Biochimica et Biophysica Acta, 423 (1976) 510–523

BBA 47049

DETERMINATION OF H^+/e^- RATIOS IN CHLOROPLASTS WITH FLASHING LIGHT

CHARLES F. FOWLER and BESSEL KOK

Martin Marietta Laboratories, 1450 South Rolling Road, Baltimore, Md. 21227 (U.S.A.)

(Received August 19th, 1975)

SUMMARY

Using a rapid pH electrode, measurements were made of the flash-induced proton transport in isolated spinach chloroplasts. To calibrate the system, we assumed that in the presence of ferricyanide and in steady-state flashing light, each flash liberates from water one proton per reaction chain. We concluded that with both ferricyanide and methylviologen as acceptors two protons per electron are translocated by the electron transport chain connecting Photosystem II and I. With methyl viologen but not with ferricyanide as an acceptor, two additional protons per electron are taken up due to Photosystem I activity. One of these latter protons is translocated to the inside of the thylakoid while the other is taken up in H_2O_2 formation. Assuming that the proton released during water splitting remains inside the thylakoid, we compute H^+/e^- ratios of 3 and 4 for ferricyanide and methyl viologen, respectively.

In continuous light of low intensity, we obtained the same H^+/e^- ratios. However, with higher intensities where electron transport becomes rate limited by the internal pH, the H^+/e^- ratio approached 2 as a limit for both acceptors.

A working model is presented which includes two sites of proton translocation, one between the photoacts, the other connected to Photosystem I, each of which translocates two protons per electron. Each site presents a $\approx$ 30 ms diffusion barrier to proton passage which can be lowered by uncouplers to 6–10 ms.

INTRODUCTION

According to the chemi-osmotic hypothesis [1] and its particular application to photophosphorylation, strict stoichiometry should exist between the number of protons released within the thylakoid space, the electrons transported and ATP synthesized [2–4].

Among these stoichiometries the H^+/e^- ratio has proven to be one of the most difficult to obtain. Typical numbers have ranged from 1 to 5 depending upon the measuring procedure [5–9] and the pH of the suspending medium [10]. The possible reasons for such large variances have been reviewed elsewhere and will not be discussed here [4].

In this paper, we describe a systematic H^+/e^- measurement in flashing light using an improved glass electrode technique. This electrode has adequate sensitivity and time response to measure the extent and the kinetics of pH changes induced by single turnovers of the electron transport system.

MATERIALS AND METHODS

Chloroplasts were isolated daily from freshly harvested greenhouse spinach by methods previously described [11]. They were kept in stock suspensions containing between 2 and 5 mg/ml total chlorophyll in a solution containing 50 mM sodium tricine buffer, pH 7.3, 50 mM NaCl and 0.4 M sucrose. For each measurement an aliquot of the stock solution was placed into a medium containing 50 mM NaCl and 0.4 M sucrose to a final concentration of approx. 50 μg/ml chlorophyll. The final pH of the suspension was between 6.7 and 7.0 except when otherwise specified. The non-cyclic electron acceptors methyl viologen or $K_3Fe(CN)_6$ and uncouplers were added as described in the text.

The equipment and the techniques used for the pH measurement have been described in a previous paper [12]. Fig. 1 shows the arrangement of the electrode assembly. The time response of the system was checked by giving flashes to a mixture of phenazine methosulfate (200 μM) and cytochrome *c* (1 mM) in tricine buffer (pH 7.4). The flash-induced pH changes as recorded by our equipment were completed in less than a millisecond. A half-silvered mirror was included in the light path to allow both continuous and flash illumination. *P*-700 measurements were made as previously described [13].

RESULTS

Calibration of flash-induced pH changes

Figs. 2a and 2b show the flash-induced pH changes induced by sequences of intensity saturated flashes spaced 1 s apart. The suspension contained 1.0 mM ferricyanide serving as the electron acceptor (results were independent of ferricyanide concentration from 0.05 to 2 mM) and 30 mM methylamine serving to allow free

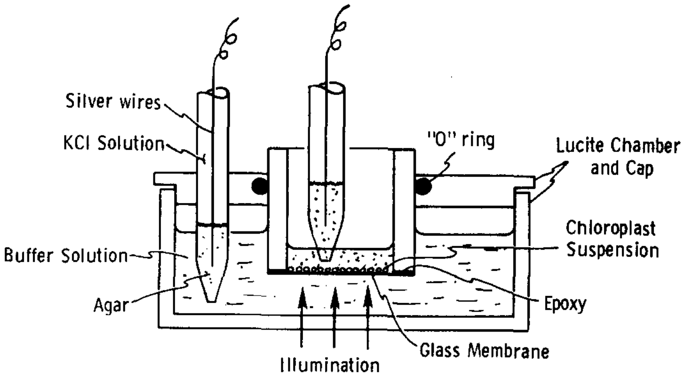

Fig. 1. Diagram of the electrode assembly for measuring flash-induced pH changes in spinach chloroplast suspensions.

