation rate. Nitrite, for example, may inhibit because its reduction, by not consuming ATP, supports establishment of a rate-limiting H^+ gradient [7,14]; deaeration produces the same effect by promoting coupled cyclic electron flow [16]. Poisoning by H_2O_2 [13] and the export of triose phosphate at high external P_i levels [15] are well known to decrease carbon

flow through that portion of the Calvin cycle responsible for regeneration of ribulose 5-phosphate, a substrate for phosphorylation by ATP. The ensuing decline in ATP consumption rate could then lead to a higher transthylakoid H^+ gradient; low levels of antimycin or uncoupler would be required to decrease the gradient and restore high assimilation rates, as observed in the present work. These same reagents, however, inhibit assimilation under control conditions (Table II).

Lilley et al. [26] gave a theoretical maximum estimate for CO_2 fixation of 219 μmol/mg chlorophyll per h in totally intact chloroplasts. This value was 81% of the average electron flow rate obtained with uncoupled thylakoid preparations from the same chloroplasts. In Fig. 4, the electron flow rate at the optimum ΔpH for CO_2-dependent O_2 evolution (approx. 3.9 units, see Fig. 5) is also approx. 80% of the maximum (fully uncoupled) rate for the same preparation. These observed upper limits reflect the need for the thylakoid to maintain a proton gradient adequate to generate ATP at a rate determined by the stoichiometry of CO_2 assimilation.

Photosynthesis in vivo (in ambient O_2 and CO_2) is perhaps always limited by the relative activities of ribulose-1,5-bisphosphate carboxylase as a carboxylase and oxygenase [27]. Enzymatic regulation of photosynthesis in vitro has been documented from studies of chloroplasts incubated with saturating concentrations of HCO_3^- [6,21,28,29]; however, recent work of Leegood and Walker [30] showed that the Calvin cycle enzymes are sufficiently active at or shortly after commencement of illumination to support the maximal rate of photosynthesis under these conditions.

An alternative suggested by the present work is that an excessive ΔpH can in some cases limit the rate of in vitro CO_2 assimilation at saturating HCO_3^- levels. This occurs because the chloroplast seems unable to compensate for overproduction of ATP by changing the efficiency for utilization of chemiosmotic potential. In vivo, regulating the proportion of cyclic to linear electron flow presumably allows the chloroplast to optimize the ATP/NADPH ratio; this is important during the induction period of photosynthesis [31] for example, in supplying additional ATP to restore pools of sugar phosphate intermediates. Experimental conditions in vitro, however, can enforce an ATP/NADPH ratio which is inappropriate for CO_2 assimilation. These inhibitory conditions are associated with abnormally high or low values of ΔpH.

Acknowledgements

Thanks are due to Catherine Chia for technical assistance and to Drs. D. Crowther and J.P. Houchins for suggesting improvements to the manuscript. This work was supported by grant 5901-0410-9-0382-0 to R.E.S. from the Competitive Research Grants Office of the U.S. Department of Agriculture. It was carried out at Brookhaven National Laboratory under the auspices of the United States Department of Energy, with program support to G.H. under contract DE-AC02-76CH00016.

References

1 Krogmann, D.W., Jagendorf, A.T. and Avron, M. (1959) Plant Physiol. 34, 272—277
2 Portis, A.R. and McCarty, R.E. (1976) J. Biol. Chem. 251, 1610—1617
3 Hind, G., Mills, J.D. and Slovacek, R.E. (1977) in Photosynthesis 77: Proc. 4th. Int. Congr. on Photosynthesis (Hall, D.O., Coombs, J. and Goodwin, T.W., eds.), pp. 591—600, The Biochemical Society, London
4 Pick, U., Rottenberg, H. and Avron, M. (1974) in Proc. 3rd. Int. Congr. Photosynthesis (Avron, M., ed.), pp. 967—974, Elsevier, Amsterdam
5 Heber, U. (1973) Biochim. Biophys. Acta 305, 140—152
6 Werdan, K., Heldt, H.W. and Milovancev, M. (1975) Biochim. Biophys. Acta 396, 276—292
7 Tillberg, J.E., Giersch, C. and Heber, U. (1977) Biochim. Biophys. Acta 461, 31—47
8 Purczeld, P., Chon, C.J., Portis, R.A., Heldt, H.W. and Heber, U. (1978) Biochim. Biophys. Acta 501, 488—498
9 Schacter, B.Z., Gibbs, M. and Champigny, M.L. (1971) Plant Physiol. 48, 443—446
10 Schacter, B.Z. and Bassham, J.A. (1972) Plant Physiol. 49, 411—416
11 Walker, D.A., Slabas, A.R. and Fitzgerald, M.P. (1975) Biochim. Biophys. Acta 440, 147—162
12 Wolosiuk, R.A. and Buchanan, B.B. (1977) Nature 266, 565—567
13 Kaiser, W. (1976) Biochim. Biophys. Acta 440, 476—482
14 Slovacek, R.E. and Hind, G. (1980) Plant Physiol. 65, 526—532
15 Walker, D.A. (1976) in The Intact Chloroplast (Barber, J., ed.), pp. 235—278, Elsevier/North-Holland, Amsterdam
16 Slovacek, R.E. and Hind, G. (1977) Plant Physiol. 60, 538—542
17 Nishimura, M., Ito, T. and Chance, B. (1962) Biochim. Biophys. Acta 59, 177—182
18 Mills, J.D., Slovacek, R.E. and Hind, G. (1978) Biochim. Biophys. Acta 504, 298—309
19 Schuldiner, S., Rottenberg, H. and Avron, M. (1972) Eur. J. Biochem. 25, 64—70
20 Heldt, H.W., Werdan, K., Milovancev, M. and Geller, G. (1973) Biochim. Biophys. Acta 314, 224—241
21 Portis, A.R., Chon, C.J., Mosbach, A. and Heldt, H.W. (1977) Biochim. Biophys. Acta 461, 313—325
22 Slovacek, R.E., Crowther, D. and Hind, G. (1979) Biochim. Biophys. Acta 547, 138—148
23 Shoshan, V., Shahak, Y. and Shavit, N. (1980) Biochim. Biophys. Acta 591, 421—433
24 Bassham, J.A. (1964) Annu. Rev. Plant Physiol. 15, 101—120
25 Warncke, J. and Slayman, C.L. (1980) Biochim. Biophys. Acta 591, 224—233
26 Lilley, R.McC., Fitzgerald, M.P., Reinits, K.G. and Walker, D.A. (1975) New Phytol. 75, 1—10
27 Siegelman, H.W. and Hind, G. (eds.) (1978) Photosynthetic Carbon Assimilation, Plenum, New York
28 Kelly, G.J., Zimmerman, C. and Latzko, E. (1976) Biochem. Biophys. Res. Commun. 70, 193—199
29 Huber, S.C. (1978) FEBS Lett. 92, 12—16
30 Leegood, R.C. and Walker, D.A. (1980) Arch. Biochem. Biophys. 200, 575—582
31 Slovacek, R.E., Crowther, D. and Hind, G. (1980) Biochim. Biophys. Acta 592, 495—505