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ANALYSIS OF LIGHT-INDUCED PROTON UPTAKE IN ISOLATED CHLOROPLASTS

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SUMMARY

1. A simple kinetic analysis of light-induced proton uptake into chloroplasts is presented. It is derived from a model of the reaction in which the incoming proton is obligatorily bound by an intra-chloroplast component, and allows quantitative analysis of the effect into parameters of light and dark rate constants and the availability of the chloroplast component.

2. The effect of the following agents on the derived parameters has been measured: electron and energy transfer inhibitors, uncouplers, NaCl concentration, light intensity and pH.

3. A maximal ratio of 4 protons taken up per electron transported has been observed, using ferricyanide as an electron acceptor.

4. A stimulation of light-induced proton uptake by phosphate or arsenate, ADP and Mg has been observed. It was not sensitive to concentrations of Dio-9, which eliminated ATP synthesis.

5. The results are seen as inconsistent with the chemiosmotic theory of energy coupling as presently presented. It is suggested that they may be interpreted in terms of a model in which the function of the proton pump is to enable co-transport into the chloroplasts of the negatively charged complex of phosphate, ADP and Mg.

INTRODUCTION

The basic characteristics of the reversible light-induced pH rise (proton uptake) in unbuffered chloroplast suspensions have been well documented[1,2], and discussed in terms of the possible relationship of the phenomenon to mechanisms of energy coupling[3].

In the original reports certain effects were observed, such as a lag in response on illuminating the suspension, and an "overshoot" in alkalinization after turning off the illumination. With the use of a continuous flow method[4] it was clearly demonstrated that these phenomena were indeed artifacts arising from the inherent response lag of the pH electrode used for making the measurement, as previously suggested[5].

Abbreviations: BDHB, *n*-butyl-3,5-diiodo-4-hydroxybenzoate; DCMU, (3,4-dichlorophenyl)-1,1-dimethylurea; diquat, 1,1'-ethylene-2,2'-dipyridylium dibromide; FCCP, carbonyl cyanide *p*-trifluoro-methoxy-phenylhydrazone; HQNO, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide; PMS, phenazine methosulfate.

The kinetic analysis described here was developed as a simple method of quantitatively analysing the light and dark stages of the reaction in terms of a few defined kinetic parameters. It also avoids measurement in the initial parts of pH response curves, where electrode artifacts predominate.

Studies of the proton to electron ratio and of the effects of phosphorylating conditions on the light-induced proton uptake were undertaken to test the validity of the "chemiosmotic"[6,7] and "chemical"[8,9] theories of energy coupling.

Maximal H^+/e^- ratios previously reported were $5H^+/e^-$, $6H^+/e^-$ and $5H^+/h\nu$ using chloranil[10], diquat[11], or pyocyanine[12], respectively, as electron acceptors or carriers. A strong variation of H^+/e^- with pH was noted[10,11].

It was previously shown that phosphorylating conditions not only failed to abolish the light-induced proton uptake[13], but in fact stimulated the steady-state yield of proton uptake, 3–4-fold[11]. Dio-9, at concentrations which eliminated ATP synthesis, did not seriously affect the stimulatory effects of the phosphorylating conditions. These data were considered consistent with a model in which the prime function of the proton pump is to allow co-transport into the intralamellar space of the metabolic substrates, phosphate, ADP and Mg^{2+}.

METHODS

Chloroplasts from lettuce leaves (*Lactuca sativa*) were prepared essentially as previously described[1]. Chlorophyll was determined as in ref. 14. All reactions were run at room temperature, in a total volume of 2.5 ml. The initial pH of the reaction mixture was brought to the desired value by addition of 0.01 M HCl or NaOH. pH changes were measured with a Radiometer GK 2024C pH electrode, and recorded on a Photovolt Model 43 Recorder. The pH scale was calibrated by addition of aliquots of standard sulfuric acid, 10^{-3} M, to the reaction system.

