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EVIDENCE FOR CHEMIOSMOTIC COUPLING OF ELECTRON TRANSPORT TO ATP SYNTHESIS IN SPINACH CHLOROPLASTS

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SUMMARY

In spinach chloroplasts it has been shown that (1) the size of the proton gradient under phosphorylating conditions is smaller than under non-phosphorylating conditions; (2) ADP, ATP or Dio-9, added under non-phosphorylating conditions, decrease the rate of electron transport but increase the size of the proton gradient; (3) ADP, ATP or Dio-9 inhibit not only electron transport but also the rate of decay of the proton gradient; (4) the H^+/e^- ratio under non-phosphorylating conditions is 1.0. It is not affected by ADP, ATP or Dio-9.

These results show that protons pass out of the thylakoids at the site of ATP synthesis and that this leakage is inhibited by ADP, ATP or Dio-9, compounds that interact with the site of ATP synthesis. As these compounds do not alter the H^+/e^- ratio the formation of the proton gradient must be an intermediate between electron transport and ATP synthesis. These data are in support of the chemiosmotic theory of coupling of electron transport to ATP synthesis.

INTRODUCTION

Electron transport in chloroplasts is coupled both to ATP synthesis, and to the translocation of protons into the thylakoids that results in the formation of a pH gradient across the thylakoid membrane[1-3]. It has been shown that a pH gradient produced by rapidly transferring chloroplasts from acidic to basic conditions can provide the energy for ATP synthesis[4]. The mechanism by which electron transport is coupled to ATP synthesis and to proton translocation is unknown but two main hypotheses, the chemical hypotheses and the chemiosmotic hypothesis, have been proposed to explain this coupling.

Scheme A, based on the chemical hypothesis[5], assumes that electron transport is coupled to the formation of a high energy chemical compound (~) which "drives" either ATP synthesis or proton translocation. In this scheme (~) is an intermediate between electron transport and the alternative reactions of ATP synthesis and proton translocation.

Scheme A

$$\text{ET} \rightleftharpoons (\sim) \rightleftharpoons \text{ATP synthesis}$$
$$\Updownarrow$$
$$\Delta\text{pH}$$

Scheme B, the chemiosmotic hypothesis[6], assumes that electron transport is stoichiometrically coupled to the translocation of protons across the thylakoid membrane, that a pH gradient is formed which can "drive" phosphorylation through a proton translocating ATPase situated in the thylakoid membrane and that the pH gradient is an intermediate between electron transport and ATP synthesis.

Scheme B

$$ET \rightleftharpoons \Delta pH \rightleftharpoons ATP\ synthesis$$

There have been many attempts to obtain evidence in favour of one or other of these two hypotheses (see GREVILLE[7]) and in several cases it has been concluded that the results obtained require a more complex interpretation than is supplied by either Scheme A or Scheme B[8,9].

The rate of electron transport in chloroplasts has been shown to be controlled by the phosphorylation reaction[10–12]. Electron transport also appears to be controlled by the proton gradient since an increase in the rate of electron transport is observed on addition of agents which dissipate the proton gradient[13]. Control of electron transport by both the phosphorylation reaction and the proton gradient is consistent with the operation of either scheme A or scheme B.

The effect of varying conditions at the site of ATP synthesis may be predicted on the basis of either Scheme A or B.

According to Scheme A, a change in the rate of ATP synthesis (*e.g.* going from non-phosphorylating to phosphorylating conditions) would alter the relative contribution of (~) to proton translocation and ATP synthesis. Inhibition of the ATP synthesising reaction (*e.g.* by addition of an energy transfer inhibitor, or when all the ADP present in the reaction mixture has been phosphorylated to ATP) should increase the contribution of (~) to proton translocation. The increased rate of proton influx would result in an increase in the size of the proton gradient and thus increase the controlling effect of the proton gradient. The rate of electron transport would then be decreased.

According to Scheme B, a decrease in the rate of ATP synthesis (*i.e.* the rate of proton efflux, through the ATPase) would decrease the overall rate of proton efflux. The proton gradient would increase in size and the rate of proton influx would be decreased. The decrease in the rate of proton influx would be accompanied by a stoichiometric decrease in the rate of electron transport.

