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PHOTOPHOSPHORYLATION ASSOCIATED WITH SYNCHRONOUS TURNOVERS OF THE ELECTRON-TRANSPORT CARRIERS IN CHLOROPLASTS

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Two saturating single-turnover flashes spaced 100 ms apart are sufficient to achieve ATP formation in isolated chloroplast thylakoids. Two turnovers of the electron carriers result in the accumulation of about 7 nmol H^+/mg chlorophyll. Under the same conditions (i.e., $\Delta G_{ATP} = 38$ kJ/mol) a solitary flash is inadequate to produce ATP. The electron flux from the third or any subsequent flash is coupled to ATP formation as efficiently as is observed in continuous light (i.e., ATP/2e > 1.0) and produces 0.8 molecules of ATP per coupling factor on each turnover. The yield of ATP per flash increases with declining temperature being largest near 4°C, the lowest value tested. The number of H^+ accumulated per flash is independent of temperature so the greater yields of ATP near 4°C indicate that fewer H^+ are exiting the membrane via nonproductive pathways. The yield of ATP per flash near 4°C is largely independent of flash frequency between 1 and 30 Hz. When the formation of an electrical potential difference is prevented by adequate amounts of valinomycin and potassium the accumulated effects of about eight flashes are required before ATP formation is achieved (i.e., about 26 nmol H^+/mg chlorophyll), indicating an average ΔpH/flash in excess of 0.3 units. In the presence of the exchange carrier nigericin, the electrical component of the driving force for ATP formation is enhanced at the expense of the ΔpH. In this case, ATP formation is efficiently coupled to electron flux only at flash frequencies rapid enough to allow a summation of the electrical field. These results clearly demonstrate that any processes which are prerequisites for ATP synthesis (i.e., activation of coupling factor or generation of Δp) are fulfilled by a remarkably small number of charge separations.

Introduction

There are a great many reasons for believing that light-driven electron transport in chloroplasts results in the formation of a transmembrane electrochemical potential difference of H^+ (Δp) which is energetically competent to account for the phosphorylation of ADP. Nevertheless, a detailed mechanism of energy conservation has not emerged. In the case of chloroplasts a great deal more needs to be known about the events involved in the transition from the dark-adapted state to the fully activated, fully coupled state. During this transition period the electrochemical potential of accumulating H^+ must surpass the energetic threshold for ATP synthesis and the coupling factor-enzyme complex must become activated.

The amount of electron transport-driven H^+ uptake which must occur to surpass the energetic threshold for ATP formation will be dependent on

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Abbreviations: Tricine, *N*-tris(hydroxymethyl)methylglycine; Mops, 3-(*N*-morpholino)propanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Taps, *N*-tris(hydroxymethyl)-methyl-3-aminopropanesulfonic acid; Chl, chlorophyll; Δp, proton-motive force; PS, photosystem.

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the electrical capacitance and H^+-binding capabilities of the membrane. The larger the capacity of the photosynthetic membrane to store charge and bind H^+, the greater the number of turnovers of the electron-transport carriers necessary to bring about ATP formation. The nature and size of these pools is a portion of the information necessary to understand the events involved in coupling.

In addition to the formation of an adequately energetic electrochemical potential to phosphorylate ADP, the development of the transmembrane potential is likely to have regulatory effects on electron and H^+ transfer as well as on the formation of ATP. The phenomenon of control of electron transfer by the development of the transmembrane electrochemical potential of H^+ is well recognized but the mechanism is as yet unknown. There are those who contend that the acidity of internal chloroplast space [1,2] is a more significant element of control of electron transfer than the magnitude of transmembrane pH difference [3,4]. Among those who favor the notion that electron transfer is controlled by the magnitude of the electrochemical potential there is uncertainty whether the membrane potential and H^+ concentration difference are equivalent in this regard [5]. The suggestion has been made that the stoichiometry of H^+ transfer to electron transfer may also be regulated by magnitude of the Δp [6]. Some evidence now exists that under certain conditions the plastoquinol : plastocyanin oxidoreductase of chloroplasts may support a cycling of electrons through the plastoquinone pool allowing for additional H^+ translocation across the membrane (often referred to as a Q-loop or Q-cycle [6–8]). The free energy of the involved electron-transfer reactions may permit such a cycle to occur only when the Δp is small [6]. Lastly, there may be regulation of ATP formation by the magnitude of Δp from the standpoint of the activation state of the coupling factor complex. Hangarter and Good [9] demonstrated from acid-base transition experiments that for Δp values at or below the threshold value for ATP formation, exceedingly little if any reversibility of the reaction occurred. This observation indicates that the coupling factor enzyme achieves an active state only after the Δp has surpassed the energetic threshold for net synthesis of ATP.