Light was provided by a 500-W slide projector. Where phenazine methosulfate (PMS) or pyocyanine were used, either a Corning c.s. 2–64 filter or a 700-mμ interference filter of 30 mμ half-band width was fitted; with ferricyanide or 1,1'-ethylene-2,2'-dipyridylium dibromide (diquat) as electron acceptors, a Corning c.s. 2–73 filter or a 580-mμ interference filter of 12 mμ half-band width was used. The intensity of illumination was about $1.0 \cdot 10^5$ erg·cm^{-2}·sec^{-1} (interference filters).

RESULTS

Kinetic analysis of light-induced proton uptake

A standard light-dark cycle of pH changes is shown in Fig. 1 and is assumed to represent uptake into and release from the chloroplasts, respectively, of free protons. The steady-state yield has been suggested previously to result from a balance between simultaneous formation and decay processes[1].

The model proposed below supposes that, after being taken into the chloroplast or bound at its outer surface, the incoming proton is buffered by a substance, YH, within the chloroplast. For the purpose of writing down rate equations of a simple pseudo first-order process, the reaction is represented by the equilibrium equation

$$YH + H^+ \underset{k_d}{\overset{k_f}{\rightleftharpoons}} YH_2^+ \qquad (1)$$

As will be shown, one can derive from this equation a forward rate constant (k_f), a decay rate constant (k_d), and the capacity to bind protons at $t = 0$, (YH_0). The experimental rate constants k_f and k_d are presumably functions of several variables such as light intensity, rate of electron transport and membrane characteristics[1,2]

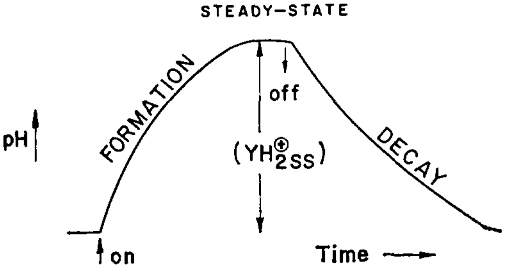


Fig. 1. Reversible light-induced proton uptake.

and refer to the real process, which must be complex, but contain, nevertheless, a pseudo first-order limiting step in both formation and decay stages. Within this convention, the concentration of YH_2^+ represents the yield of proton uptake at any time and ($YH_2{}^+{}_{ss}$) is the steady-state yield. For the sake of simplifying the derivation it was assumed that the pH of the medium remains essentially constant during any one experiment. Thus, for the formation or light stage:

$$\frac{d(YH_2^+)}{dt} = k_f(YH_0 - YH_2^+) - k_d(YH_2^+) \qquad (2)$$

At steady state:

$$\frac{d(YH_2^+)}{dt} = 0 \quad (YH_2{}^+{}_{ss}) = \frac{k_f}{k_f + k_d}(YH_0) \qquad (3)$$

For the decay or dark process:

$$-\frac{d(YH_2^+)}{dt} = k_d(YH_2^+) \qquad (4)$$

Integration of Eqns. 2 and 4 leads to the following expressions, respectively:

$$\log(YH_2{}^+{}_{ss} - YH_2^+) = \log(YH_2{}^+{}_{ss}) - \frac{k_f + k_d}{2.3}t \qquad (5)$$

$$\log(YH_2^+) = \log(YH_2{}^+{}_{ss}) - \frac{k_d}{2.3}t \qquad (6)$$

The straight line plots from Eqns. 5 and 6 enable one to obtain the kinetic parameters k_d, k_f and from Eqn. 3, (YH_0). It is assumed here that the k_d in the light is the same as in the dark; a similar assumption was made previously in studies on the high-energy intermediate, "Xe" (ref. 15).

Analysis of the effect of electron transport inhibitors, uncouplers and salt

The method of analysis enables inspection of the effect of different conditions and agents on the formation (k_f) and decay (k_d) stages, separately. It can be seen

TABLE I

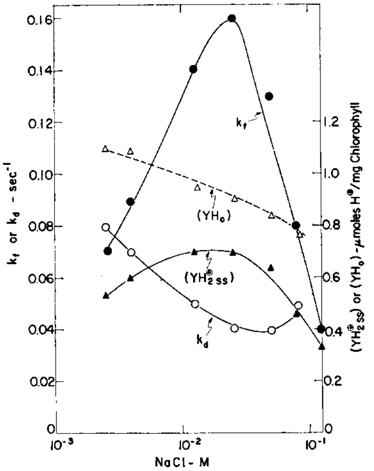
EFFECT OF ELECTRON TRANSPORT INHIBITORS AND PHOTOPHOSPHORYLATION UNCOUPLERS ON THE KINETIC PARAMETERS k_d, k_f, YH_0.