According to either Schemes A or B, the proton gradient would therefore be expected to be smaller under phosphorylating conditions than under non-phosphorylating conditions. SCHWARTZ[14] showed that the proton gradient is smaller under phosphorylating conditions than under non-phosphorylating conditions. KARLISH AND AVRON[8] had earlier reported that the proton gradient was increased in size by phosphorylating conditions and concluded that neither scheme correctly described the coupling mechanism. The discrepancy between these two reports may be due to the fact that the measurement of the size of the proton gradient under phosphorylating conditions is complicated by the occurrence of another increase in pH, due to the consumption of protons during the phosphorylation of ADP to ATP.

Experimentally it should be possible to distinguish between the two schemes because there is an essential difference between Scheme A and Scheme B. In Scheme B

the H^+/e^- ratio is an unvarying whole number under all conditions, while in Scheme A the H^+/e^- ratio varies as the relative contribution of ($\sim$) to ATP synthesis and proton translocation is altered.

The values reported for the H^+/e^- ratio under non-phosphorylating conditions are extremely variable[8,14-18]. This is in part due to the difficulty in obtaining accurate measurements of rapid changes in pH using a glass electrode[17] and in part due to the difficulty in measuring the initial rate of electron transport during the establishment of the proton gradient.

DILLEY[19] has measured the H^+/e^- ratio under both non-phosphorylating conditions and phosphorylating conditions in the presence and absence of energy transfer inhibitors. He found that phosphorylating conditions decreased the H^+/e^- ratio while addition of energy transfer inhibitors increased the H^+/e^- ratio. Although these results might be interpreted as supporting Scheme A, DILLEY[19] proposed a more complex explanation.

We have investigated the effect of various conditions on the rate of electron transport, on the size of the proton gradient, on the initial rate of proton efflux and on the H^+/e^- ratio and we have obtained results consistent with the operation of Scheme B rather than Scheme A.

METHODS

Broken washed chloroplasts (P_1S_1) were isolated essentially as described by WHATLEY AND ARNON[1] from greenhouse grown spinach. The chlorophyll concentration was measured by the method of ARNON[20].

Pseudocyclic electron transport catalysed by methyl viologen was measured as an uptake of oxygen in a Rank oxygen electrode cell. Changes in pH were measured with a Radiometer pH meter (PH M26) and a Radiometer combined glass electrode (GK 2321C). The glass electrode was inserted through the lid of the oxygen electrode cell so that simultaneous measurements of oxygen and pH changes could be recorded on a dual channel recorder.

The reaction mixture was maintained at a temperature of 20° and was illuminated by two slide projectors (300 W each) through 3 cm water and a filter transmitting light between 540 and 740 nm.

The pH of the reaction mixture was initially adjusted to approx. pH 8.3 with KOH. The pH scale used was calibrated in terms of proton equivalents by the addition of standard amounts of HCl at the end of each experiment. There was no significant difference between the buffering characteristics of the chloroplasts in the light or in the dark (see POLYA AND JAGENDORF[21]).

Measurement of the H^+/e^- ratio

The H^+/e^- ratio was calculated from measurements of the initial rate of proton efflux, after turning off the light, and of the steady state rate of electron transport, before turning off the light.

The glass electrode used in these experiments was found to respond rather slowly to rapid changes in pH, *i.e.* an "overshoot" was observed after short periods of illumination (see JAGENDORF AND NEUMANN[13]). In order to measure the initial rate of proton efflux it was necessary to apply the following correction procedure.

Chloroplasts were illuminated and the apparent kinetics of the pH rise were recorded as shown in Fig. 1. The light was then turned off for a 2-sec dark period. It can be seen that the observed pH decrease in the dark continued in the subsequent light period for almost 2 sec before it increased again to the original pH value observed in the light. The pH minimum of the overshoot curve represents the real pH value at that time because there is no change in pH and the glass electrode is able to respond accurately.