We feel the most effective way to investigate both the energetic and possible regulatory roles of the proton-motive force is by making measurements during the period when the Δp is forming. Saturating xenon flashes brief enough to allow only single turnovers of the electron carriers can be used to great advantage in such a study, since once the appropriate chlorophyll-to-electron carrier ratios have been established, both electron and H^+ transfer can be tabulated from the number of flashes delivered. In this paper, we report on the detailed nature of ATP formation associated with single turnovers of the electron carriers. We were able to establish the relative importance of the membrane potential versus the ΔpH in energizing ATP during flashing light. We were also able to quantitate the size of the 'pools' which must be filled in order to achieve a threshold Δp and to measure a value for the average ΔpH/flash during the formation of the threshold Δp. These data form a foundation for future investigations of the role of Δp in regulating electron and H^+ transfer and ATP formation.

Materials and Methods

Chloroplasts. Chloroplasts (intact, naked lamellae) were isolated from fresh market spinach (*Spinacea oleracea* L.) as described elsewhere [10].

Measurement of ATP formation. Photophosphorylation reactions were carried out in a water-jacketed chamber with the temperature regulated within 0.2°C of the indicated value. The sample volume was either 2 or 4 ml. Samples were excited with 6-μs half-width saturating flashes (11 J total discharge energy) from a xenon lamp (Model FX-193 Flashtube, EG & G *, Salem, MA). The light was either filtered through a red cut-off filter (Corning 2-58) or used unfiltered. The total time of exposure of the chloroplasts to the reaction medium was constant for each experiment regardless of the number of flashes or the frequency at which the flashes were delivered. The time was

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maintained constant by varying the dark interval prior to the first flash. The phosphorylation reaction was terminated by the addition of 1.5 ml of 1.0 M trichloroacetic acid solution containing 10 mM EDTA.

Precise measurement of the ATP produced from single-turnover flashes was accomplished by adsorbing the nucleotides on charcoal and finally eluting mono-, di- and triphosphate adenine nucleotides sequentially by acid treatment of a Dowex AG 1×4 anion-exchange column. The exact procedure is described in detail elsewhere [10,11].

Measurement of the electrochromic absorption band shift. The flash-induced electrochromic absorption band shift was measured as the change in absorbance at 518–540 nm in a 10×10 mm cuvette maintained at 5°C. The actinic light was supplied from the same xenon flash lamp used in the phosphorylation experiments and was filtered through a Corning 2-58 red cut-off filter. The single-beam spectrophotometer and signal-averaging equipment were described previously [10].

Measurement of flash-induced pH change. Changes in H^+ concentration of the reaction mixture were detected with a glass electrode (Orion 9103) connected to a Keithley 610 C electrometer. The output of the electrometer was recorded on an Esterline-Angus strip chart recorder. Changes in pH were monitored with a scale expansion of 0.06 pH unit full scale on the recorder. In these experiments, reactions were run in a final volume of 4.0 ml in a thermostatically controlled vessel at 4°C with continuous stirring. Prior to illumination the reaction mixture was adjusted to pH 8.1 with dilute HCl. The actinic light was from the same xenon lamp used in the phosphorylation experiments and was filtered through a Corning 2-58 red cut-off filter. At the completion of each flash series the pH changes registered on the chart paper were translated into H^+ equivalents by titrating the reaction mixture with 20 nmol HCl.

Further details of reaction conditions are given in the legends of the figures.