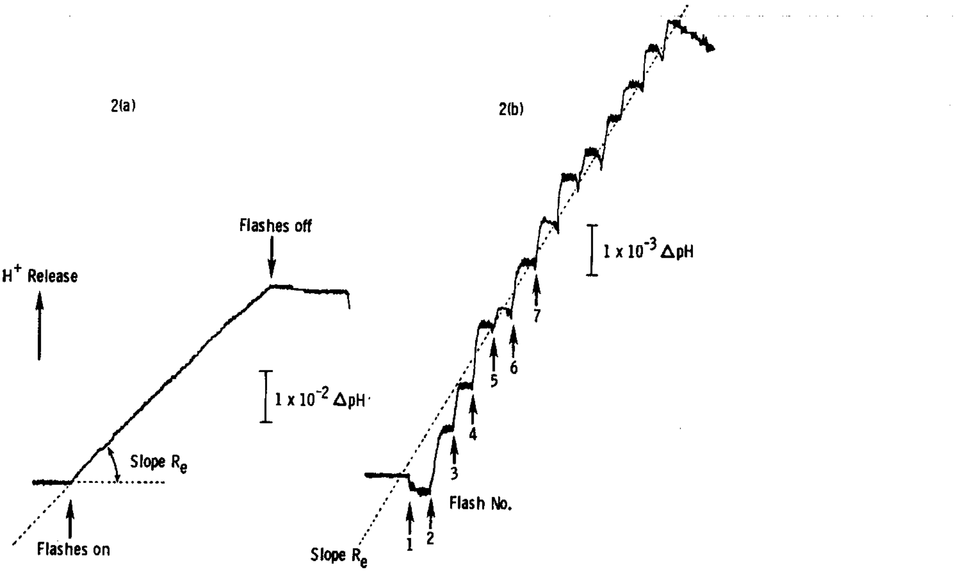

Fig. 2. Changes of pH induced by short saturating flashes in spinach chloroplast suspensions containing 1.0 mM $K_3Fe(CN)_6$ as acceptor. Chloroplasts were suspended in 0.4 M sucrose and 0.05 M NaCl, and 0.03 M methylamine. Chlorophyll concentrations were between 10 and 20 μg/ml. Flashing rate was 1 per s. (a) Linear pH change induced by 50 flashes. (b) pH change induced by individual flashes. Gain was increased 10 times and time base expanded five times compared to a.

movement of protons across the thylakoid membrane. In this case, the following reaction takes place:

$$1/2\ H_2O + Fe(CN)_6{}^{3-} \xrightarrow{h\nu} 1/4\ O_2 + H^+ + Fe(CN)_6{}^{4-} \qquad (1)$$

In the steady state each flash releases 1/4 O_2 and one proton per System II trapping center and moves one electron through each electron transport chain to ferricyanide.

Fig. 2a shows that a sequence of 50 flashes induces a linear decrease of pH. The same experiment was repeated in Fig. 2b with higher sensitivity and increased time resolution so that the time course and amplitude of individual flashes could be observed. The half-time of the proton release was dependent upon the concentration of the uncoupler (either methylamine or gramicidin d) but was never shorter than 50 ms. The proton yields are not released monotonically as a function of flash number, but show a damped oscillation.

This oscillatory behavior will be discussed in more detail in a later paper. It is not important for the objectives of this paper because the average of the first few flashes was equal to the steady-state yield obtained after 12 or more flashes. The average values of ΔpH/flash calculated from Figs. 2a and 2b were identical. We denote this average as "ΔpH_e" and assume it equals 1 H$^+$ per reaction chain and per electron moved from water to ferricyanide as shown in Eqn. 1. This number was measured for each sample and served as the calibration for all quantitative analyses of other flash-induced pH changes.

ΔpH_e, usually of the order of 10^{-3}, is a relative number, affected by the buffer capacity and chloroplast content of the medium. In our experiments, the buffering capacity was maintained at a level high enough so that the pH changes were linearly related to H^+ concentration.

Flash yields of proton uptake with ferricyanide and methyl viologen

Figs. 3a and 3b show experiments similar to those of Figs. 2a and 2b, except that no uncoupler was added to the suspension. The flash sequences were given after a dark time of 5 min. As shown in Fig. 3a, a net uptake of protons occurs during an initial phase, which lasts for about 10 flashes. Fig. 3b shows that the amount of this uptake remains constant and independent of flash number. We denote the average single flash uptake as ΔpH_f. (Again we neglect at this moment a slight oscillation with a period of two which is apparent in the data). During the (1 s) dark times between the flashes, there occurs a back-reaction (acidification) the rate of which increases with flash number. Ultimately, after approx. 20 flashes, as shown in Fig. 3a, this acidification predominates and the balance of uptake and release leads to slope R_e and a ΔpH_e value identical to the one seen in the steady state in Figs. 2a and 2b. The zero time intercept of the extrapolated line R_e (see Fig. 3a) represents the maximum pool size H_f obtained with the given flashing rate.

The ratio between ΔpH_f and ΔpH_e was measured in 10 experiments and was found to be 2.0 ($\sigma = 0.2$). This leads to the conclusion that in the presence of ferricyanide 2.0 protons were taken up from the suspension medium (translocated) per electron transferred through the reaction chain.