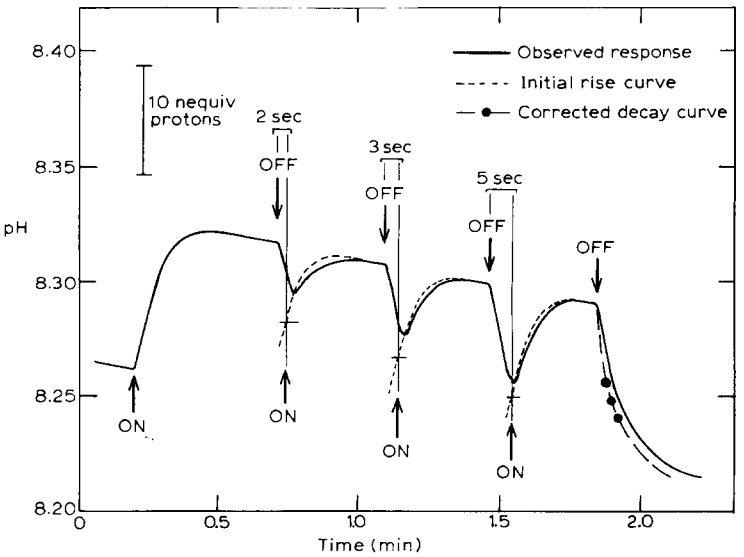


Fig. 1. Estimation of the real pH decay curve by analysis of overshoot curves obtained during brief periods of darkness. See text for details of this technique. The reaction mixture contained in a total volume of 3 ml: chloroplasts equivalent to 250 μg chlorophyll; 150 μmoles KCl; 6 μmoles $MgCl_2$; 6 μmoles sodium azide; 0.275 nmoles methyl viologen and 1.5 μmoles potassium phosphate (pH 8.3). Reaction conditions were as described in METHODS.

The dotted line represents the initial pH rise curve which has been replotted so that it passes through the point at which the pH has reached its minimum value. The point at which the pH rise curve intersects the time at which the light was turned on again is assumed to be the real value of the pH after 2 sec darkness. This procedure was repeated twice after 2-, 3-, 4-, 5- and 6-sec dark periods.

Using the values, obtained by this method, for the true pH at the end of each dark period a corrected pH decay curve was constructed (dashed line). In Fig. 1 both the observed and the corrected decay curves are shown. The rates of decay of the proton gradient at 1-sec intervals from 2 to 6 sec were then measured from the corrected curve.

As the decay of the proton gradient has been shown to obey first order kinetics[17] the initial rate of proton efflux can be obtained from a semi-logarithmic plot of the rate of decay against dark time. The initial rate of proton efflux, *i.e.* the rate of decay of the proton gradient at dark time zero, and the steady rate of electron transport measured as an oxygen uptake during the period of illumination can then be used to calculate the H^+/e^- ratio.

RESULTS

Fig. 2 shows the change in pH observed under non-phosphorylating and phosphorylating conditions. Addition of ADP, to an otherwise complete phosphorylating medium initiates a linear rate of increase in pH due to the uptake of protons during ATP formation as well as the proton gradient formation, seen in the absence of ADP. A limiting concentration of the electron acceptor methyl viologen was used because of the slow response of the glass electrode to rapid changes in pH such as are found in the presence of a non-limiting concentration of electron acceptor. When the rate of electron transport is limited by the acceptor concentration both ATP synthesis and proton gradient formation are decreased to such an extent that it is possible to distinguish between the rapid proton gradient formation and the slower pH change due to ATP synthesis. Thus the size of the pH change due to proton gradient formation could be measured. Under these conditions the rate of electron transport, measured simultaneously as an oxygen uptake, was not significantly affected by the addition of ADP.

As previously stated the glass electrode used in the experiments reported here was found to respond slowly to rapid changes in pH (see IZAWA AND HIND[17]). It may therefore be assumed that although in Fig. 2 the proton gradient appears to require almost 15 sec before steady state conditions are established this is a reflection of the slow response of the glass electrode and that the proton gradient was fully established in a shorter time.

