THE DEPENDENCE OF PHOTOPHOSPHORYLATION IN CHLOROPLASTS ON ΔpH AND EXTERNAL pH

Uri PICK, Hagai ROTTENBERG* and Mordhay AVRON
Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel

Received 11 September 1974

1. Introduction

The rate of photophosphorylation in broken chloroplasts is strongly dependent on medium pH with an optimum around 8.3 [1]. Several factors may contribute to this pH dependence: (a) the pH dependence of the phosphorylating enzyme complex itself; (b) the pH dependence of electron transport which drives the phosphorylation, and (c) since it has been demonstrated that in chloroplasts a correlation exists between the size of the pH gradient maintained across the thylakoid membrane (ΔpH) and the efficiency of energy conversion [2,4], it would be expected that the rate of ATP formation will depend on the magnitude of ΔpH, which itself is strongly pH dependent [3,11].

It was previously shown [5] that the rate of electron transport was mostly dependent on internal pH, but also on ΔpH and external pH. If the driving force for phosphorylation is provided by the electrochemical gradient of protons across the chloroplasts membrane [6], one should expect that the membrane potential could also act as an energy source for phosphorylation. Evidence was presented recently [7,8] which indicated that under the condition of limiting ΔpH, an externally induced diffusion potential could indeed drive phosphorylation. Nevertheless, we have previously concluded that during light-dependent phosphorylation, the size of the membrane potential was too small to play any significant role in energy conversion [3]. It has been recently claimed that phosphorylation can be detected when chloroplasts are illuminated by single-turnover flashes at low frequency and that under these conditions it was solely a membrane potential which was driving the phosphorylation.

It this communication, we describe (a) that the rate of phosphorylation has a very definite dependence on the size of the ΔpH. No phosphorylation is observed below a critical ΔpH, and there exists a sharp dependence of the rate of phosphorylation on the size of the ΔpH beyond the critical level. (b) At constant ΔpH, phosphorylation is maximal at low pH values (around 7); ΔpH is maximal at much higher pH (around 9.5), and the customarily observed optimum (around 8.3) is suggested to be the result of both and (c) a sizeable ΔpH can be observed under illumination with single-turnover flashes at low frequencies.

2. Materials and methods

Lettuce chloroplasts were prepared essentially as previously described [10]. ΔpH values were calculated from the distribution of 9-aminoacridine as previously described [11]. Photophosphorylation was followed either by pH changes according to Chance and Nishimura [12] or according to Avron [13]. Actinic light was provided by a 24 V halogen lamp, filtered through a Schott RG 645 filter, which provided an incident light intensity of $3–6 \times 10^5$ erg $\times cm^{-2} \times sec^{-1}$.

Light flashes were produced by an ILC L-268 xenon flash tube kindly provided by Drs Hardt and Malkin [14]. The energy of one flash was approx. 9 J and its duration 17 μsec.

* Present address: Dept. of Biochemistry, Tel-Aviv University, Israel.

North-Holland Publishing Company – Amsterdam

3. Results and discussion

Fig. 1A shows the dependence of the rate of ATP formation and of ΔpH on external pH. As was previously described [1,3,11], photophosphorylation is maximal around pH 8.3, whereas ΔpH is maximal above pH 9.0. When phosphorylation is plotted as a function of ΔpH (fig. 1B), two conclusions stand out clearly; (a) at high external pH, when ΔpH increases beyond about 3.7, the rate of phosphorylation decreases; and (b) as ΔpH decreases below about 2.7 (at low external pH) essentially no phosphorylation can be observed. This behavior is essentially the same for cyclic phosphorylation (pyocyanine), and non-cyclic phosphorylation (ferricyanide, diquat).

The dependence of photophosphorylation and ΔpH on uncoupling by ammonium salts or FCCP is shown in fig. 2A. In this set of experiments, under *constant* external pH (8.0), the addition of uncoupler decreased phosphorylation and ΔpH. Here again, as can be seen more clearly in fig. 2B where phosphorylation is plotted as a function of ΔpH, no phosphorylation was observed below a threshold ΔpH. ATP formation was detected only above a certain minimal ΔpH (about 2.6) and rose sharply with increase of ΔpH (at constant external pH). The dependence of the rate of ATP formation on the magnitude of ΔpH was independent of the type of uncoupler used.