The reaction mixture consisted of: chloroplasts, containing 30 µg chlorophyll/ml; diquat, 3 µM; and NaCl, 20 mM. The pH was 6.0. The inhibitors and uncouplers were added at concentrations giving about 50% inhibition of the extent of the proton uptake process, ($YH_2{}^+{}_{ss}$).

Inhibitor	*Concentration* (*µM*)	*% of control*		
		k_d	k_f	YH_0
DCMU	0.17	90	40	120
HQNO	3.3	95	20	90
BDHB	1.5	100	40	90
NH_4Cl	600.0	170	50	120
FCCP	2.0	145	40	120
Atebrin	10.0	240	95	110

from Table I that electron transport inhibitors ((3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide (HQNO), *n*-butyl-3,5-diiodo-4-hydroxybenzoate (BDHB)) and photophosphorylation uncouplers (NH_4Cl, carbonyl cyanide *p*-trifluoro-methoxy-phenylhydrazone (FCCP), atebrin), at concentrations giving intermediate levels of inhibition, affected only the rate constants and not the capacity. The detailed effects were very similar to those on "two-step phosphorylation"[15,16]; thus electron transport inhibitors inhibit the formation constant only, atebrin increases the decay constant only, while NH_4Cl or FCCP both inhibit the formation and increase the decay constants.

Fig. 2 demonstrates the effect of NaCl on the kinetic parameters and shows that k_f is the most affected by different NaCl concentrations; other experiments at



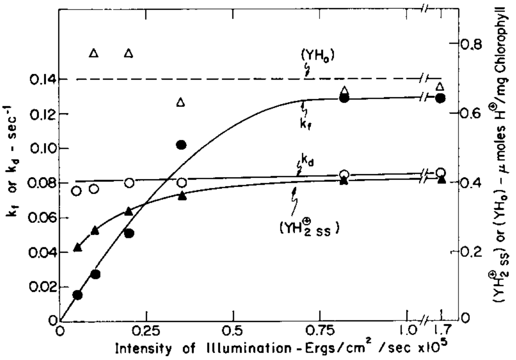


Fig. 2. Effect of NaCl on the parameters k_f, k_d, ($YH_2{}^+{}_{ss}$), (YH_0). Conditions as in Table I, except that NaCl concentration was varied as indicated.

Fig. 3. Effect of light intensity on the parameters k_f, k_d, ($YH_2{}^+{}_{ss}$), (YH_0). Conditions as in Table I, except that light intensity was varied as indicated, with the use of calibrated wire gratings.

pH 7.8 showed a similar variation of k_f with NaCl concentration. The effect on k_f may reflect the variation in rate of electron transport, that has been observed on changing the osmolarity of the medium[17].

Light intensity

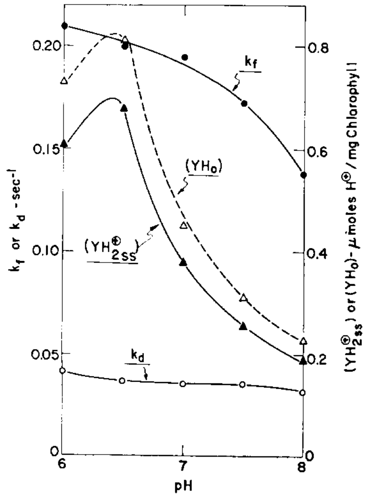
Fig. 3 shows that the only parameter seriously affected by light is k_f, and, as predicted by Eqn. 3, the variation in the equilibrium yield ($YH_2{}^+{}_{ss}$) is much less than that of k_f.