Results

Near maximum yields of ATP were reached at a flashing frequency of 3 Hz and as Fig. 1 shows the

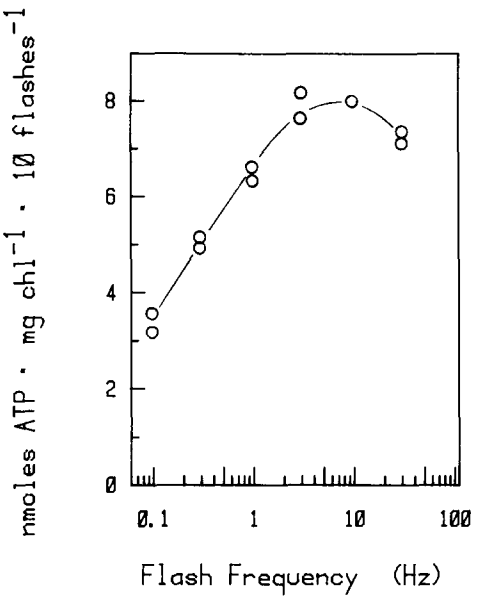

Fig. 1. Dependence of ATP formation on the frequency of single-turnover flashes. Chloroplasts containing 30 μg Chl were suspended in 2 ml of reaction mixture containing 50 mM sorbitol, 50 mM Tricine-KOH (pH 8.0), 5 mM $MgCl_2$, 0.1 mM methyl viologen, 0.1 mM ADP and 0.5 mM $Na_2H^{32}PO_4$ (15 μCi). The reaction was continuously stirred and thermostatically maintained at 4°C.

yield of ATP from a sequence of ten flashes did not change significantly for flash frequencies between 3 and 30 Hz. Yields fell at very low flash frequencies because a solitary flash was insufficient to make ATP (see Fig. 4) and long intervening dark periods prevented the buildup of an optimal electrochemical potential of accumulated H^+. Yields should fall at high flash frequencies when dark times are not sufficient to allow the slowest steps of electron transport to be completed. A flash frequency of 30 Hz, even at 4°C, provided adequate dark time since electron transfer of 17–18 nequiv./mg Chl per 10 flashes was measured at 3, 10 and 30 Hz.

Fig. 2 shows that the yield of ATP from a sequence of 20 flashes given at the rate of 1 Hz increased steadily as the reaction temperature was decreased. The smaller yields of ATP per flash at room temperature resulted from a decreased yield per flash and not an increase in the number of flashes in which no ATP was made (data not shown). Similar temperature dependences have been reported for postillumination and acid-base

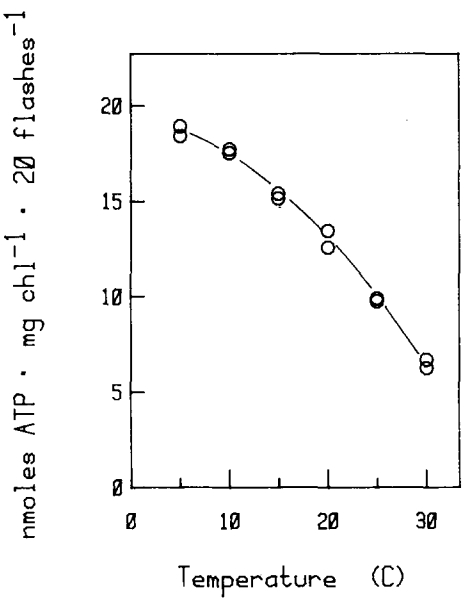

Fig. 2. Temperature dependence of flash-induced ATP yield. Reaction conditions were the same as given for Fig. 1. The flashing frequency was 1 Hz.

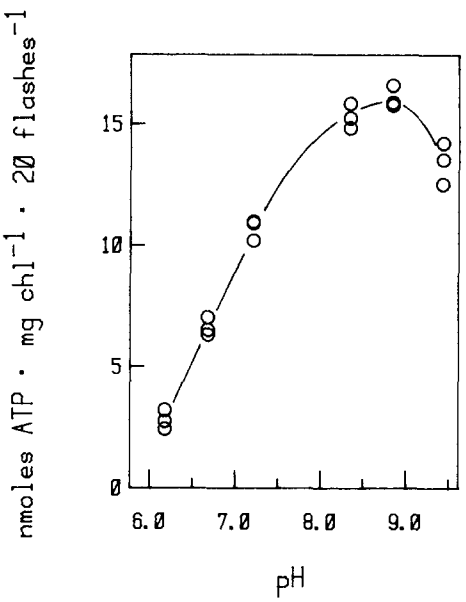

Fig. 3. The effect of the pH of the reaction on flash-induced ATP yield. Except for the H^+ buffer present the reaction conditions were the same as those given for Fig. 1. The pH range was spanned using the buffers Mes, Mops, Tricine and Taps. The flash frequency was 3 Hz. Data points are plotted at the 4°C pH value calculated from the dependence of pK_a on temperature reported by Good and Izawa [27].