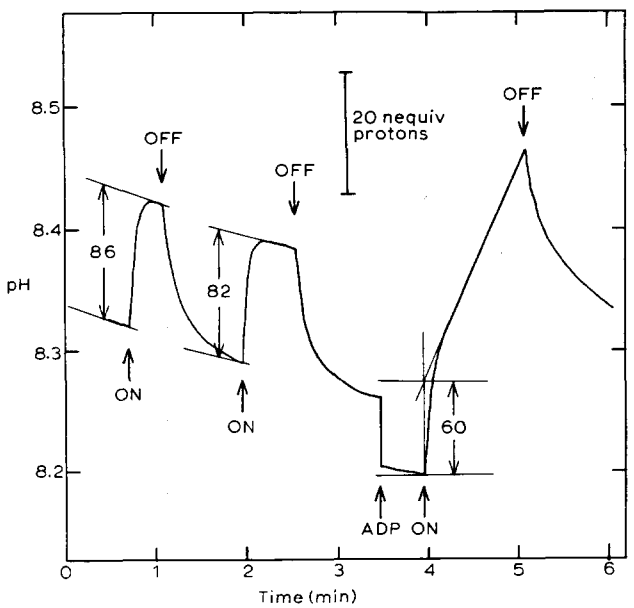
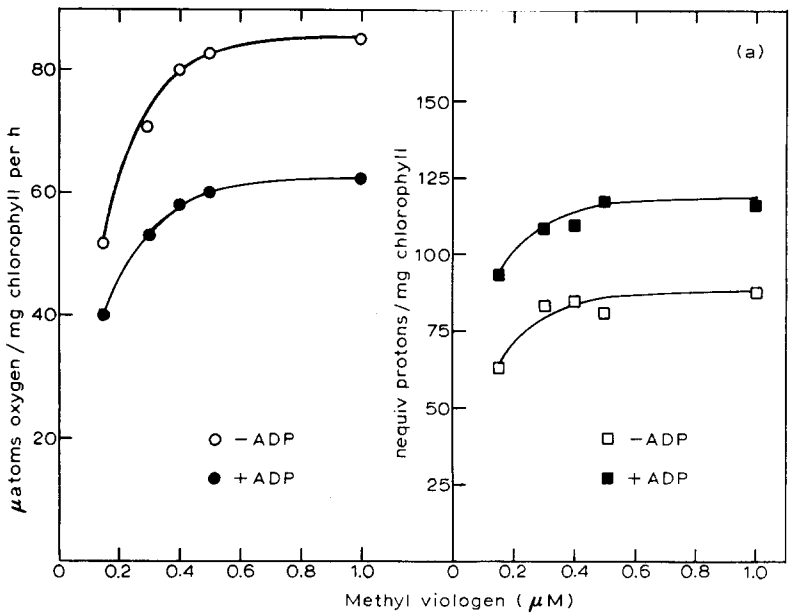
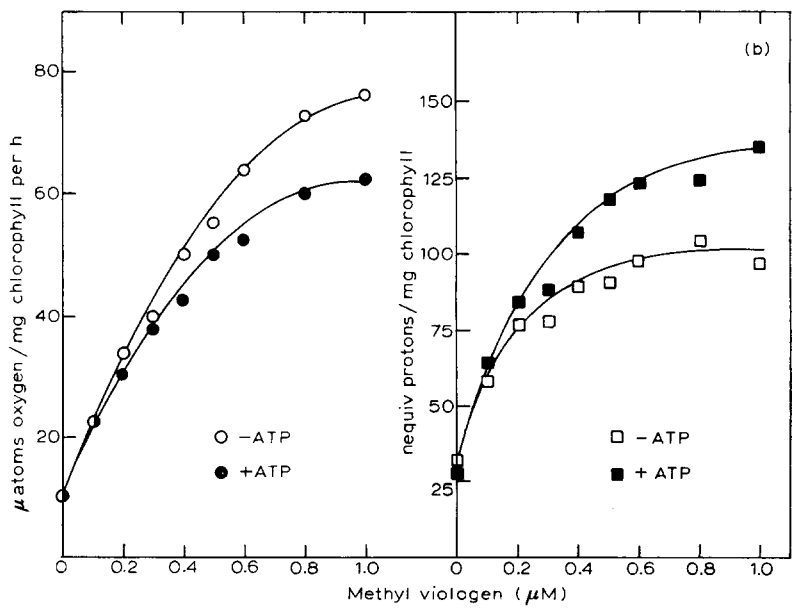


Fig. 2. Comparison of light driven proton gradient formation in chloroplasts under either phosphorylating or non-phosphorylating conditions. The reaction mixture contained in a total volume of 3 ml: chloroplasts equivalent to 200 μg chlorophyll; 150 μmoles KCl; 6 μmoles $MgCl_2$; 3 μmoles sodium azide; 0.375 nmole methyl viologen and 1.5 μmoles potassium phosphate (pH 8.3). 0.08 μmole ADP (pH 8.2) were added as indicated. Construction lines show the extent of the pH change due to proton gradient formation taking into account the general decrease in pH seen both in the light and the dark. Reaction conditions were as described in METHODS.

If the assumption is made that a linear rate of phosphorylation is initiated immediately on illumination, it can be seen that the change in pH due to the formation of the proton gradient is smaller under phosphorylating conditions than under non-phosphorylating conditions (60 nequiv protons/mg chlorophyll as compared with approx. 84 nequiv protons/mg chlorophyll).