Figs. 4a–4c show similar experiments in which methylviologen was used as the electron acceptor. In this case, the following reactions are induced by the light:

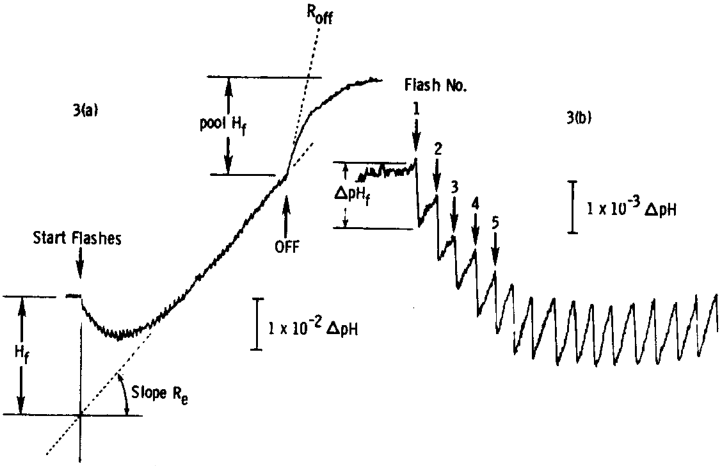

Fig. 3. Changes of pH induced by short saturating flashes in a spinach chloroplast suspension containing ferricyanide as acceptor. The suspending medium and chlorophyll concentration was the same as Fig. 1. (a) pH change induced by a sequence of 50 flashes. (b) pH changes with individual flashes. Gain was increased 10 times and time base expanded five times compared to a.

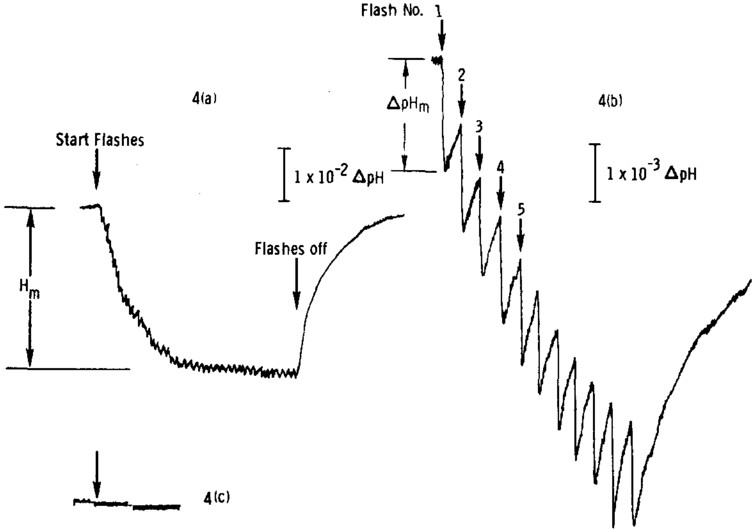

Fig. 4. Change of pH induced in a sample containing methyl viologen (100 μM) as acceptor (conditions otherwise same as Fig. 1). (a) pH change occurring with a sequence of 45 flashes. (b) pH change following individual flashes. Gain was 10 times and the time base expanded 5 times compared to a. (c) pH change induced by a sequence of flashes following the addition of the uncoupler methylamine (0.03 M). Gain and time span was the same as Fig. 3a.

$$1/2\ H_2O \xrightarrow{h\nu} 1/4\ O_2 + e^- + H^+ \tag{2}$$

$$H^+ + e^- + 1/2\ O_2 \xrightarrow{\text{methyl viologen}} 1/2\ H_2O_2 \tag{3}$$

$$\overline{1/2\ H_2O + 1/4\ O_2 \longrightarrow 1/2\ H_2O_2} \tag{4}$$

According to Eqn. 4, there is no net steady-state release of protons in the light. Indeed, in the presence of an uncoupler the flashes do not induce any pH change (Expt. Fig. 4c). In the absence of methylamine (Fig. 4a) the steady-state pH change is also zero, but initially, during the first $\approx$ 20 flashes, there occurs a strong net H^+ uptake and pool formation.

Fig. 4b shows that, as was observed with ferricyanide, each flash induces an immediate uptake of protons with a halftime of approx. 30 ms (see also Fig. 9). The amount of this uptake, denoted ΔpH_m, was again independent of flash number. A progressively accelerating backreaction (H^+ release) decreased the net proton exchange until it became zero in the steady state.

Using the described calibration procedure (Fig. 2), a number of measurements (15) show that with viologen, the rapid flash-induced proton uptake was twice as large as seen with ferricyanide. It amounted to 4 (3.9, $\sigma = 0.5$) protons per electron transferred from water to viologen.

Analysis of H^+/e^- ratios in flash sequences

From the flash yields of proton uptake, as determined above we can calculate the H^+/e^- ratio: the number of protons which accumulated inside the thylakoid per flash, i.e. per electron transfer through the chain. With both acceptors a proton is released per flash inside the thylakoid due to the O_2 evolution process [12]. In the presence of ferricyanide this proton is observed immediately in the presence of an uncoupler (Figs. 2a and 2b) and after steady state is attained in the absence of an uncoupler (Figs. 3a and 3b).

This extra proton from the O_2 system must be added to the two protons which are taken up from the suspension medium and translocated (see above). Therefore per flash three protons accumulate within the chloroplast membrane.