ATP formation [12,13]. In each of these cases the ATP-synthesizing reactions are competing with dissipative processes for the stored energy. The higher yields of ATP at low temperature indicate that nonproductive H^+ leakage and perhaps membrane-depolarizing ion movements are more strongly affected by temperature than is the slowest step in ATP synthesis. A positive correlation of ATP formation to temperature would not be expected until the illumination regime allowed electron transport to become the rate-limiting parameter.

The pH dependence of flash-induced ATP yield (Fig. 3) has a profile which is similar to the pH dependence of phosphorylation efficiency ($ATP/2e^-$) measured in continuous light [14]. It differs only with respect to the pH optimum which was shifted about one-half unit in the alkaline direction for flash-induced ATP formation. This alkaline shift is reminiscent of the data of Saha et al. [15] in which ATP synthesis was measured at low light intensity. Saha et al. found that the pH optimum for ATP synthesis at low light intensity was shifted in the alkaline direction from that found at high light intensity. The profile shown in Fig. 3 is easily distinguished from the pH dependence of ATP-formation rate measured in continuous light which declines more precipitously at acid pH values, falling to only 15% of maximum rate at pH 7.0 [14].

Fig. 4 shows that two saturating single-turnover flashes spaced 100 ms apart were sufficient to achieve ATP formation in isolated chloroplast thylakoids. Under these conditions (i.e., ΔG_{ATP} = 38 kJ/mol) a solitary flash was inadequate to produce ATP. The efficiency of coupling between electron transfer and ATP formation can be determined once the amount of electron transfer per flash is measured. The amount of flash-induced electron transfer was determined from measurements of flash-induced net acidification of the reaction media by H^+ release associated with water oxidation (Fig. 5). We found that from 1.60 to 1.70 nmol H^+/mg Chl were released per flash with ferricyanide as the electron acceptor and the uncoupler nigericin present to promote rapid movement of H^+ across the membrane to the

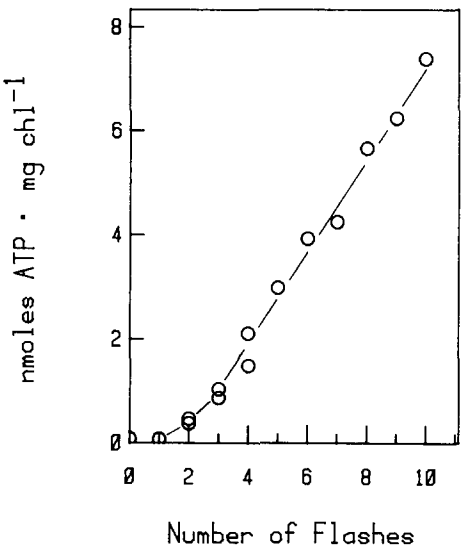

Fig. 4. Dependence of ATP formation on the number of single-turnover flashes. Chloroplasts containing 64 μg Chl were suspended in 4 ml of reaction mixture containing 40 mM sorbitol, 5 mM Tricine-KOH (pH 8.1), 2 mM $MgCl_2$, 20 mM KCl, 1 mg/ml bovine serum albumin, 0.1 mM methyl viologen, 0.1 mM ADP, and 0.5 mM $Na_2H^{32}PO_4$ (15 μCi). Chloroplasts (1.6 mg Chl/ml) were dark adapted for at least 60 min at 2°C in a solution containing the first five ingredients listed above. The reaction temperature was 4°C and the flash frequency 10 Hz.