Even if it is assumed that phosphorylation is not initiated until the proton





gradient has been established it would be expected to begin after no more than 2 sec illumination (SCHWARTZ[14]). Fig. 2 shows that after 2 sec illumination the proton gradient is still smaller under phosphorylating conditions than under nonphosphorylating conditions (64 nequiv protons/mg chlorophyll as compared to approx. 84 nequiv protons/mg chlorophyll).

Figs. 3a, 3b and 3c respectively show the effect of ADP, ATP and Dio-9 (and energy transfer inhibitor[22]) on the non-phosphorylating rate of electron transport and size of the proton gradient, over a range of methyl viologen concentrations. The low rate of electron transport and the small proton gradient established in the absence of added electron acceptor is due to the presence of an endogenous electron acceptor that catalyzes a pseudocyclic flow of electrons to oxygen.

Figs. 3a, 3b and 3c show that all three compounds, ADP, ATP and Dio-9 decrease the rate of electron transport and at the same time increase the size of the proton gradient, except at very low concentrations of electron acceptor.

Table Ia shows the effect of ADP, ATP and Dio-9 on the size of the proton gradient, the initial rate of proton efflux and the first order rate constant of the decay reaction. In these three experiments the size of the proton gradient is increased by addition of all three compounds while the initial rate of proton efflux and the first order rate constant are decreased. In all our experiments addition of these compounds caused an increase in the size of the proton gradient and a decrease in the rate constant of proton efflux. In some experiments the rate of proton efflux was, however, higher

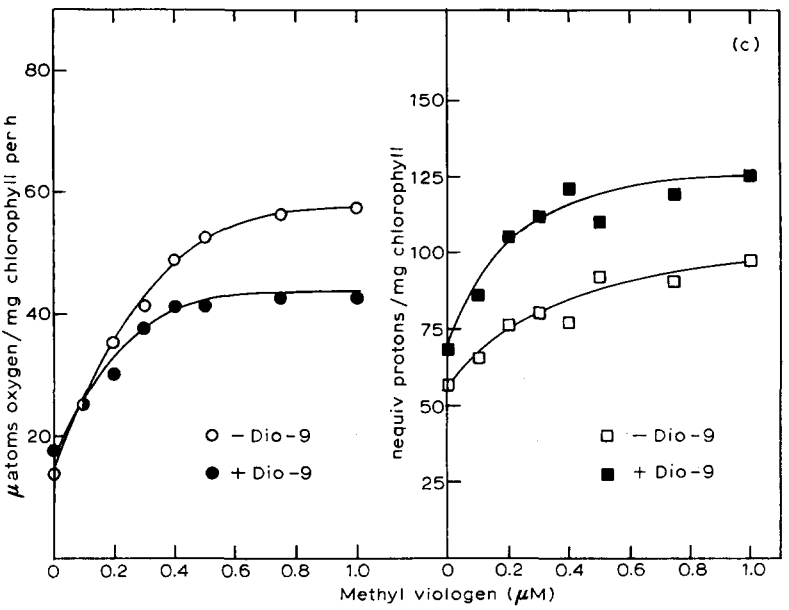


Fig. 3. Effect of electron acceptor concentration on the rate of electron transport and the size of the proton gradient in chloroplasts under non-phosphorylating conditions in the presence and absence of (a) ADP; (b) ATP; (c) Dio-9. The reaction mixtures were as described in Fig. 2, except that phosphate was omitted, methyl viologen was added in the concentrations indicated and 0.12 μmole ADP, 0.06 μmole ATP or 10 μg Dio-9 were added as indicated. Reaction conditions were as described in METHODS.

TABLE Ia

THE EFFECT OF ADP, ATP AND Dio-9 ON THE SIZE OF THE PROTON GRADIENT, THE INITIAL RATE OF PROTON EFFLUX AND THE FIRST ORDER RATE CONSTANT OF THE DECAY REACTION

Expt. No.	*Additions*	*Size of proton gradient (μequiv H^+/mg chlorophyll)*	*Initial rate of proton efflux (μequiv H^+/mg chlorophyll per h)*	*First-order rate constant*
i	—	0.145	105	722
	ADP	0.183	94	513
ii	—	0.127	94	740
	ATP	0.143	87	610
iii	—	0.132	154	1170
	Dio-9	0.170	124	730

TABLE Ib

THE EFFECT OF ADP, ATP AND Dio-9 ON THE MEAN VALUE OF THE INITIAL RATE OF PROTON EFFLUX

The reaction mixtures contained in a total volume of 3 ml: chloroplasts equivalent to 200 μg chlorophyll; 150 μmoles KCl; 6 μmoles $MgCl_2$; 3 μmoles sodium azide and in Table Ia 1.8 nmoles methyl viologen. In Table Ib the concentration of methyl viologen was varied between 0.4 and 1.0 μM. 0.12 μmole ADP, 0.06 μmole ATP and 10 μg Dio-9 were added as indicated. Other details were as described in METHODS.