Fig. 3A shows the dependence of phosphorylation on ΔpH, when varied by the addition of FCCP, at different constant external pH's, and fig. 3B shows a similar experiment in which DCMU was used to control the rate of phosphorylation and size of ΔpH. It is clear that in either case, just as in the cases illustrated in fig. 2B, a threshold ΔpH of around 2.7 is evident. Similar threshold values are observed also in post-illumination and acid base type experiments [7,8]. In addition, when one compares the rate of phosphorylation as a function of external pH (fig. 3C)

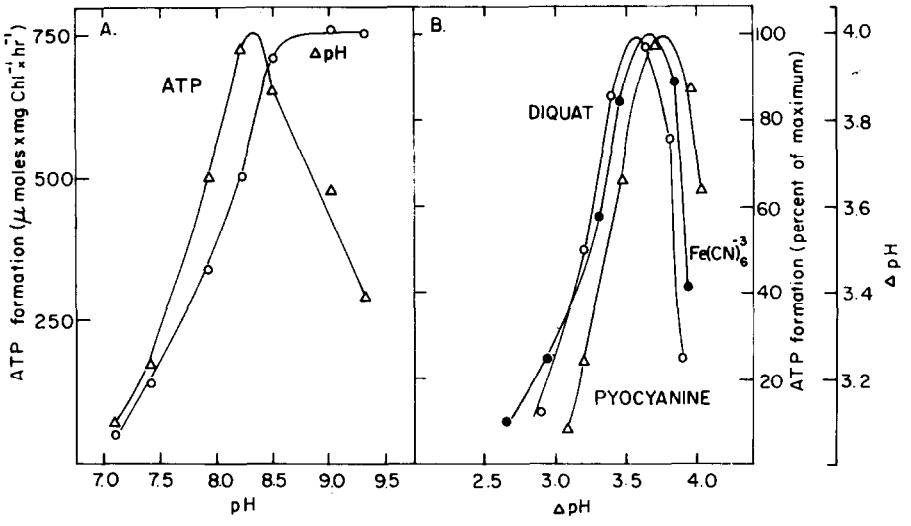

Fig. 1A. The dependence of the rate of photophosphorylation and the magnitude of ΔpH on external pH. The reaction medium contained in 3 ml: KCl, 60 mM; $MgCl_2$, 1 mM; inorganic phosphate, 2.3 mM; ADP 0.4 mM; 9-aminoacridine, 2–4 μM; pyocyanine, 20 μM, and chloroplasts containing 15–30 μg chlorophyll. Photophosphorylation was measured by the pH electrode technique [12]. In no case did the total change in pH during measurement exceed 0.1 pH units. B. The dependence of the rate of photophosphorylation on ΔpH. As described above, except that where indicated, diquat, 15 μM plus NaN_3, 1 mM replaced pyocyanine. Photophosphorylation coupled to ferricyanide reduction was measured with ^{32}P [13] in a reaction medium containing in 3 ml tricine-maleate - NaOH, or tricine-glycine - NaOH, 30 mM; $MgCl_2$, 1 mM; ADP, 2 mM; inorganic phosphate, 2.3 mM (containing P^{32}, 2×10^6 cpm/μmole); ferricyanide, 1 mM; 9-aminoacridine, 4 μM; and chloroplasts containing 110 μg chlorophyll. Samples were taken out after 1–5 min and analyzed for ATP^{32}. 100% activity in μmoles ATP formed × mg chlorophyll^{-1} × hr^{-1} was 750, 260, 245 with pyocyanine, diquat and ferricyanide, respectively.

