Stoichiometry of Proton Uptake in Isolated Pea Chloroplasts Under Different Light Intensities

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Abstract

The number of protons released inside the chloroplast thylakoids per electron which is transferred through the electron transport chain (H^+/e^- ratio) was measured in isolated pea chloroplasts at pH 6.0 under continuous illumination and with methyl viologen as an electron acceptor. At saturating light intensity ($200\,W \cdot m^{-2}$) ("strong" light) the H^+/e^- ratio was 3. At low intensity ($0.9\,W \cdot m^{-2}$) ("weak" light) the H^+/e^- ratio was 2 with dark-adapted chloroplasts, but it was close to 3 with chloroplasts that were preilluminated with strong light. It is shown that the presence of azide in the reaction mixture leads to errors in the determination of the H^+/e^- ratio due to underestimation of the initial rate of H^+ efflux on switching off the light. To explain the above data, we assume that transformation of the electron transport chain occurs during illumination with strong light, namely, the Q cycle becomes operative.

Key Words: Photosynthetic electron transport chain; proton transport; H^+/e^- ratio; azide; light intensity, Q cycle.

Introduction[2]

Since Neumann and Jagendorf (1964) established that the photosynthetic electron transport is accompanied by proton uptake by isolated chloroplast thylakoids, many attempts have been made to measure the stoichiometry of this coupling. The experiments performed at Witt's laboratory resulted in the widely known scheme in which the transfer of one electron from water to the PS I acceptor was accompanied by a release of two protons within the

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[2] Abbreviations: ETC, electron transport chain; PS I and PS II, photosystems I and II: MV, methyl viologen; FeCN, ferricyanide; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid.

thylakoid space with one proton being released due to the splitting of the water and the other being translocated across the membrane by plasto-quinone at the ETC section connecting PS II and PS I (Schliephake *et al.*, 1968; Witt, 1971). This model was a result of the studies in which a suspension of chloroplasts was illuminated with short saturating light flashes. It was later supported by a number of other experiments following essentially the same procedures (see review by Witt, 1979; Hope and Morland, 1979). However, there is evidence that the overall H^+/e^- ratio may be greater than 2 even under these conditions of the electron transport excitation (Fowler and Kok, 1976; Velthuys, 1978, 1980a; Graan and Ort, 1983).

The H^+/e^- ratios obtained under conditions of continuous illumination show a wide range of values, from 1 to 6 (Ivanov and Muzafarov, 1974; Jagendorf, 1977). The results of the determinations of the H^+/e^- ratio often gave rise to doubts due to possible methodological errors. Most of the sources of errors were discussed by Jagendorf (1977) and Saphon and Crofts (1977). A steady interest in the stoichiometry of proton–electron coupling is explained by researchers' belief that the true H^+/e^- value will lead to understanding of the structural and functional organization of ETC. In this paper we present the data obtained with MV as an acceptor which received electrons on a reducing side of PS I. The results show that the overall H^+/e^- ratio for photosynthetic ETC may be 2 or 3 depending on the way it operates (either in a mode of rare electron transfer or in a mode of saturating electron flow, with the latter mode resulting, as we propose, in transformation of ETC).

Materials and Methods

Chloroplast Preparation

Chloroplasts were isolated from fresh leaves of the Rannij-301 two-week-old pea plants grown in a lumino- and thermostatically controlled chamber. The leaves were homogenized in a MPW-302 homogenizer (Poland) for 15 sec at 0°C. The grinding medium contained 0.4 M sucrose, 50 mM KCl, 10 mM NaCl, 5 mM $MgCl_2$, and 30 mM MES (pH 6.6). The homogenate was filtered through five layers of cheesecloth and centrifuged for 3 min at 150 × *g*. The supernatant was then centifuged for 10 min at 1400 × *g*. The chloroplast pellet was washed once with 75 ml of 50 mM/10 mM/2 mM $MgCl_2$ medium and resedimented at 1400 × *g* for 10 min. The pellet was then finally resuspended in 2–3 ml of 0.1 M sucrose, 50 mM KCl, 10 mM NaCl, 2 mM $MgCl_2$, and 30 mM HEPES, pH 7.2, to a concentration of 1.5–2.5 mg Chl/ml. Suspensions were kept on ice in the dark and used in

experiments only for 3–4 hrs following isolation. During this period of time the light-dependent activities of the preparation remained stable. The chlorophyll concentration was determined by the method of Arnon (1949).