With methyl viologen as the acceptor one proton is taken up per electron passing through the chain in the terminal formation of peroxide (Eqn. 3). Therefore of the four protons which are taken up three are translocated. In addition one proton from the O_2 evolution is released inside the membrane. Therefore in the presence of viologen the H^+/e^- ratio is 4.

In addition to considering the pH changes induced by individual flashes we can also analyze the pH changes induced by the entire sequence, i.e. the envelopes of the curves in Figs. 2a, 3a and 4a. A similar analysis was done previously for observations in continuous light [9]. In Expt. Fig. 3a, we notice, at the end of the illumination, an accelerated acidification with an initial rate R_{off}. It decays in a first-order fashion to a final pH level. Evidently, we observe the draining of the internal proton pool (designated H_f in Fig. 3a) which was built up during the light. In Fig. 3a the straight line, R_e, (which operationally is the same as ΔpH_e) representing the steady-state rate of acidification (electron transport) can be extrapolated to the moment the flash sequence was initiated. This intercept also reveals the pool H_f.

With ferricyanide the flashes induce (a) proton release inside the membrane from water splitting (1 H^+ per flash per chain) which we denote process a, (b) proton translocation from outside to inside (process b), (c) proton efflux due to a leak process c. After reaching a steady state in the light, influx b is cancelled by an equal efflux c_b and the efflux represents exclusively process a, $c_a = a = R_e$ as defined in Eqn. 1. At the end of the illumination a and b stop and the initial efflux rate $R_{\mathrm{off}} = c_a + c_b$. Thus $R_{\mathrm{off}}/R_e = (c_a + c_b)/c_a$ and the ratio between proton translocation b and proton release a is: $c_b/c_a = (R_{\mathrm{off}}/R_e) - 1$. Measurement of the R_{off}/R_e ratio in 10 independent flash sequences yielded a value of 2.9 ($\sigma = 0.2$).

This result is identical to that computed above from the individual flash induced events: per electron transferred through the chain and per proton released from water, two protons are translocated across the membrane, and the H^+/e^- ratio is 3.

The same reasoning can be applied to the data of Fig. 4a, obtained with viologen. When the illumination stops, we again notice an acidification: a first-order process with a half-time of approx. 4 s which results in the return of the pH to the original value before illumination. The rate constant of the dark decay process is identical to that obtained with ferricyanide, which makes it possible to compare the steady-state pools accumulated inside the thylakoid with the two acceptors. With viologen, this pool H_m proved to be 25–30 % larger than with ferricyanide (H_f) while also the dark efflux rate, R_{off}, is approx. 30 % faster and amounts to 4.0 ($\sigma = 0.2$) times R_e in Fig. 3a.

This result again agrees with the analysis of the individual flash yields observed with methylviologen (Figs. 3b and 2b).

Observations in continuous light, H^+/e^- ratio as a function of intensity

The determination of H^+/e^- ratios from R_e and R_{off} can also be made with continuous light which offers a wider intensity range. Examples of such measurements are shown in Fig. 5. Flashing light with known flash spacings was used to calibrate the intensity of the continuous beam in terms of hits per reaction chain and per s. In weak enough intensities the results obtained in the two types of illumination are identical. We measured a number of R_{off}/R_e ratios in weak continuous light. The H^+/e^- values computed from these data center on 3.0 for ferricyanide and 4.0 for methyl viologen.

All previous experiments were done with weak light where no rate limitation of electron transport occurred. We have extended such measurements over a wide range of intensities. In Fig. 6a, we have plotted R_e and R_{off} as a function of intensity. R_{off} was divided by 3 to normalize the two plots for weak intensities. We also plotted ΔR, the difference between R_e and $R_{off}/3$. Fig. 6b shows a plot of the ratio R_{off}/R_e, which is a measure of the H^+/e^- ratio. These data show that R_e increased proportionally with intensity only in very weak light and was half maximal at 8 hits/s per trap. This early rate limitation presumably results from the proton pool formed inside the chloroplast membrane during illumination [13]. Note however that compared to R_e, R_{off} saturates more abruptly and remains more constant in stronger light. Also the pool size H_f (not shown) saturated abruptly and remained rather constant. As a consequence (Fig. 6b) at higher intensities the H^+/e^- ratio decreased and approached 2 as a limit.

In addition, the rates R_{off} and the pools H_f in ferricyanide and H_m in methyl-

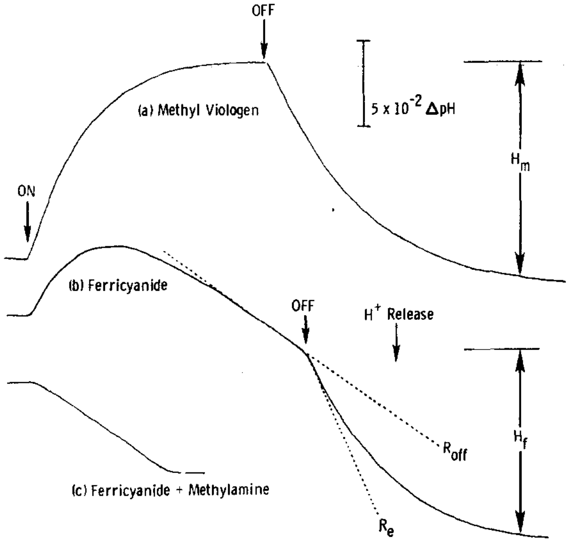

Fig. 5. Changes in pH induced in a suspension of spinach chloroplasts by weak continuous illumination. Intensity was adjusted to induce a change equal to the change induced by saturating 3-μs flash spaced 200 ms apart. (a) The sample contained methyl viologen (100 μM) as an acceptor. (b) The sample contained ferricyanide (1.0 mM) as an acceptor. (c) Sample contained ferricyanide and 0.03 M methylamine.