external solution. This measurement shows that there were about 670 Chl molecules per active PS II center. The independence of H^+ release on flash frequency from 1 to 30 Hz (Fig. 5) demonstrates that the intervening dark times were, in all cases, adequate to allow for complete regeneration of the PS II centers to their photochemically active state. Moreover, the fact that the same result was obtained when the flash energy was increased 25% (▲ symbols in Fig. 5) establishes that the flashes were saturating. Using the value of 1.65 nequiv./mg Chl per flash, an ATP/2e$^-$ ratio calculated using the slope of the line from 3 to 10 flashes in Fig. 4 shows that an efficiency in excess of 1.0 was reached after only three flashes which means that 0.8 molecules of ATP were produced per coupling factor on each turnover subsequent to the third (using the value of 860 Chl/coupling factor [16]). Thus, using the ratio of 2 H^+ accumulated per electron transferred through the chain, ATP formation was observed to begin after the accumula-

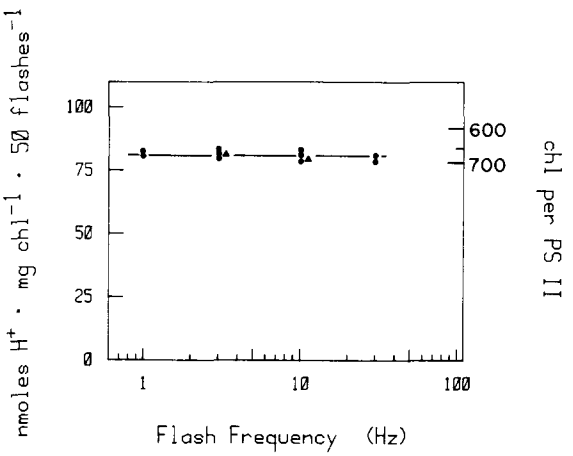

Fig. 5. H^+ release associated with flash-induced oxidation of water. Measurement of H^+ release from a series of 50 flashes was made with a pH-sensitive glass electrode in a 4 ml reaction volume consisting of 100 mM sorbitol, 25 mM KCl, 2 mM $MgCl_2$, 0.5 mM Tricine-KOH (pH 8.1), 0.2 mM ferricyanide, 0.2 mM 2,5-dimethylquinone, 1.5 μM nigericin and chloroplasts equivalent to 160 μg Chl. The xenon flashes were filtered through a Corning CS 3-71 sharp yellow cut-off filter. The flash energy (total discharge energy) was either 10.5 J (●) or 13 J (▲). The number of chlorophyll molecules per active PS II center was calculated using an average molecular weight for Chl *a* and Chl *b* of 900.

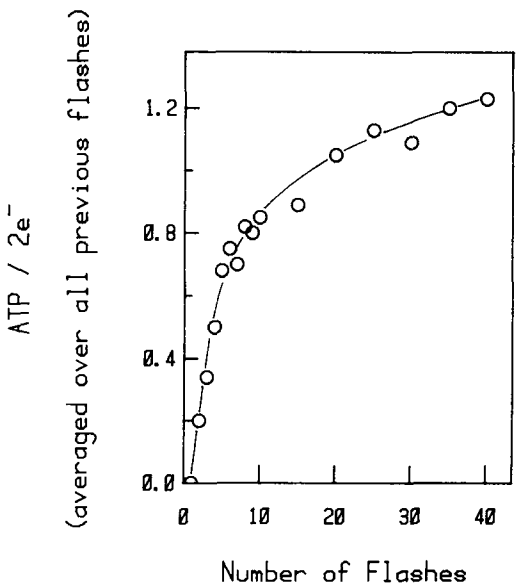

Fig. 6. The efficiency of flash-induced electron transfer in supporting ATP formation (ATP/2e) as a function of the number of flashes. The reaction conditions were identical to those given for Fig. 4. The value for flash-induced electron transfer of 1.7 nequiv./mg Chl per flash used in the calculation of the ATP/2e ratio was taken from the data in Fig. 5.

tion of only 7 nmol H^+/mg Chl and had reached nearly maximum efficiency (ATP/$2e^-$ of 1.0 vs. the maximum value of 1.2 measured in continuous light) after the accumulation of only 10.5 nmol H^+/mg Chl. If a Q-cycle involving plastoquinone and the plastoquinol : plastocyanin oxidoreductase is operative under these conditions then these threshold pool sizes would have to be increased in accord with the increase in the H^+/e^- ratio. In Fig. 6 the average ATP/$2e^-$ ratio, which includes the poorly coupled initial flashes, is plotted as a function of the number of flashes. This figure shows that the efficiency in flashing light attained fully the steady value of 1.2.