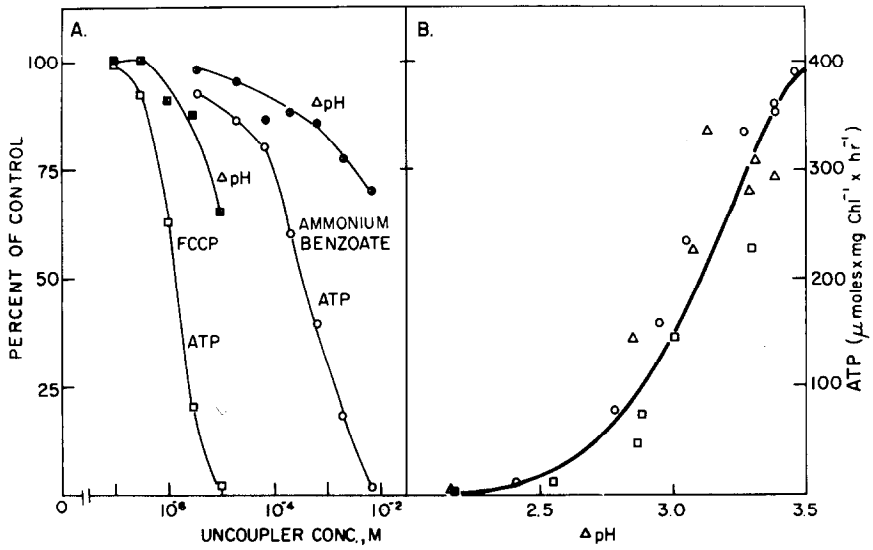

Fig. 2A. Inhibition of photophosphorylation and ΔpH by ammonium benzoate and FCCP. The reaction mixture contained in 3ml: Na-tricine pH 8.0, 60 mM; magnesium (acetate)$_2$, 2 mM; inorganic phosphate, 1 mM (containing P^{32}, 2×10^{-6} cpm/μmole); ADP, 1 mM; pyocyanine, 30 μM; 9-aminoacridine, 1 μM; chloroplasts containing 20 μg chlorophyll and uncouplers at the indicated concentrations. ATP formation and ΔpH were measured as described under Materials and methods [13,11]. 100% activities were 3.30 and 3.45 ΔpH units and 330 and 390 μmoles ATP formed × mg chlorophyll^{-1} × hr^{-1} for FCCP and ammonium benzoate, respectively. B. The dependence of the rate of photophosphorylation on ΔpH. Photophosphorylation rate is plotted as a function of ΔpH for ammonium benzoate (○–○–○), FCCP (□–□–□) and dianemycin (△–△–△).

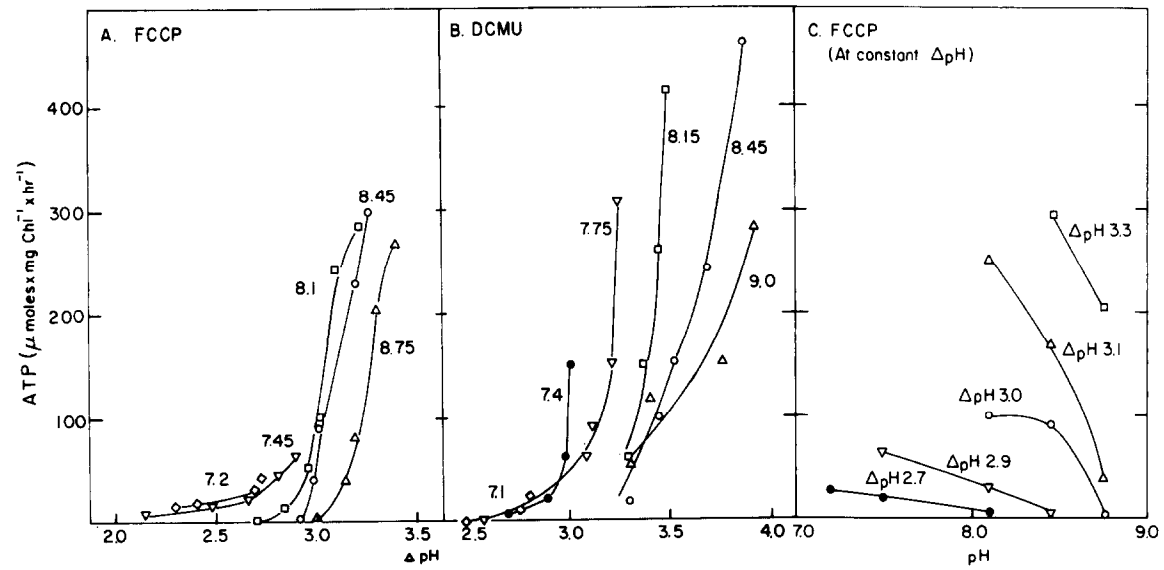

Fig. 3A, B. The dependence of photophosphorylation on ΔpH at various external pH values. The reaction medium was essentially as described under fig. 2, but contained diquat, 15 μM; and NaN_3, 1 mM in place of pyocyanine. Photophosphorylation and ΔpH were controlled by the additions of FCCP 0.1–2 μM (A) or DCMU, 3 nM–600 nM (B). C. The dependence of the rate of photophosphorylation on external pH at constant ΔpH. The data are taken from fig. 3A.

it can be seen that at *constant ΔpH,* the lower the external pH, the higher the rate of phosphorylation. Thus, at constant ΔpH, there exists a pH optimum, possibly that of the phosphorylating complex, of around 7.