viologen become identical in strong light. These results imply that for both acceptors the H$^+$/e$^-$ ratio declines and approaches 2 when electron transport becomes limited by the proton pool. Apparently one of the two translocation sites operative with ferricyanide and two of the three sites operative with viologen become ineffective or are bypassed when the proton pool becomes filled.

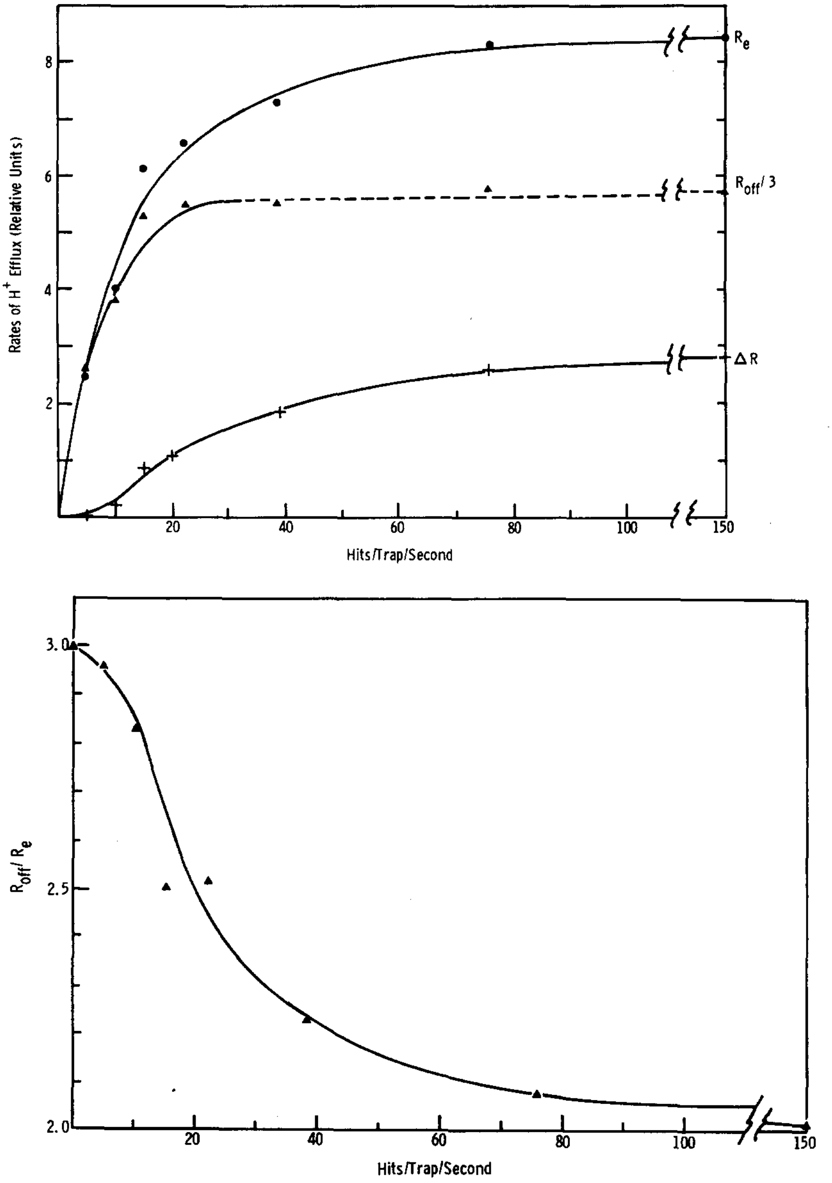

Fig. 6. (a) Plot of R_e and R_{off} and ΔR as a function of intensity. ΔR is equal to $R_{off}/3$ minus R_e. Sample contained 1.0 mM ferricyanide as external acceptor. (b) Plot of R_{off}/R_e a direct measure of H$^+$/e$^-$ as a function of intensity.

Location of the proton translocation sites

The experiment shown in Fig. 7 gives some additional information about where in the electron transport chain the various proton translocation sites might occur. This figure shows two experiments similar to those in Figs. 3b and 4b made with ferricyanide and viologen, respectively. In this case, the flash sequence was preceded by a 1-min exposure to a 720 nm light. Note that this preillumination did not at all affect the flash-induced proton uptake in the presence of ferricyanide. However, in the viologen system the first flash induced only half the normal uptake, i.e. the same as seen with ferricyanide. The third flash, however, induced the full uptake of $4H^+/e^-$.