The capacity of the chloroplast lamellar membrane to store charge (i.e., electrical capacitance) is believed to be small in comparison to its capacity to bind protons (i.e., H^+-buffering capacity). Consequently, the development of the two components of the electrochemical potential should show a different dependence on flash number and this should be reflected in the dependence of ATP formation on flash number. Valinomycin-facilitated movement of K^+ through the lamellar membrane acts to oppose the formation of the electric potential of accumulated H^+. In fact, so long as the internal K^+ reservoir remains adequate, valinomycin should prevent the formation of any delocalized electric potential altogether. Fig. 7 shows the effect of valinomycin and K^+ on the dependence of ATP formation on flash number.

The formation of ATP was abolished by valinomycin plus K^+ for a period of 7–8 flashes regardless of whether the flash frequency was 1 or 10 Hz (Fig. 7). We have shown previously that the concentrations of both valinomycin and K^+ were in excess [17]. In these experiments the chloroplasts were dark incubated in the presence of the indicated amount of KCl or KCl plus valinomycin for at least 1 h to ensure equilibration. These data indicate that about 26 nmol H^+/mg Chl are required to establish a ΔpH that is energetically adequate to drive efficient ATP formation in the absence of any membrane potential. If a ΔpH of 2.7 units is required in order to initiate ATP formation [9], then the average ΔpH per flash during the first eight flashes would be in excess of 0.3 units. The ΔpH/flash would not be expected to be linear with flash number, since the internal buffer is weaker in the alkaline range [18], thus the

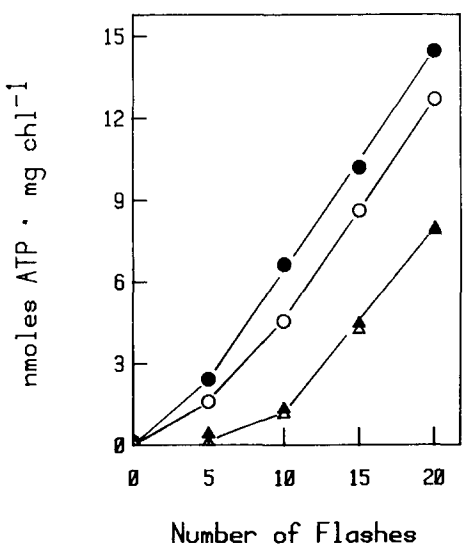

Fig. 7. The effect of valinomycin and K^+ on the dependence of ATP formation on flash frequency and flash number. The reaction conditions were the same as those given for Fig. 4. (○——○) 1 Hz, (●——●) 10 Hz, (△——△) 1 Hz plus 0.4 μM valinomycin, (▲——▲) 10 Hz plus 0.4 μM valinomycin.

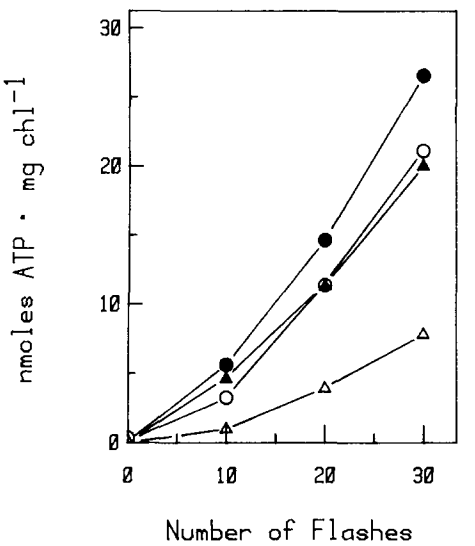

Fig. 8. The effect of nigericin and K^+ on the dependence of ATP formation on flash frequency and flash number. The reaction conditions were the same as those given for Fig. 4. Identification of the symbols is as follows: (○——○) 1 Hz, (●——●) 10 Hz, (△——△) 1 Hz plus 0.1 μM nigericin, (▲——▲) 10 Hz plus 0.1 μM nigericin.