Additions	*No. of determinations*	*Mean value of initial rate of proton efflux (μequiv H^+/mg chlorophyll per h)*	*S.D.*
—	13	99	24
ADP	5	108	29
ATP	5	92	18
Dio-9	6	115	11

in the presence of these compounds. Table Ib shows the mean values for the rate of proton efflux from a number of experiments and shows that the addition of ADP, ATP or Dio-9 does not significantly affect the rate of proton efflux.

In Fig. 4 the rate of decay of the proton gradient during the first 6 sec of darkness is plotted semi-logarithmically against the dark time. Figs. 4a, 4b and 4c show respectively the effect of addition of ADP, ATP and Dio-9 under non-phosphorylating conditions. Addition of these compounds decreases the rate of decay of the proton gradient (represented by the slope of the curve) and also slightly decreases the initial rate of proton efflux (the rate of proton efflux at dark time zero).

Fig. 4 also shows the effect of ADP, ATP and Dio-9 on the H^+/e^- ratio. The H^+/e^- ratios are calculated from the initial rate of proton efflux and the steady state rate of electron transport (measured before turning off the light). The value of the H^+/e^- ratio is increased by all three compounds ADP, ATP and Dio-9. Table II which gives the mean value of a number of determinations of the H^+/e^- ratio, shows that the increase in the H^+/e^- ratio varies between 12 % and 40 %.

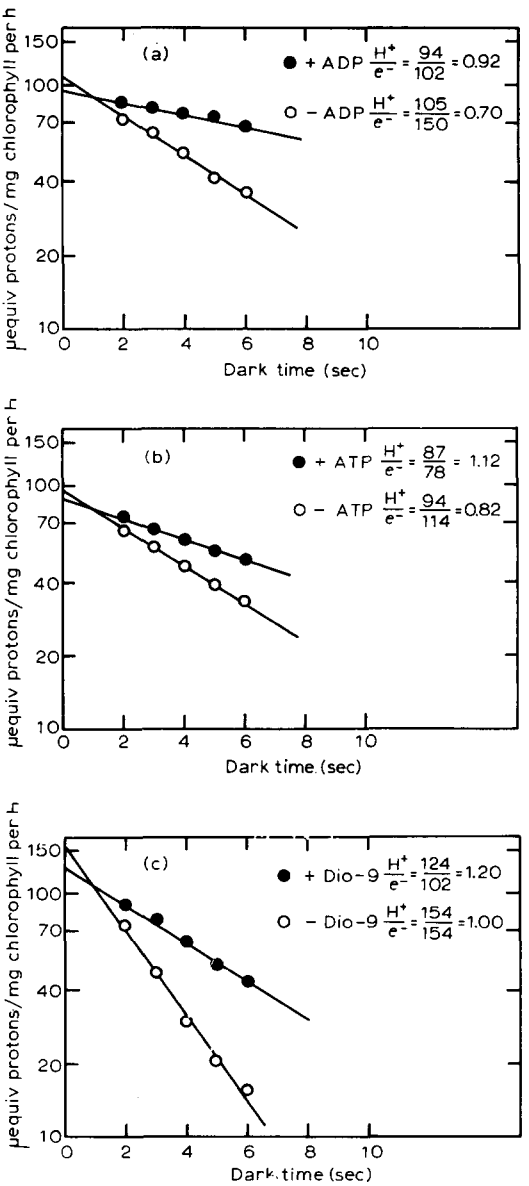


Fig. 4. Semilogarithmic plot of the rate of proton efflux as a function of dark time, showing the effect of (a) ADP; (b) ATP; (c) Dio-9. The H^+/e^- ratios have been calculated from the initial rate of proton efflux (*i.e.* rate of proton efflux at dark time zero-expressed as μequiv protons/mg chlorophyll per h) and the steady state rate of electron transport (measured before turning off the light and expressed as μequiv electrons/mg chlorophyll per h). The reaction mixtures were as described in Table Ia.