Parallel observations were made of the behavior of *P*-700. These showed that (1) after far red (720 nm), *P*-700 was more than 90 % oxidized with either acceptor. Subsequently it was almost totally reduced after three flashes. (2) Additions of ferricyanide to our chloroplast suspension, as used for the pH measurement did not oxidize *P*-700 in the dark. (3) Light flashes in a sequence with a 1-s dark time induced full turnover of *P*-700 with ferricyanide as well as viologen.

The uptake of only two protons induced by the first flash after far red in the presence of either viologen or ferricyanide locates two uptake sites before System I (which does not turnover in this first flash), probably in the electron transfer chain from Q^- to P^+. Of the two additional uptakes observed in the presence of viologen one is obviously due to reduction of viologen by X^- (Eqn. 3) while the second is being translocated to the inside by an as yet unspecified process.

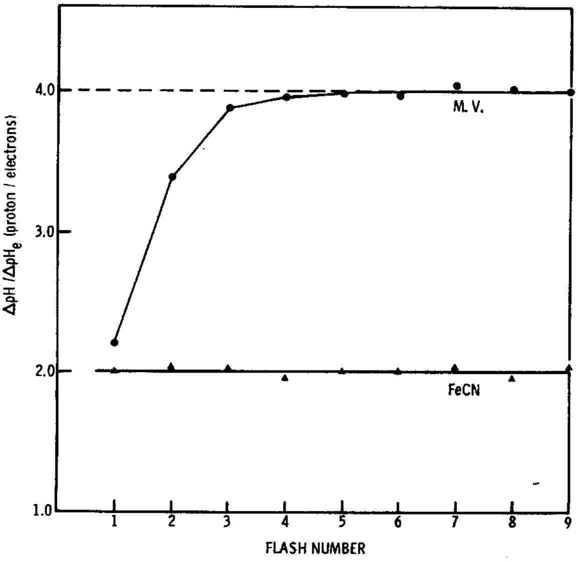

Fig. 7. Proton uptake induced by a sequence of flashes in a chloroplast suspension containing either ferricyanide (▲) or methyl viologen (●) following a 1 min 720 nm preillumination. In each case the ΔpH change was divided by ΔpH_e, the average pH change per trap per electron moved from water to ferricyanide (see Eqn. 1). ΔpH_e is assumed to be equivalent to 1 H^+/e^-.

Effect of pH on H^+/e^-

The H^+/e^- ratio has been reported to vary with pH [6, 10]. For instance, 4 H^+/e^- at pH 6–1 H^+/e^- at pH 8 have been reported with ferricyanide as an acceptor [10]. We repeated the experiments of Figs. 2b and 2c with ferricyanide as an acceptor over a pH ranging from 6.3 to 8. The H^+/e^- ratio was found to vary little, if at all, over this range.

DISCUSSION

Under conditions that the rate was not limited, we have measured H^+/e^- ratios of 3 and 4 with $K_3Fe(CN)_6$ and methyl viologen, respectively, in single turnovers of the electron transport system, independent of pH between 6.3 and 8.0.

These numbers are larger than have been reported by some workers [8, 9] who observed $H^+/e^- = 2$ regardless of the electron acceptor, but below the high (and pH dependent) values found by others [5–7]. In particular, Junge and Auslander [25] obtained H^+/e^- ratios of 2 and 1, respectively, for benzyl viologen and ferricyanide in single turnovers of the photosystem using a rapid pH indicator dye technique. The numbers differ from ours probably because of the different techniques used for measuring the pH change. Dyes can be partitioned into the chloroplast membrane and may indicate pH changes at specific sites. The glass electrode on the other hand, views the outside exclusively. The different results might indicate a site of proton pumping between the "outside" of the chloroplast and the location of the indicator dye.

In the context of the chemi-osmotic theory, our values are in good agreement with the recently published values for the ATP potentials in illuminated chloroplasts [16, 17], H^+/ATP ratios of 3 and 4 [18, 19] and ATP/2 e^- ratios of 2 [20, 21], but contrast with ATP/2e^- ratios near one by others [22, 23]. These matters have been extensively reviewed in the literature [4], and we will restrict this discussion to a few aspects of direct pertinence to the observations reported above.

Evidence has been presented for two additional proton translocations not normally predicted by the linear photosynthetic electron transport scheme, one entirely dependent upon Photosystem I and the other upon Photosystem II. In the following, we consider two possible schemes to account for these results. The first of these shown in Fig. 8, places all five loci of proton exchange in a linear arrangement of the electron transport component across the thylakoid membrane. It is essentially Mitchell's scheme [1] expanded by two additional loops. One of these loops (No. 3) occurs between the photosystem and the other is at the reducing end of Photosystem I (Site No. 4). This model is not satisfactory because of the low midpoint potential of methyl viologen, the absence of known electron carriers beyond X (bound ferredoxin) [24] and the lack of known proton carriers in addition to plastoquinone between the photosystems.