Electron and Proton Transport Assays

A water-jacketed thermostated glass reaction vessel (18°C) was used, into which both Clark-type O_2 and glass pH electrodes were inserted. This allowed simultaneous measurements and recording of both parameters. The 1.7-ml reaction mixture contained 50 mM KCl, 10 mM NaCl, 2 mM $MgCl_2$, 0.5 mM MES, 0.1 mM MV, and chloroplasts with routine concentrations corresponding to $30 \mu g$ chl $\cdot$ ml^{-1}. During experiments the mixture was vigorously stirred with a magnetic stirrer. The pH was adjusted to 5.9–6.1. Actinic light was provided by a 150-W slide projector using a KS-14 red cutoff filter ($\lambda > 620$ nm) and an SZS-24 (heat) filter. The light was focused on the reaction vessel, its intensity being $200 W \cdot m^{-2}$ ("strong" light). Neutral-density filters were used to reduce light intensity to $0.9 W \cdot m^{-2}$ ("weak" light). The response half-time for the pH-measuring system was on the order of 0.5–0.6 sec. When necessary the curves charted on a strip recorder could be arranged according to the method by Izawa and Hind (1967) to obtain the real time course of pH change. At the end of each experiment the pH changes were translated into H^+ equivalents by titrating the reaction mixture with a known amount of 0.002 M HCl.

Determination of H^+/e^- Ratio

In the absence of irrelevant influences the H^+/e^- ratio, under continuous illumination, should be equal to the ratio of the proton efflux rate estimated from the initial rate of acidification of the medium when the light was switched off (V_{H^+}) to the stationary electron transport rate (V_{e^-}) (Schwartz, 1968). The quantity V_{H^+} was determined both as the slope of the tangent line to the postillumination pH change curve at the light-off point and as the value of the product of $K_d \cdot \Delta H^+$, where K_d is an apparent first-order reaction rate constant for postillumination pH decrease, and ΔH^+ is the amount of protons leaving thlyakoids after the light was switched off. The fact that the kinetics of this process is of the first order was established by the plotting pH change in the dark on logarithmic scale against time; values of K_d were calculated from the slopes of the straight lines obtained.

The electron transport rate in the steady state, V_{e^-}, was measured as the rate of oxygen uptake (V_{O_2}) in MV-catalyzed reactions. It was calculated as four electrons that moved per oxygen molecule converted to H_2O_2. To avoid error in the estimation of the V_{H^+}/V_{e^-} ratio, which might be a consequence of superposition of errors in two independent calibrations of pH and pO_2

electrodes, the following procedure was used. For each chloroplast sample the amounts of protons and oxygen molecules evolved during FeCN reduction (FeCN concentration 0.7 mM, illumination period 1–2 min), designated $\Delta \bar{H}_{FeCN}^{+}$ and $\Delta \bar{O}_{2,FeCN}$, were simultaneously measured, and the V_{H+}/V_{e-} ratio was derived from the formula

$$V_{H+}/V_{e-} = (V_{H+}/V_{O_2})(\Delta \bar{O}_{2,FeCN}/\Delta \bar{H}_{FeCN}^{+})$$

where $\Delta \bar{O}_{2,FeCN}$ and $\Delta \bar{H}_{FeCN}^{+}$ are expressed in millimeters (according to recorder tracings) and V_{H+} and V_{O_2} are expressed in millimeters per time unit, with the same sensitivities of the pH and pO_2 measuring system in both experiments with MV and FeCN. The total biffer capacity of the reaction mixture in our experiments was 0.8–1 μeqv H^{+}/pH unit. The light-induced change of the buffering capacity of chlorophyll may be estimated from the data of Walz et al. (1974) and Opanasenko et al. (1978) as equal to $10^{-2} \mu$eqv H^{+}/pH unit. Thus, this change constitutes less than 1.5% of the total buffer capacity.