pH optimum of light-induced proton uptake

An analysis of the pH optimum of light-induced proton uptake[1], shown in Fig. 4, demonstrates the effect of pH on the ($YH_2{}^+{}_{ss}$) and on the parameters which determine its value. Over the pH range 6–8 the value of the rate constants k_f and k_d did not vary sufficiently to seriously affect the extent of the reaction while the capacity (YH_0) did vary almost parallel to the observed extent. It was noted that the percentage utilization of the capacity (YH_0) to form $YH_2{}^+$ in different chloroplast preparations varied rather widely between 30 and 80 %. The observation in Fig. 4 that k_d is essentially a constant over the relevant pH range distinguishes the proton uptake effect from that of "Xe" formation, since in the latter effect the decay constant was shown to change greatly over the pH range 6–8 (ref. 18).

The surprising observation of Fig. 4 that the capacity to accept protons is strongly pH dependent with a dumb-bell-shaped pH-dependence curve may be explained by suggesting that a material within or on the surface of the chloroplast which constitutes the capacity (*i.e.* YH) ionizes according to the following:

$$Y^- + H^+ \overset{K_2}{\rightleftharpoons} YH + H^+ \overset{K_1}{\rightleftharpoons} YH_2{}^+ \tag{7}$$



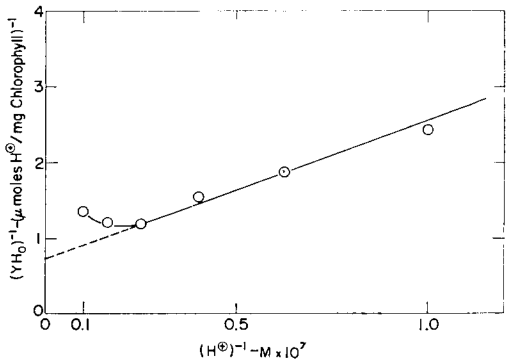


Fig. 4. Effect of pH on the parameters k_f, k_d ($YH_2{}^+{}_{ss}$), (YH_0). The reaction mixture consisted of chloroplasts containing 65 µg chlorophyll/ml; NaCl, 20 mM; and PMS, 3 µM. The pH was altered before the reaction by addition of acid or alkali.

Fig. 5. Plot of $1/H^+$ against $1/YH_0$. See Eqn. 8 and text for details.

YH is assumed to be the only species directly involved in binding incoming protons. The concentration of YH would thus determine the upper limit of proton uptake, and the variation of the latter with pH therefore reflects the variation in concentration of YH. This interpretation rests on an assumption that Y^- is converted to YH at a slow rate, and does not, therefore, contribute to the capacity for proton uptake in short-term experiments.

From Eqn. 7, the following relationship between the YH and H^+ can be formulated:

$$\frac{1}{(YH)} = \frac{1}{(Y_T)} + \frac{H^+}{K_1 \cdot (Y_T)} + \frac{K_2}{H^+ \cdot (Y_T)} \tag{8}$$

where:

$$Y_T = Y^- + YH + YH_2^+ \tag{9}$$

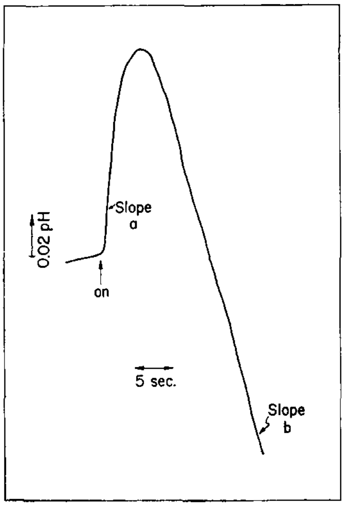
On the alkaline side, the second term of Eqn. 8 will be negligible and therefore a plot of $1/(YH)$ *versus* $1/(H^+)$ should be a straight line with an intercept equal to $1/(Y_T)$. Fig. 5 demonstrates such a plot; from the intercept and slope the following values were obtained: $(Y_T) = 1.34$ μmoles H^+ per mg chlorophyll; $pK_2 = 6.7$.

Consistent results on the acid side were unobtainable, probably due to a variable denaturation of the chloroplasts. An approximate value of pK_1 would be 5.5 judging from the curve of Fig. 4 and other similar ones.