We may conclude, therefore, that the rate of photophosphorylation is a function of both this optimal external pH around 7, which controls the rate of the ATP synthesizing complex and of ΔpH (optimal around pH 9.5, [3,11], which indicates the magnitude of the driving force for this reaction. The normally observed optimal pH of about 8.3 (fig. 1A) is a balance between these two optima.

It has been previously concluded [3] that even the maximal observed ΔpH values (about 4) are insufficient to account by themselves for ATP formation, if one accepts a constant stoichiometry of $2H^+/ATP$ [6] and a phosphate potential of 15–17 Kcal/mole [15]. This conclusion becomes even stronger when one considers the threshold ΔpH values of about 2.7 determined in this paper which requires that no phosphorylation would occur when $\Delta G'$ of phosphorylation is above 7.5 Kcal/mole. However, some doubt can be raised as to the validity of employing such arguments for systems whose phosphate potential was not determined. We have therefore, attempted to measure the effect of varying the phosphate potential on the dependence of phosphorylation on ΔpH. Fig.4 illustrates that changing the phosphate potential by a factor of 1:400 had no detectable effect on the threshold value or on the dependence of the rate of phosphorylation on ΔpH. A threshold value of 2.7 was observed, even when the $\Delta G'$ was as high as 13 Kcal/mole.

It was recently reported [9] that with low frequency flash illumination, ATP formation can be observed. Under these conditions it was suggested that no significant ΔpH can be maintained. We have followed directly the development of ΔpH as a function of the frequency of single-turnover flashes. Fig. 5 shows the tracing of the quenching of 9-aminoacridine fluorescence (which indicates the formation of ΔpH, [11] at several flash frequencies. Table 1 shows the ΔpH values calculated from these and similar experiments. Even at one flash per sec ΔpH values above 2.0 units were maintained. Moreover, phosphorylating conditions clearly reduced the steady state ΔpH, indicating the utilization of this energy for phosphorylation, as was previously observed under saturating light [16].

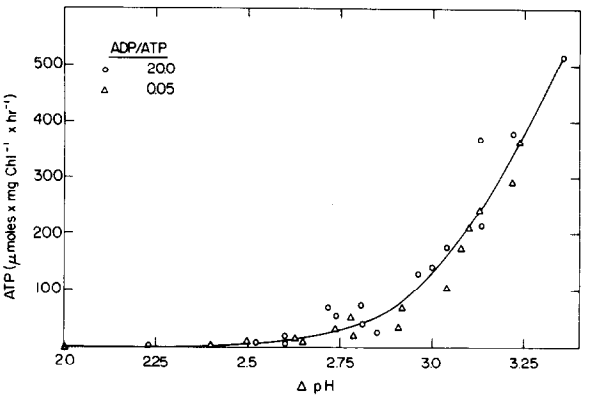

Fig. 4. The effect of phosphate potential on the dependence of photophosphorylation on ΔpH. The reaction medium contained in 3 ml; KCl, 30 mM; Na-tricine pH 8.3, 30 mM; $MgCl_2$, 5 mM; inorganic phosphate, 1.33 mM (containing P^{32}, 6×10^6 cpm/μmol); pyocyanine 30 μM; 9-amino acridine 1 μM; and chloroplasts containing 14 μg chlorophyll. The initial concentrations of ADP and of ATP were 2.0 mM and 0.1 mM or 0.1 mM and 2 mM respectively. The rate of phosphorylation and the magnitude of ΔpH were varied by changing the light intensity (red light) from $1.3 \times 10^3 - 3.5 \times 10^5$ erg $\times$ cm^{-2} $\times$ sec^{-1}. Maximal changes in $\frac{ADP}{ATP}$ ratios during the reaction in the light were between 17–20 and 0.04–0.05. ATP and ΔpH were measured as described under Materials and methods [13,11].