Results

A series of attempts were made to determine the H^{+}/e^{-} value with low-potential acceptors under continuous illumination. Dilley (1970), Telfer and Evans (1972), and Chow and Hope (1977), using MV as an acceptor, found the value of the H^{+}/e^{-} ratio to be 0.6–0.8 at pH 8.0–8.2 in the absence of valinomycin. The dependence of the H^{+}/e^{-} ratio on pH was studied when anthraquinone (Karlish and Avron, 1971) and methyl viologen (Ivanov et al., 1980) were used as electron acceptors. The H^{+}/e^{-} value decreased in both cases from about 1.5–2.0 to about 0.2–0.6 with pH rising from 6.0–6.5 to 8.1–8.4. In all the above studies the H^{+}/e^{-} ratio was determined as the V_{H+}/V_{e-} ratio, and the experiments were carried out in the presence of sodium azide (NaN_3, pK of HN_3 is 4.7), which was added to the reaction mixture to inhibit possible activity of endogenous catalase. As the electron transport rate was usually measured as the rate of oxygen uptake, the catalase decomposition of H_2O_2 formed in the presence of low potential acceptors could violate the stoichiometry of $e:O_2 = 4$ and lead thereby to an underestimated V_{e-} value.

Table I shows that the presence of 0.6 mM NaN_3 in the reaction mixtures has little influence on the velocity of oxygen uptake. The addition of azide raised the V_{O_2} value by only 5–10% in all our experiments. In our opinion, however, this increase in V_{O_2} was mainly the result of the increase in the rate of outward proton translocation from the thylakoids during illumination when azide is present (see below).

Experiments with FeCN (Table II) as an electron acceptor provide evidence that lower of the V_{H+}/V_{e-} ratio in the presence of azide (Table I) is

Table I. Effect of Sodium Azide, CCCP, and Illumination Conditions on the Rate of Oxygen Uptake (V_{O_2}), Steady-State Level of Proton Accumulation (ΔH^+), Rate Constant (K_d) and Rate (V_{H+}) of the Proton Efflux, and V_{H+}/V_{e-} Ratio[a]

Additions	Illumination conditions	V_{O_2}, μmol O_2 (hr · mg chl)$^{-1}$	ΔH^+, μmol H^+ (mg chl)$^{-1}$	K_d, sec^{-1}	V_{H+}, μmol H^+ (hr · mg chl)$^{-1}$	V_{H+}/V_{e-}
—	Strong light	6.7	0.35	0.066	83.2	3.1
—	Weak light preceded by strong light	2.2	0.10	0.070	25.2	2.8
Sodium azide 0.6 mM	Strong light	7.2	0.24	0.070	60.5	2.1
Sodium azide, 0.6 mM	Weak light preceded by strong light	2.2	0.03	0.075	8.1	0.9
CCCP, $5 \cdot 10^{-7}$ M	Strong light	10.0	0.27	0.120	116.6	2.9

[a]The composition of the basic reaction mixture and the strong and weak light intensities were as described under Materials and Methods; illumination with weak light preceded by strong light was given after two to three illuminations with strong light, each continued for 1–1.5 min followed by 1–1.5 min of darkness. The table shows the data for the third illumination with strong light and for the first illumination with weak light preceded by a strong one.

due to the effect of this substance on proton exchange in the thylakoids rather than to the inhibition of catalase activity. According to the data by Shroder *et al.* (1972) and Ivanov and Muzafarov (1974), the V_{H+}/V_{e-} ratio measured with FeCN in the absence of azide is close to 2, whereas its addition to the reaction mixture in a concentration similar to that with MV reduces this ratio to about 1 to 1.3 in different experiments (Table II).

A slight raising of the H^+ permeability of the thylakoid membrane by low concentrations of CCCP results in an increase of V_{e-} and decrease of ΔH^+, while it does not affect the magnitude of the V_{H+}/V_{e-} ratio (Table I).

Table II. Effect of Sodium Azide on Electron Transport, Proton Translocation, and V_{H+}/V_{e-} Ratio with FeCN as the Acceptor[a]

Sodium azide, 0.6 mM	V_{e-}, μeq(hr · mg chl)$^{-1}$	ΔH^+, μmol H^+ (mg chl)$^{-1}$	K_d, sec^{-1}	V_{H+}, μmol H^+ (hr · mg chl)$^{-1}$	V_{H+}/V_{e-}
—	48	0.41	0.063	92.0	1.94
+	52	0.28	0.065	65.5	1.26

[a]Reaction mixture as described under Materials and Methods except MV was omitted and 0.7 mM FeCN was added; illumination with strong light.