Ratio of protons taken up to electrons transported

Fig. 6 shows the typical light-induced pH changes in a system with ferricyanide as the electron acceptor. Ferricyanide reduction is associated with release of one proton per electron transported according to the equation:

$$1/2\ H_2O + Fe(CN)_6^{3-} \longrightarrow Fe(CN)_6^{4-} + 1/4\ O_2 + H^+ \tag{10}$$



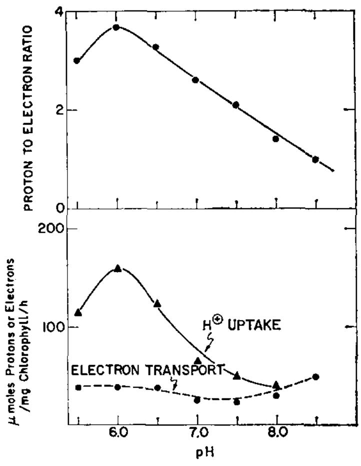


Fig. 6. Light-induced pH changes with ferricyanide. Conditions as in Table I, except ferricyanide, 0.3 mM, replaced diquat.

Fig. 7. Variation with pH of proton and electron transport and the H^+/e^- ratio. Conditions as in Fig. 6, except for the pH which was varied as indicated. See text for details.

We assumed that slope (a) represents the sum of light-induced proton uptake and proton release due to ferricyanide reduction, while slope (b) measures release of protons due to reduction only. Hence, the rate of light-induced proton uptake, electron transport, and thus H^+/e^- were obtained from the same curve (see Fig. 7). The ratio of H^+/e^- varied with pH and had a maximum value of 4 at pH 6.0. Similar results were previously reported with diquat as the electron acceptor[11]. Different chloroplast preparations demonstrated H^+/e^- ratios, at pH 6.0, in the range 1.5–4.0. The variation may depend on the structural state of the chloroplasts. We have found, for example (unpublished results), that bubbling of argon or air for 1–2 min through the usual reaction mixture is sufficient to abolish the light-induced proton uptake whilst leaving electron transport intact. Concentrations of ferricyanide higher than 0.8 mM have also been found to uncouple the chloroplasts. Uncoupling by ferricyanide has been previously reported[19].

Effect of phosphorylating conditions on light-induced proton uptake

The previously noted[11] stimulation of the steady-state extent of proton uptake, in the presence of phosphorylating conditions, is summarized in Table II. In the absence of $MgCl_2$ (0.1 mM EDTA was added to chelate the endogenous Mg^{2+}) a regular, reversible light-induced proton uptake was observed (see Fig. 1). ATP synthesis was initiated by addition of $MgCl_2$. In the presence of $MgCl_2$ two phases of the pH changes were observed in the light. The first corresponded to the light-induced proton uptake, and was followed by the irreversible proton consumption accompanying ATP synthesis[11,13]. The rates of ATP synthesis (2nd column) induced by the added $MgCl_2$ concentrations (1st column) were calculated from the slope of the second phase, assuming 0.93 proton consumed per ATP synthesized at pH 7.8 (ref. 20).

The extent of reversible proton accumulation during phosphorylation (5th column) was recorded on turning off the illumination. Phosphate concentrations which are suboptimal for ATP synthesis were used deliberately to maintain the buffer

TABLE II

EFFECT OF PHOSPHORYLATING CONDITIONS ON THE LIGHT-INDUCED PROTON UPTAKE

The reaction mixture consisted of: chloroplasts, containing 60 μg chlorophyll/ml; pyocyanine, 12 μM; NaCl, 16 mM; ADP, 0.24 mM; phosphate, 0.48 mM; EDTA, 0.1 mM; $MgCl_2$ at different concentrations was added after a control run (—Mg). The pH was 7.8.