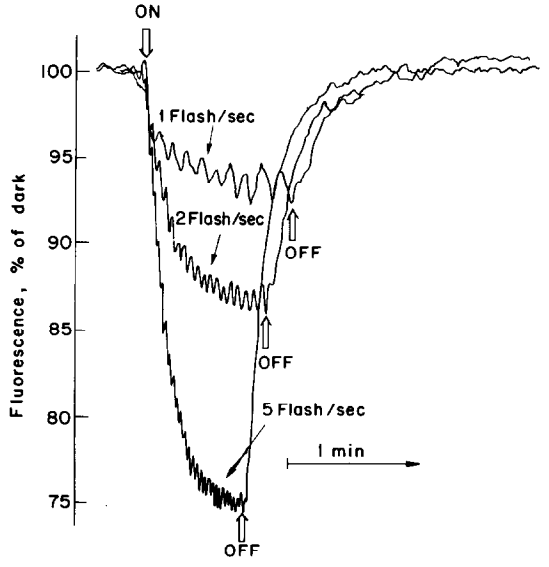

Fig. 5. Effect of the frequency of single-turnover flashes on ΔpH. The reaction medium contained in 3 ml: KCl, 40 mM; tricine-glycine-NaOH, pH 8.0, 30 mM; 9-aminoacridine, 8 μM; pyocyanine 20 μM and chloroplasts containing 60 μg chlorophyll.

Table 1
Dependence of ΔpH on the frequency of saturating single-turnover flashes

Flash frequency (flashes/sec)	Phosphorylating reagents	Quenching (%)	ΔpH
0.5	–	3	1.8
1.0	–	7	2.2
1.0	+	4	1.9
2.0	–	14	2.5
2.0	+	7	2.2
5.0	–	25	2.8
continuous light	–	63	3.5

The reaction medium was as described under fig. 5. The phosphorylating reagents added were 2 mM P_i, 2 mM $MgCl_2$ and 0.7 mM ADP.

In conclusion: It was demonstrated that the rate of photophosphorylation has a very definite dependence both on the magnitude of the ΔpH formed across the thylakoidmembrane and on the external pH. At a constant pH the rate of photophosphorylation rises with the increase in ΔpH, as expected, but there exists a definite threshold of about 2.7 pH units below which no significant phosphorylation can be observed. Surprisingly, this threshold value is insensitive to significant changes of the phosphate potential. At a constant ΔpH, optimal photophosphorylation occurs around pH 7, which is probably the pH optimum of the phosphorylating complex.

References

[1] Good, N., Izawa, S. and Hind, G. (1966) Cur. Topics Bioenergetics 1, 65–112.
[2] Jagendorf, A. T. and Uribe, E. (1966) Brookhaven Symp. Biol. 19, 215–245.
[3] Rottenberg, H., Grunwald, T. and Avron, M. (1972) Eur. J. Biochem. 25, 54–63.
[4] Avron, M. (1972) Proc. 2nd International Congress Photosynthesis, Vol. 2, pp. 861–871, Junk, The Hague.
[5] Bamberger, E., Rottenberg, H. and Avron, M. (1973) Eur. J. Biochem. 34, 557–563.
[6] Mitchel, P. (1961) Nature 191, 144–148.
[7] Schuldiner, S., Rottenberg, H. and Avron, M. (1972) FEBS Letters 28, 173–176.
[8] Schuldiner, S., Rottenberg, H. and Avron, M. (1973) Eur. J. Biochem. 39, 455–462.
[9] Boeck, M. and Witt, H. T. (1972) Proc. 2nd. International Congress Photosynthesis, Vol. 2, pp. 903–911, Junk, The Hague.
[10] Avron, M. (1960) Biochem. Biophys. Acta 40, 257–272.
[11] Schuldiner, S., Rottenberg, H. and Avron, M. (1972) Eur. J. Biochem. 25, 64–70.
[12] Chance, B. and Nishimura, M. (1967) Methods in Enzymology, Vol. 10, pp. 641–645.
[13] Avron, M. (1961) Anal. Biochem. 2, 535–543.
[14] Hardt, H. and Malkin, S. (1973) Photochem. Photobiol. 17, 433–440.
[15] Kraayenhof, R. (1969) Biochem. Biophys. Acta 180, 213–215.
[16] Pick, U., Rottenberg, H. and Avron, M. (1973) FEBS Letters 32, 91–94.