DISCUSSION

It has been shown in Fig. 2 that, under conditions when the rate of electron transport is limited by the concentration of electron acceptor, the size of the proton

TABLE II

THE EFFECT OF ADP, ATP AND Dio-9 ON THE MEAN VALUE OF THE H^+/e^- RATIO

The reaction conditions were as described in Table I, except that the methyl viologen concentration varied between 0.1 and 1.0 μM.

Additions	*No. of determinations*	*Mean value of H^+/e^- ratio*	*S.D.*
—	23	0.77	0.18
ADP	9	0.87	0.18
ATP	9	1.02	0.15
Dio-9	9	1.09	0.20

gradient is smaller under phosphorylating conditions than under non-phosphorylating conditions. This shows the close relationship between proton gradient formation and phosphorylation (as in Scheme B).

This experiment corroborates the report of SCHWARTZ[14] that phosphorylation decreases the size of the proton gradient. SCHWARTZ[14] showed that the proton gradient was decreased in size by phosphorylation when the electron transport rate was not limited at the level of the electron transport chain. In his experiments, initiation of ATP synthesis would have released the controlling effect on the electron transport rate that was imposed under non-phosphorylating conditions.

Fig. 3 shows that addition of ADP, ATP or Dio-9 under non-phosphorylating conditions decreases the rate of electron transport and increases the size of the proton gradient. It has been known for some time that both ADP and ATP inhibit the non-phosphorylating rate of electron transport[23] and GROMET-ELHANEN[24] showed that Dio-9 inhibits not only the phosphorylating rate of electron transport but also the non-phosphorylating rate of electron transport to the same low rate of electron transport. However, it has not previously been shown that this decrease in the rate of electron transport is accompanied by an increase in the size of the proton gradient. The effect, on the rate of electron transport and the size of the proton gradient, observed on addition of ADP, ATP or Dio-9 is the reverse of the effect observed on initiation of phosphorylation.

The effect of these compounds suggests that under non-phosphorylating conditions there is normally some breakdown of the high energy intermediate (either ($\sim$) or ΔpH) at the site of phosphorylation. Our results show that ADP, ATP and Dio-9 inhibit this breakdown.

The most likely site of action of ADP, ATP and Dio-9 is the site of phosphorylation itself.

According to Scheme A addition of any of these compounds would result in a decrease in the rate of ($\sim$) breakdown at the phosphorylation site, an increase in the rate of proton influx and therefore an increase in the size of the proton gradient which would ultimately cause a decrease in the rate of electron transport. On the other hand according to Scheme B there would be a decrease in the rate of efflux of protons causing an increase in the size of the proton gradient, a decrease in the rate of influx of protons and a decrease in the rate of electron transport.

It therefore follows that the two Schemes, A and B, cannot be separated by the effect of these compounds on the steady state extent of the proton gradient or on the rate of electron transport. However, the two schemes should be distinguished by the effect of these compounds on the rate of proton efflux and on the H^+/e^- ratio.

It has been shown in Tables Ia and Ib that although the size of the proton gradient is increased the initial rate of proton efflux is not significantly altered by the addition of ADP, ATP or Dio-9. However, Table Ia and Fig. 4 show that the rate constant of the decay reaction is decreased by the addition of ADP, ATP or Dio-9. Thus these compounds must inhibit proton efflux. This is consistent with Scheme B.

A large increase in the initial rate of efflux would have been expected if Scheme A were in operation, as the initial rate of proton efflux should increase exponentially with increase in size of the proton gradient. If the rate constant of the decay reaction were unaffected, as would be consistent with Scheme A, the expected increase in the H^+/e^- ratio may be calculated as follows from the rate constant, obtained in the absence of additions. On the basis of the increase in the size of the proton gradient and decrease in the rate of electron transport, caused by addition of ADP, ATP or Dio-9, the H^+/e^- ratio would be expected to be increased by 80–100 %. Table II shows that the observed increases in the H^+/e^- ratio were between 15–40 %. It is therefore suggested that the observed increases in the H^+/e^- ratio obtained on addition of ADP, ATP or Dio-9 are not significant.

We propose that phosphorylation is coupled to electron transport by a chemiosmotic mechanism, as shown in Scheme B, and not by a chemical mechanism, as shown in Scheme A.