In Fig. 10 we suggest an alternate scheme which attempts to account for presented data in a different way. The reasoning for it follows: On first sight, one would expect locus No. 4 in Fig. 8 (the proton uptake associated with the autooxidation of reduced viologen) to be a simple collision process, occurring in the suspension medium, i.e. entirely unrelated to energy storage in a gradient across the membrane. However, our observations imply that this may not be the case. We

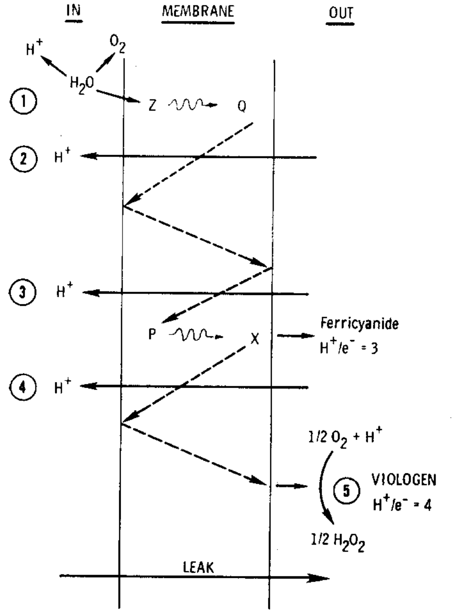

Fig. 8. Model for active proton accumulation inside the thylakoid by electron transport carriers as suggested by Mitchell. The numbers 1–5, show the location of sites which would be involved in proton exchange.

reported earlier [12] that in the presence of an uncoupler, the proton uptake by reduced viologen after a flash occurs within 6–10 ms. Similar times have been observed with the pH indicator dye technique [15, 25]. Fig. 9 shows that in the absence of an uncoupler, no 8-ms component can be discerned; a simple first-order (30 ms) time course of proton uptake is observed. These proton uptake times proved to be independent of viologen concentration between 10 and 100 μM. We thus consider the hypothesis that under "coupled" conditions, (1) all proton uptake occurs via a specific (30 ms halftime) translocation site, T, as shown in Fig. 10, (2) Photoreductant X is located inside in the membrane structure close to T, which protonates the reduced acceptor, either directly or, as drawn in Fig. 10, via another intermediate Y. (3) To account for the observation that for every proton taken up by viologen, one proton is added to the inside pool, we assume that the 30-ms site T translocates protons from out to inside the membrane only in pairs. One proton of the pair goes to reduced viologen, actually H_2O_2 formation, the other is added to the proton pool. This operational scheme attempts to combine loci Nos. 4 and 5 in Fig. 8 in one and the same process. It apparently does not operate with the non-protonated electron acceptor ferricyanide for which our data show a H^+/e^- ratio of 3 rather than 4.

The scheme given in Fig. 10 assumes a similar process for sites 2 and 3 between Q and P as was assumed for the System I sites 4 and 5 discussed above, except that A is substituted for X. Since in this case we are dealing with electron flow between the photosystems, both protons are discharged in the interior. The actual mechanism by which one site translocates two protons remains obscure.

The variation in H^+/e^- observed with higher illumination intensities is

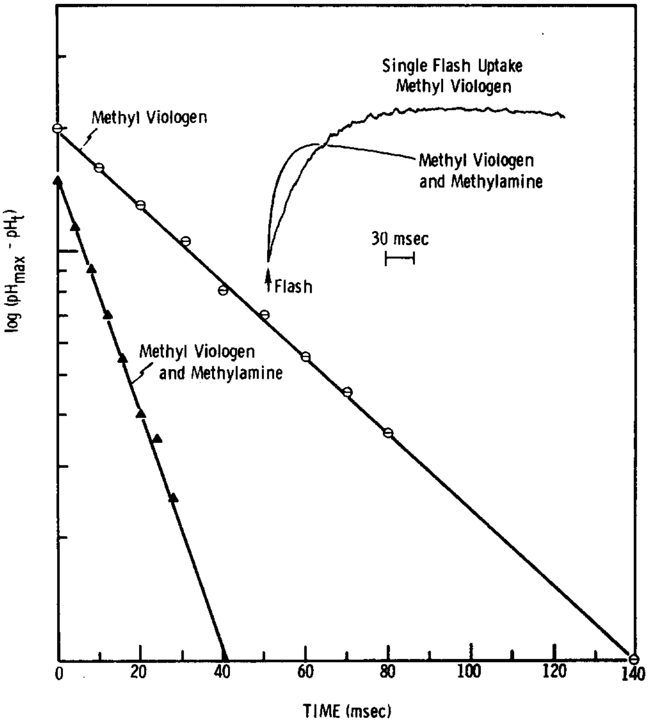

Fig. 9. Rise curves induced by a single flash in a sample containing either methylviologen or both viologen and methylamine. (a) Insert, actual rise curves with methylviologen (mv) and methylviologen and methylamine. (b) Plot of $\Delta pH_m - \Delta pH(t)$ as a function of time. $\Delta pH = \Delta pH_m (1 - e^{-Kt})$