This fact indicates that it is the specific influence of azide upon the proton exchange that leads to underestimated H^+/e^- ratios. In accordance with the pH gradient in the light the distribution of HN_3 and N_3^- molecules across the thylakoid membrane should be such that $[HN_3]_{in} = [HN_3]_{out}$ and $[N_3^-]_{in} <$ $[N_3^-]_{out}$, with the subscripts "in" and "out" standing for concentrations "inside" and "outside" of the thylakoids, respectively. The N_3^- ions can move inward down their gradient as the permeability of the thylakoid membranes for anions of weak acids is not very low (Strotman, 1972; Molotkovskii and Jakovleva, 1980). This may be especially true for such a relatively lipophilic anion as azide. The N_3^- ions leaving the outer phase combine with protons released both by water oxidation and plastohydroquinone oxidation. HN_3 molecules thus formed move outward down their established gradient carrying protons with them. The protonation of N_3^- ions can occur in the inthrathylakoid space and at the inner side of the membrane near the sites where the above proton-releasing reactions take place.

Proton binding by N_3^- and outward movement of HN_3 molecules will reduce ΔH^+ and result in increased V_{e^-} values, as we see in Table I. However, the fast proton efflux caused by the HN_3 outlet is not presumably registered by the pH electrode at the instant the light is extinguished since HN_3 efflux is expected to drop abruptly to zero because of the low concentrations of N_3^- ions in the inner phase and within the thylakoid membrane, in particular, at the first illuminations. This may explain why the K_d values in experiments with and without azide do not differ essentially (Table 1)—even though in the former they reflect the efflux of protons into the medium without an additional H^+ outlet which operates in the light. Thus, the measured V_{H+} value in the experiments with azide does not correspond to the true proton efflux rate in the light.

It may also be assumed that V_{H+} in the presence of azide is lower than in the light because of binding by N_3^- ions of protons entering the outer phase after the light is turned off followed by an inward movement of HN_3 molecules. Brand *et al.* (1976) and Pozzan *et al.* (1979) analyzed this source of error in the determination of the H^+/e^- ratio in experiments on mitochondria when the proton ejection into the medium was also estimated. However, it is easy to show that under the conditions of our experiments where the pH change of the medium caused by illumination is below 0.02 pH unit, the inward HN_3 translocation can take only about 1–2% of the protons that leave thylakoids after switching off the light. Yet, one cannot exclude the possibility that this process may reduce just the initial rate of acidification of the medium upon cessation of illumination, i.e., V_{H+}.

The effect of azide on the proton exchange in thylakoids is particularly pronounced under illumination with weak light provided after illumination with strong light (Table I). In saturating light, increased V_{e^-} results in

Table III. Changes in V_{O_2}, ΔH^+, V_{H+}, and K_d in Successive Illuminations of the Chloroplast Suspension[a]

Number of illumination	V_{O_2}, μmol O_2 (hr · mg chl)$^{-1}$	ΔH^+, μmol H^+ (mg chl)$^{-1}$	K_d, sec^{-1}	V_{H+}, μmol H^+ (hr · mg chl)$^{-1}$
1	6.1	0.34	0.045	55.1
2	7.0	0.32	0.053	61.1
3	7.3	0.26	0.065	60.8
4	7.5	0.22	0.075	59.4

[a]Basic reaction mixture as described under Materials and Methods except sodium azide was added; illumination with strong light for 1–1.5 min with an intermediate dark period for 1–1.5 min.

increased H^+ influx which in turn partly impedes a large drop in ΔH^+, while in limiting light an additional loss of protons cannot be counterbalanced by an increase in V_{e-}. Moreover, the increase in K_d in the successive illuminations (Table III) indicates that the permeability of the thylakoid membranes to ions rises during incubation in the reaction mixture, particularly after the illumination. Table III presents the results of experiments carried out in the presence of azide; similar, though less fast, K_d growth and ΔH^+ drop were observed in the cases without azide. Apparently, the permeability to N_3^- ions rises as well. In addition, an increase in the incubation time leads to an increase in the concentration of azide inside the thylakoids. These factors cause not only a large drop in ΔH^+ in the presence of azide in weak light after a strong one (Table I), but also enhance underestimation of V_{H+} under such conditions. This may account for a very low V_{H+}/V_{e-} ratio observed in this case.