ΔpH/flash on the first few flashes may be substantially greater than the average. Tiemann et al. [19] arrived at the value of 0.2 pH units/flash from the measurements with neutral red which is lower than our value but much more in line than the value of 0.06 reported by Junge et al. [20] or the value of 0.0001 reported by Grunhagen and Witt [21].

Development of a transmembrane ΔpH can be inhibited by nigericin, which catalyzes an electrically neutral exchange of H^+ for K^+ or certain other alkali metal ions. In contrast to valinomycin, the effect of nigericin on ATP synthesis under flashing light was strongly frequency dependent (Fig. 8). The inhibition of ATP formation by 100 nM nigericin, which uncouples steady-state ATP formation by about 50% at this pH, was substantial at 1 Hz (approx. 70%) but minimal at 10 Hz (approx. 15%). Measurements of H^+ uptake made under the same conditions show that while substantial net H^+ uptake occurred in the presence of 100 nm nigericin, the half-time of the decay was decreased from 42 to 13 s by the uncoupler (data not presented).

The data in Fig. 9 indicate that nigericin acts to enhance the electrical component of the electrochemical potential difference at the expense of the transmembrane pH difference. The top trace in Fig. 9 shows the absorption change (518–540 nm) from a series of 15 flashes delivered at 10 Hz in the absence of ionophores. The addition of 100 nM nigericin caused an increase in the extent of the absorbance change (measured after the fourth flash) by 50%. An increase in the extent of the absorption change by nigericin was clearly evident after the second flash and possibly even after the first flash. Thus, the frequency dependence of nigericin can be largely accounted for by the enhanced electric potential that builds up at higher frequency in the presence of nigericin thereby replacing the ΔpH which had been partially dissipated. At a flashing frequency of 1 Hz the intervening dark period allowed complete relaxation of the electrical field as measured by the 518 nm absorption change (data not shown) so a compensatory increase in the electric field was not possible.

Fig. 9 also shows the elimination of the flash-induced electrochromic absorption change by

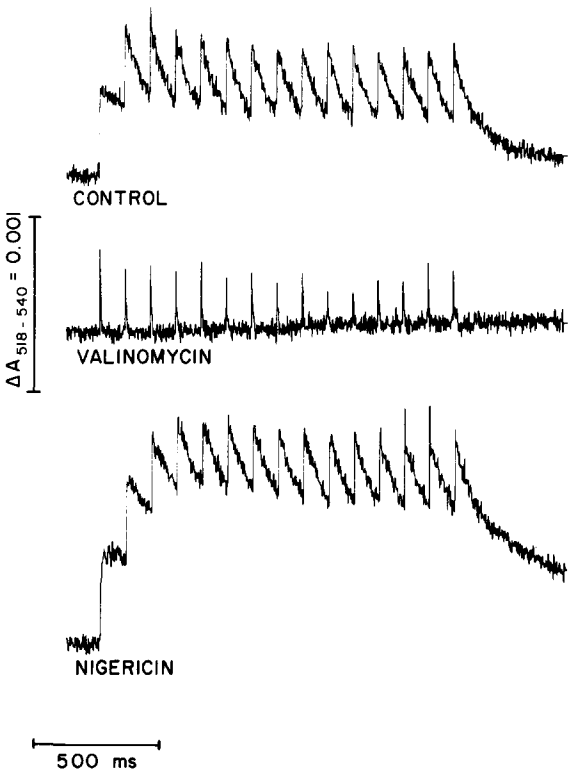

Fig. 9. The effect of valinomycin and K^+ or nigericin and K^+ on the flash-induced electrochromic absorption band shift (518–540 nm). Reaction conditions as in Fig. 4. Sequences of 15 flashes at 10 Hz were delivered to the sample at 60-s intervals. The absorbance changes resulting from four sequences of 15 flashes were averaged at each wavelength. Trace A, no additions; trace B, plus 0.4 μM valinomycin; trace C, plus 0.1 μM nigericin.

valinomycin-facilitated movement of K^+. The half-time for the decay of the absorption change was decreased from 220 to 1.7 ms by 0.4 μM valinomycin at 1.0 Hz (data not shown). Fig. 9 shows clearly that there was no summation of the absorption change at 10 Hz in the presence of 0.4 μM valinomycin.