Mg^{2+} concentration (mM)	*ATP synthesis (μmoles/mg chlorophyll per h)*	*Yield ($YH_2{}^+{}_{ss}$)*		
		Expected		*Observed (μmoles H^+/mg chlorophyll)*
		Assuming $2H^+/ATP$ (μmoles H^+/mg chlorophyll)	*Assuming $1H^+/ATP$ (μmoles H^+/mg chlorophyll)*	
0.00	0	0.13	0.13	0.13
0.06	15	0.01	0.08	0.18
0.09	60	—0.01	0.05	0.27
0.16	120	—0.10	0.00	0.38
0.32	200	—0.34	—0.08	0.58
0.64	235	—0.39	—0.11	0.75

capacity of the system at a level permitting the accurate determination of the pH changes. The significance of the stimulation of proton uptake induced by phosphorylating conditions is emphasized by the calculations of the yield of proton uptake which should have been expected, according to the chemiosmotic theory, assuming utilization of 1 or 2 protons from the gradient per ATP formed (3rd and 4th column).

To calculate these values we accepted the assumption, implicit in the chemiosmotic theory, that the sole effect of phosphorylation on light-induced proton accumulation would be to increase its decay rate without affecting the initial formation rate $\mathrm{YH_0} \cdot k_\mathrm{f}$, or capacity $(\mathrm{YH_0})$. If we designate the rate of ATP synthesis as R, the ratio of $\mathrm{H^+/ATP}$ as n, and the steady-state yield in phosphorylating conditions, expected by chemiosmotic theory, $(\mathrm{YH_2}^{+}{}_{\mathrm{ssPHP}})$, then the steady-state rate of proton release during phosphorylation would be $k_\mathrm{d}\ (\mathrm{YH_2}^{+}{}_{\mathrm{ssPHP}}) + R \cdot n$. Eqn. 2, which represents the rate of proton uptake into the chloroplasts in the light phase, becomes in the steady state:

$$k_\mathrm{f}(\mathrm{YH_0} - \mathrm{YH_2}^{+}{}_{\mathrm{ssPHP}}) = k_\mathrm{d} \cdot (\mathrm{YH_2}^{+}{}_{\mathrm{ssPHP}}) + R \cdot n \tag{12}$$

$$(\mathrm{YH_2}^{+}{}_{\mathrm{ssPHP}}) = \frac{k_\mathrm{f} \cdot (\mathrm{YH_0}) - R \cdot n}{(k_\mathrm{f} + k_\mathrm{d})} \tag{13}$$

The yield expected by the chemiosmotic theory can thus be calculated from the parameters k_f, k_d, $\mathrm{YH_0}$ (calculated from the control curve, —Mg), the rate of ATP synthesis (R), and the postulated values of the $\mathrm{H^+/ATP}$ ratio, $n = 1$ or 2.

Columns 3 and 4 of Table II show that rates of ATP synthesis have been observed at which negative values of $(\mathrm{YH_2}^{+}{}_{\mathrm{ssPHP}})$ are predicted by the chemiosmotic theory. In other words, the initial rate of proton uptake $(\mathrm{YH_0} \cdot k_\mathrm{f})$ is insufficient to

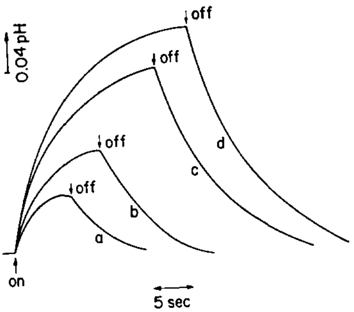


Fig. 8. Effect of arsenate, ADP and Mg on light-induced proton uptake. The reaction mixture consisted of: chloroplasts containing 60 μg chlorophyll/ml; pyocyanine, 12 μM; NaCl, 30 mM; EDTA, 0.1 mM; ADP, 0.24 mM; arsenate, 0.5 mM. The pH was 8.3. $\mathrm{MgCl_2}$, at the concentration given below, was added after a control run (—$\mathrm{MgCl_2}$): (a) 0; (b) 0.08 mM; (c) 0.13 mM; (d) 0.2 mM.