The major results of this investigation are summarized in Table IV. The table shows that under continuous illumination with strong light the measured V_{H+}/V_{e-} ratio is about 3. In continuous light of low intensity this ratio is about 2 or 3 depending on whether the chloroplasts have been kept previously in the

Table IV. The V_{H+}/V_{e-} Ratio in Chloroplasts under Different Conditions of Illumination[a]

Illumination conditions	V_{e-}, μeq (hr · mg Chl)$^{-1}$	ΔH^+, μmol H^+ (mg Chl)$^{-1}$	K_d, sec^{-1}	V_{H+}, μmol H^+ (hr · mg Chl)$^{-1}$	V_{H+}/V_{e-}
Strong light	24.0 ± 1.2	0.34 ± 0.03	0.060 ± 0.002	72.4 ± 4.2	3.1 ± 0.1 (18)
Weak light	13.6 ± 1.4	0.16 ± 0.01	0.048 ± 0.003	28.3 ± 2.5	2.1 ± 0.1 (9)
Weak light preceded by strong light	8.0 ± 0.6	0.09 ± 0.01	0.071 ± 0.005	22.8 ± 1.9	2.8 ± 0.2 (11)

[a]Conditions were similar to those for Table I. The data are shown as mean ± SD; V_{H+}/V_{e-} are the means of these values obtained in individual experiments; number of experiments is shown in parentheses.

dark or in strong light. To our mind these values of the V_{H+}/V_{e-} ratio measured in the absence of azide reflect the true H^+/e^- ratios under the given conditions.

Discussion

The H^+/e^- ratio of 2 at low light intensity for dark-adapted chloroplasts corresponds to that generally accepted for photosynthetic ETC. The H^+/e^- ratio of 3 obtained in strong light or in weak light for preilluminated chloroplasts apparently points to a transformation of ETC occurring during the high-intensity light treatment. Moreover, it points to the preservation of this "light" state of ETC for an appreciable time period in darkness at pH 6.0.

It would be simpler to assume that the H^+/e^- ratio of 3 is the result of Q cycle operation (Mitchell, 1976) in the chloroplasts ETC, i.e., the result of the translocation of two protons across the membrane per electron transferred from plastoquinol to cytochrome f, plus one proton from oxidation of water. All the proposed modified Q cycle models include a concerted two-electron reduction of the components of the cytochrome b_6–f complex, namely cytochrome b_6 and the Rieske iron–sulfur center (FeS), by plastoquinol and the fact that one of the electrons released from plastoquinol during its oxidation returns from cytochrome b_6 to a site of the plastoquinol oxidation (see review by Hauska *et al.*, 1983).

It should be noted that our present findings contradict the data obtained by Rathenow and Rumberg (1980) as well as our own (Ivanov and Ovchinnikova, 1984), where the H^+/e^- ratio was 3 in weak light, and 2 in strong light with FeCN as the electron acceptor. FeCN is usually considered to be a class 1 acceptor, but it is capable of accepting electrons before PS I.

If the H^+/e^- ratio of 3 with FeCN in weak light is thought to be accounted for by a Q-cycle operation, the H^+/e^- ratio of 2 in strong light with this acceptor may imply that under the latter conditions the following may occur: (1) a Q cycle (or any of its variants) ceases to operate (Bouges-Bocquet, 1981); (2) a proton is carried outward together with the electron destined for cytochrome b_6 (Graan and Ort, 1983); or (3) a number of electrons are accepted by FeCN before PS 1.

As was discussed by Graan and Ort (1983), it is difficult to reconcile Bouges-Bocquet's suggestion with the requirement for a concerted two-electron reduction of the cytochrome b_6–f complex. On the other hand, the proposal made by these authors also seems unlikely. Their data on an increase in the efficiency of a flash-induced proton uptake (H^+/e^-) in the presence of valinomycin (from 1.7 to 2.6) obtained at pH 8.1 with MV as well as ours on an increase in the value of the H^+/e^- ratio in continuous light in

the presence of valinomycin and/or DCCD (from 1.5 to 3) obtained with the same acceptor at pH 7.7 (Ivanov *et al.*, 1983) can be better explained by a rapid, energization-dependent H^+ efflux through the CF_0–CF_1 complex in the absence of any additions. A similar interpretation of the observed decrease in the apparent stoichiometry of flash-induced PS II proton accumulation was recently given by Flores and Ort (1984).