The results and conclusions presented here are in agreement with the results of SCHWARTZ[14] who found that phosphorylation increased the rate of proton efflux as compared to the rate in the absence of phosphorylation. SCHWARTZ[14] did not, however, measure the rate of electron transport during these experiments so the effect of phosphorylation on the H^+/e^- ratio is not known.

The results and conclusions presented here do not agree with those of DILLEY[19]. He reported that phosphorylation decreased and Dio-9 increased the H^+/e^- ratio while it has been shown here that under a variety of different conditions the H^+/e^- ratio does not alter significantly. However, the H^+/e^- ratios reported here were calculated from the initial rate of efflux of protons and steady state rate of electron transport while DILLEY[19] calculated the H^+/e^- ratios from the initial rate of influx of protons and the steady state rate of electron transport.

DILLEY[19] states that the rate of electron transport is linear after about 2 sec of illumination. It has been shown, previously, that there is an initial fast rate of electron transport before the controlling effect of the proton gradient slows the non-phosphorylating rate of electron transport to its steady state rate[25,26]. It has also been shown that the proton gradient is fully established in a short time, the duration of which is dependent on pH. SCHWARTZ[14] has shown, using an electrode with a very rapid response time, that at pH 8.0 the proton gradient is fully established in 2 sec. Thus at pH 8.2, as in the experiments of DILLEY[19], an initial fast rate of electron transport would only be expected to be observed during the first 2 sec of illumination, while DILLEY[19] measured his initial rate of electron transport "from about 2 sec" of illumination.

As control of the rate of electron transport only occurs once the proton gradient

is fully established, the initial rate of electron transport should be the same under different conditions (*e.g.* non-phosphorylating compared to phosphorylating conditions).

The H^+/e^- ratios may be re-calculated from the data of DILLEY[19] using the rate of electron transport measured under phosphorylating conditions which was the fastest rate measured and thus the rate most closely related to the fast initial rate of electron transport. If the H^+/e^- ratios are re-calculated using the rate of electron transport, measured under phosphorylating conditions in each case, and the initial rate of proton influx measured under the following different conditions: phosphorylating conditions (*i.e.* + ADP + P_i), non-phosphorylating conditions (*i.e.* −ADP + P_i) and in the presence of Dio-9 (*i.e.* + ADP + P_i + Dio-9 or −ADP + P_i + Dio-9) the values obtained are all approx. 1 and the variation observed is only 40 %. Unlike the variation of 400 % observed in the values calculated by DILLEY[19], the variation of 40 % cannot be correlated with the alteration of conditions.

Thus, the data of DILLEY[19] may be re-interpreted as showing that neither phosphorylation nor Dio-9 affect the H^+/e^- ratio significantly and that the H^+/e^- ratio is approx. 1.

If a chemiosmotic mechanism is in operation the H^+/e^- ratio must be a whole number. A wide range of values have been obtained for the H^+/e^- ratio, but of the more recent measurements, in which account has been taken of the slow response of glass electrodes to rapid changes in pH, the majority of values obtained have been between 1.0 and 2.0. 1.0 is the value obtained when the steady state electron transport rate and the initial rate of efflux of protons are used for the calculation as reported here and by RUMBERG *et al.*[18].

However, when the initial rates of electron transport and proton influx are used for the calculation the value obtained is 2.0 (ref. 17, 18). A value of 2.0 is also obtained if the steady state rate of electron transport is measured by the pH decrease observed when the electron acceptor employed releases protons on reduction and the initial rate of proton efflux is measured by the rate of decay of the proton gradient[14].

It is not known whether the H^+/e^- ratio is 1.0 as is reported here or whether, as proposed by RUMBERG *et al.*[18], the ratio is 2.0. Despite the doubt as to the true value of the H^+/e^- ratio the results presented here may be explained in the following scheme based on the chemiosmotic hypothesis.

Electron transport is coupled to stoichiometric pumping of protons across the thylakoid membrane. Protons can pass back across the membrane both non-specifically and also through the ATPase which is situated in the thylakoid membrane. Under phosphorylating conditions ATP synthesis occurs as a result of the transfer of the protons back across the membrane through the ATPase. Under non-phosphorylating conditions protons pass back across the membrane not only non-specifically but also through the ATPase. The leakage of protons through the ATPase is inhibited by ADP, ATP and Dio-9 and the control exerted by these compounds on the rate of electron transport is a result of this inhibition of proton efflux.

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