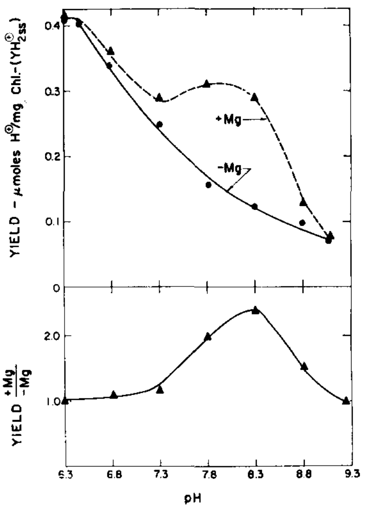


Fig. 9. pH optimum of the arsenate, ADP and Mg stimulation of light-induced proton uptake. Conditions as in Fig. 8, except that $\mathrm{MgCl_2}$ was added at 0.2 mM and the pH varied, as shown.

sustain the observed rate of ATP synthesis, R, at either of the assumed values of n.

Fig. 8 demonstrates the effect of increasing concentrations of $MgCl_2$ in the presence of arsenate and ADP. It shows that, qualitatively, the phenomenon is the same as that observed using phosphate and ADP. The optimal $MgCl_2$ concentration was 0.2 mM at pH 8.3 and 0.8 mM at pH 7.7. The optimal arsenate concentration was 1.0 mM. In Fig. 9 the pH optimum of the arsenate, ADP and Mg stimulation is seen to be around 8.3, a figure close to the optimum of phosphorylation.

Table III demonstrates the effect of the energy transfer inhibitor Dio-9 on light-induced proton uptake in the presence and absence of phosphorylating conditions. As we previously reported, Dio-9, at concentrations which eliminated ATP synthesis, did not seriously affect the stimulation by phosphate, ADP and Mg (ref. 11). However, at much higher Dio-9 concentrations, the stimulation by phosphate ADP and Mg was eliminated. At pH 8, Dio-9 also stimulated the control proton uptake (4th column), a fact which contrasts with previous observation that at pH 6.0 there was no effect[21].

TABLE III

EFFECT OF DIO-9 ON LIGHT-INDUCED PROTON UPTAKE UNDER PHOSPHORYLATION CONDITIONS

The reaction mixture consisted of chloroplasts containing 70 µg chlorophyll/ml; pyocyanine, 15 µM; NaCl, 35 mM; EDTA, 0.1 mM; ADP, 0.25 mM; phosphate, 0.25 mM and; Dio-9 at the indicated concentrations. The pH was 8.3. $MgCl_2$ at 0.8 mM was added after a control run (−Mg). $(YH_2{}^+{}_{ss})$ −Mg was measured from the control run, and the rate of ATP synthesis and $(YH_2{}^+{}_{ss})$ + Mg from the +Mg run.

Dio-9 concentration (µg/ml)	*ATP synthesis (µmoles/mg chlorophyll per h)*	$(YH_2{}^+{}_{ss})$		$(YH_2{}^+{}_{ss})$ + *Mg*
		+*Mg* (*µmoles H⁺/mg chlorophyll*)	−*Mg* (*µmoles H⁺/mg chlorophyll*)	$(YH_2{}^+{}_{ss})$ − *Mg*
0	77	0.140	0.055	2.5
1.3	54	0.155	0.068	2.3
2.6	24	0.155	0.073	2.1
4.0	7	0.175	0.088	2.0
5.5	0	0.175	0.088	2.0
8.0	0	0.160	0.095	1.7
12.0	0	0.150	0.095	1.5
20.0	0	0.120	0.088	1.3
32.0	0	0.110	0.085	1.2

TABLE IV

EFFECT OF DIO-9 ON THE PARAMETERS $YH_2{}^+{}_{ss}$, k_d, k_f, YH_0

The reaction mixture consisted of: chloroplasts containing 25 µg chlorophyll/ml; pyocyanine, 15 µM; NaCl, 35 mM; and Dio-9 added at the indicated concentrations. The pH was 8.0.

Dio-9 concentration (µg/ml)	*% of control*			
	$(YH_2{}^+{}_{ss})$	k_d	k_f	(YH_0)
0	100	100	100	100
2.5	108	115	155	95
5.0	115	135	200	100
10.0	125	135	220	95

This small stimulation considerably changes the shape of the pH changes and, as seen from Table IV, it derives from a large increase in k_f, counteracted by a smaller increase in k_d.