In the presence of FeCN at pH 8.0 the H^+/e^- ratio in high light is close to 0.8 (in contrast to 1.5 in the presence of MV at pH 7.7), and the addition of valinomycin raises it only to 2 (Schroder *et al.*, 1972; Ivanov and Muzafarov, 1974; Chow and Hope, 1977). Thus it seems that the low H^+/e^- values observed in the presence of FeCN at high light intensity are presumably a consequence of the transfer of a good many electrons to the acceptor from the components of the intersystem chain in swollen thylakoids.

It still remains to be seen, however, why the H^+/e^- ratio at low light intensity is close to 3 with FeCN, and close to 2 with MV; and why this ratio increases to 3 with the latter acceptor at high light intensity. At present we can give no unequivocal explanation of these observations, but we may argue that in our experiments with MV the overall H^+/e^- ratio is 2 in limiting light because under such conditions the Q cycle or any of its other variants resulting in the translocation of two protons across the membrane for every electron transferred from the cytochrome b_6–f complex to P700 does not operate. More specifically, there is no steady electron transfer from the reduced cytochrome b_6 to the donor side of the cytochrome complex. A possible reason for this is that under continuous illumination of weak intensity the components of the B-PQH$_2$ region of the ETC cannot oxidize the reduced cytochrome b_6 because of their redox state. Then an electron may go from the reduced cytochrome b_6 either to cytochrome f within the cytochrome b_6–f complex and subsequently to P700 or directly to the oxygen.

The H^+/e^- ratio of 3 found in this study in strong light provides strong evidence for the operation of the Q cycle in chloroplasts under the conditions of a noncyclic electron transport. According to the preceding discussion the onset of the operation of the Q cycle should be the result of changes in the chain allowing the cytochrome b_6 to take part in a steady-state reduction of plastoquinone molecules. Most of the proposed modified Q cycle models for chloroplasts were based on studies in the presence of DCMU and did not consider electron transfer from cytochrome b_6 back to the donor side of the cytochrome b_6–f complex when PS II does operate (Crowther and Hind, 1980; Chain, 1982; Selak and Whitmarsh, 1982). It should be pointed out that more rapid oxidation of cytochrome b_6 after its reduction during the flash-induced turnover was observed in the preilluminated chloroplasts in contrast to dark-adapted ones (Velthuys, 1979). Velthuys (1979, 1980b) assumed that a pair of reduced cytochromes b_6 can rereduce the plastoquinone molecule of

the pool. To our mind, oxidation of cytochrome b_6 may proceed in the reaction with plastosemiquinone that is generated in the pool. A tentative hypothesis may be proposed that the process leading to depairing of the reducing equivalents in the pool is oxidation of plastohydroquinone by cytochrome b-559 (Whitmarsh and Cramer, 1978) and that the latter, in turn, donates an electron to $P680^+$. The occurrence of this cyclic electron transport around PS II involving cytochrome b-559 precisely under the conditions of high light was suggested by Heber *et al.* (1979). Thus, we assume that under the conditions of noncyclic electron transport steady oxidation of cytochrome b_6 and consequently operation of the Q cycle depend on the oxidation of components of the B=PQ portion of the chain by cytochrome b-559. The obtained H^+/e^- of 3 in weak light in the presence of FeCN (Rathenow and Rumberg, 1980; Ivanov and Ovchinnikova, 1984) may, in terms of our proposal, be the result of occurrence of the same situation as in high light in the presence of MV. As shown by Drechsler and Neumann (1982), FeCN inhibits electron transfer between water and P680. This inhibition may lead to the reduction of a strong oxidant $P680^+$ by the electrons originating from the acceptor side of PS II even under a low quantum flow, i.e., to the cycle around PS II as discussed above.

The hypothesis is clearly very speculative, but it should be pointed out that both the influence of preillumination of chloroplasts upon the value of H^+/e^- ratio in weak light (Table IV) and our proposal as to the role of cytochrome b-559 in switching the Q cycle operation on, are comparable with the data obtained by Horton and Cramer (1976). These authors observed the ability of strong preilluminating light to stimulate the amplitude of the subsequent oxidation of cytochrome b-559 by far-red light. Oxidation by far-red light in those experiments does not prove, however, that the electrons from cytochrome b-559 transfer to PS I (Heber *et al.*, 1979). In any case it is noteworthy that the preillumination of chloroplasts created an interconnection between cytochrome b-559 and its oxidant (Horton and Cramer, 1976) and, on the other hand, changes the H^+/e^- ratio in weak light (this paper).

Acknowledgment

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