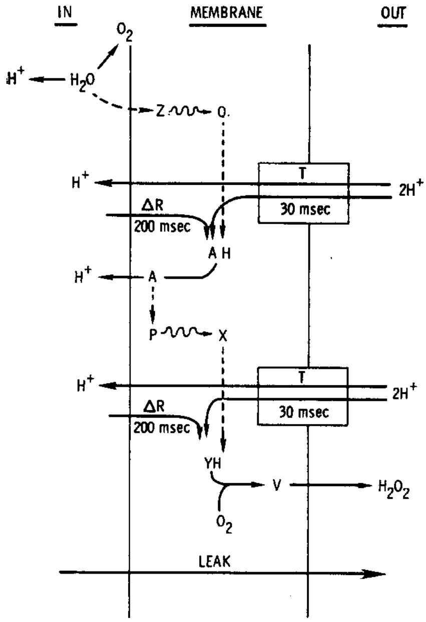

Fig. 10. Alternate model for coupling electron transport to proton transport. (See text for description.)

accommodated by the model shown in Fig. 10. We showed in Fig. 6 that, for intensities beyond $\approx$ 20 hits/s per trap, R_{off} and H_f (not shown) remain practically constant, whereas R_e still increases. The difference, ΔR, apparently reflects electron transport which does not contribute to the proton pool H_f. It amounts to about 30 % of the total flow and is equivalent to a rate of 5 equiv./s per chain or a 200-ms time constant. This observation can be explained by a competing 200 ms short circuit path in which translocation sites receive a proton from the inside pool instead of outside.

R_{off} is 63 % of maximal at approx. 10 hits/s per trap which corresponds to a translocation rate of 30 H^+/s per trap or 30 ms/H^+ (since H^+/e^- is 3). Note that this number corresponds closely to the flash-induced rate of proton uptake which can be observed directly (see Fig. 9). Independent measurements which will be published later show that the 30-ms proton uptake time is independent of (a) the state of the pool and (b) the number of protons translocated by the flash, which can vary depending upon its intensity and duration. This uptake time is shortened by uncouplers (see Fig. 9) and, as pointed out by Junge and Auslander [25], a diffusion barrier may be involved.

ACKNOWLEDGEMENTS

This work was supported in part by a grant from the Energy Research and Development Administration Contract E (11-1)-3326. This report was also prepared with the support of the National Science Foundation Grant No. BMS74-20736. Any opinions, findings, conclusions or recommendations expressed herein are those of the author(s) and do not necessarily reflect the views of N.S.F. We express our appreciation to Drs. George M. Cheniae and Richard Radmer for helpful discussions in preparing this manuscript.

REFERENCES

1 Mitchell, P. (1966) Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation, pp. 1–192, Glynn Research, Bodmin
2 Greville, G. D. (1969) Curr. Top. Bioenerg. 3, 1–72
3 Hall, D. O. and Evans, M. C. W. (1971) Sub-Cell. Biochem. 1, 197–206
4 Jagendorf, A. T. (1975) Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 413–492, Academic Press, New York
5 Dilley, R. A. and Vernon, L. P. (1967) Proc. Natl. Acad. Sci. U.S. 57, 395–400
6 Lynn, W. and Brown, R. (1967) J. Biol. Chem. 242, 426–432
7 Izawa, S. and Hind, G. (1967) Biochim. Biophys. Acta 143, 377–390
8 Rumberg, B., Reinwald, E., Schröder, H. and Siggel, W. (1968) Naturwissenschaften 55, 77–79
9 Schwartz, M. (1968) Nature 219, 915–919
10 Karlish, S. J. D. and Avron, M. (1968) Biochim. Biophys. Acta 153, 878–888
11 Schwartz, M. (1966) Biochim. Biophys. Acta 112, 204–212
12 Fowler, C. F. and Kok, B. (1974) Biochim. Biophys. Acta 357, 299–307
13 Kok, B., Joliot, P. and McGloin, M. (1969) Progress in Photosynthetic Research (Metzner, H., ed.), Vol. 2, pp. 1042–1056
14 Marsho, T. V. and Kok, B. (1970) Biochim. Biophys. Acta 223, 240–250
15 Grünhagen, H. H. and Witt, H. T. (1970) Z. Naturforsch. 25b, 373–386
16 Rottenberg, H., Grunwald, T. and Avron, M. (1972) Eur. J. Biochem. 25, 54–63
17 Pick, U., Rottenberg, H. and Avron, M. (1974) FEBS Lett. 48, 32–36
18 Junge, W., Rumberg, B. and Schröder, H. (1970) Eur. J. Biochem. 14, 582–587

19 Schröder, H., Muhle, H. and Rumberg, B. (1972) Proc. 2nd Int. Photosynthesis Congress (Forti, G., ed.), Vol. 2, pp. 919–930

20 Winget, G. D., Izawa, S. and Good, N. E. (1965) Biochim. Biophys. Res. Commun. 21, 438–443

21 Hall, D. O., Reeves, S. C. and Baltscheffsky, H. (1971) Biochem. Biophys. Res. Commun. 43, 359–366

22 Trebst, A. (1974) in Proceedings of the Third International Congress on Photosynthesis (Avron, M., ed.), p. 439, Elsevier Publishing Co., Amsterdam, The Netherlands

23 Saha, S. and Good, N. E. (1970) J. Biol. Chem. 5017–5021

24 Malkin, R. and Bearden, A. J. (1971) Proc. Natl. Acad. Sci. U.S. 68, 16–19

25 Junge, W. and Auslander, W. (1973) Biochim. Biophys. Acta 333, 59–70