DISCUSSION

The kinetic analysis shows that light-induced proton shifts in isolated chloroplasts can be quantititated, and the results are consistent with previously reported qualitative observations[1,2]. The similarity in response of the light-induced proton uptake and "Xe" to pH, uncouplers and electron transport inhibitors was taken as evidence that the two reflect the same basic phenomena[1,2]. However, similar effects of such agents on the proton gradient and on "Xe" would be expected if the two shared a part of the reaction sequence involved and were therefore in a mobile equilibrium. The data of Fig. 4, which shows that the k_d of the proton-gradient reaction is essentially pH independent, strongly contrast with the clearly established observation that the k_d of the "Xe" reaction is strongly pH dependent[16,18].

The chloroplast compartment into which protons are pumped is probably the intralamellar space rather than the total intrachloroplast space since light-induced proton uptake is also observed in chloroplast fragment preparations. Studies on ion uptake and release[22], and osmotic properties[23] of chloroplasts have suggested the existence of fixed negative charges within intralamellar spaces which bind protons and other cations. These may be identical with the presently postulated "proton binding capacity" (YH). It has been claimed that the intralamellar volume can accommodate only a very small fraction of the large amount of protons pumped inside as free ions. Hence the large proton uptake observed must be accommodated by essentially complete proton binding.

On the basis of the chemiosmotic theory the H^+/e^- ratios reported[10–12] predict between 4 and 6 sites of energy coupling in both non-cyclic and cyclic electron transport paths. Such high numbers are difficult to accept in view of current knowledge.

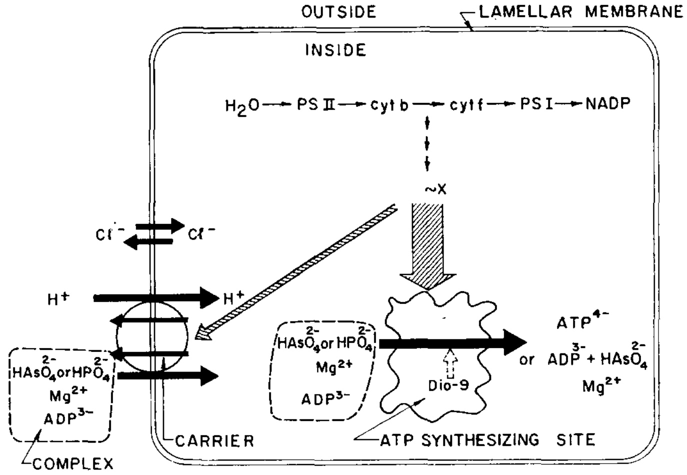


Fig. 10. A hypothetical model describing the proposed function of light-induced proton uptake in chloroplasts. See text for details.

If we accept current formulations which suggest that there are 1 or 2 sites of energy coupling in non-cyclic electron transport, several protons must be taken up per site. One could envisage several YH molecules per site, or one protein molecule capable of binding and transporting several protons. A number of models of ion-transporting systems have been proposed recently, in which binding and transport of ions are the result of energy-dependent conformational changes in the protein transport system[12,24,25].

The stimulatory effects of phosphate or arsenate, ADP and Mg on light-induced proton uptake, and the lack of inhibition by Dio-9, were reported in a preliminary paper[11]. A model was drawn up (Fig. 10) in which the primary function of the proton uptake is to allow co-transport into the chloroplasts of the negatively charged substrate complex of phosphate, ADP and Mg. The model permits the stimulatory effect of phosphate, ADP and Mg to remain, despite complete inhibition of ATP synthesis by Dio-9. The inhibition shown here of the stimulation by phosphate, ADP and Mg at Dio-9 concentrations 6–7 times higher than required to eliminate ATP synthesis (Table III) does not invalidate the model. It may mean that Dio-9 acts at the transport site, as well as the ATP synthesis site, but with a lower affinity. Protons which entered the chloroplast with ADP and phosphate must eventually leave it, possibly with the ATP synthesized.

The stimulation of the control light-induced proton uptake by Dio-9 is not surprising. One would expect the large observed increase in k_f due to the availability of more energy for proton pumping (Fig. 10). It is more difficult to visualize why k_d should increase, if indeed the increase observed is significant.

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