Discussion

There are two earlier reports regarding [^{32}P]ATP formation by chloroplasts in which single-turnover flashes were used as the actinic light source. The results of Harris and Crofts [22] are different in two respects from the results reported here. First, we observed approximately an order of magnitude

more ATP per flash and second, the optimum yield of ATP per flash was reached at a frequency of 3 Hz in our hands whereas Harris and Crofts reported a requirement for a frequency of 50 Hz for optimum yield. We have found that a general feature of ATP formation driven by single-turnover flashes is its extraordinary sensitivity to the conductance of the membrane to ions and, in particular, to H^+. For instance, levels of weakly basic amines which uncouple phosphorylation in continuous light hardly et all (i.e., less than 10%) decreased the ATP yield per flash at 10 Hz nearly 80%. Since ATP formation associated with single turnover flashes may be driven by near-threshold Δp levels this high sensitivity to uncouplers is not unexpected. If the chloroplast preparations employed by Harris and Crofts were slightly uncoupled during isolation it would account for the low ATP yields and the requirement for high flash frequencies. The flash yields and the frequency dependence of the ATP synthesis reported by Gräber and Witt [23] are in closer agreement with our data. Their optimum ATP/2e value was about 0.6 (calculated from their ATP data assuming 1.65 μequiv./mg Chl per flash) at 10 Hz but they observed a drastic decrease in the yield of ATP per flash as the frequency dropped below 5 Hz. It is likely that the low reaction temperature we employed contributed significantly to the ATP synthesis which we observed at low flash frequencies.

The measurement of flash-induced electron transfer shown in Fig. 5 was performed in a fashion very similar to the determination of 'photosynthetic unit size' described by Emerson and Arnold [24]. In contrast to Emerson and Arnold's measurement of oxygen evolved per saturating single-turnover flash, Fig. 5 shows the H^+ released from water oxidation per flash. Since four H^+ are released from the water oxidation apparatus for each molecule of O_2 evolved, the value of 670 Chl per water-oxidation H^+ (taken from Fig. 5) results in a value of 2680 Chl per O_2 evolved. This value of 2680 Chl per O_2 evolved for spinach is remarkably similar to the value of 2480 reported by Emerson and Arnold for *Chlorella pyrenoidosa*. The dependence of coupled electron transfer on flash frequency reported in the discussion of Fig. 1 was determined in a similar fashion. However, in this case, the uncoupler was omitted and hexokinase and glucose were included in the reaction mixture to convert newly formed ATP to glucose 6-phosphate avoiding the H^+ consumption associated with net ATP formation.

We reported previously that ATP synthesis during the initial period of continuous illumination exhibits an absolute requirement for a membrane potential (Refs. 10 and 25, see also Refs. 23 and 26). In its absence the onset of ATP formation is delayed for a period of 40 ms (at saturating light intensity and ΔG_{ATP} of 38 kJ/mol). These data do not provide precise information about the number of turnovers of the electron-transport carriers necessary to initiate photophosphorylation in the absence of an electrical field. Employing saturating single-turnover flashes we found that a minimum of eight turnovers of the electron carriers was necessary (Fig. 7) to establish a ΔpH adequate to initiate ATP synthesis in the absence of an electric potential (Fig. 9) and against a ΔG_{ATP} of 38 kJ/mol. At flash frequencies below 1 Hz the number of turnovers required to initiate ATP synthesis under these conditions increases.

The inhibition of photophosphorylation by valinomycin is independent of flash frequency between 1 and 10 Hz (Fig. 7). The lack of effect of frequency on the yield of ATP in the presence of valinomycin plus K^+ suggests that there is no significant loss of the accumulating high-energy state when the flashes are spaced 1 s apart as compared to 0.1 s apart. This is consistent with the notion that a slowly decaying ΔpH is the driving force for ATP synthesis in the presence of valinomycin plus K^+. Our measurements of the decay of the H^+ gradient under our reaction conditions (i.e., 4°C and pH 8.1) showed a half-time of 20 s which would allow only 3.5% of the H^+ to leak out between flashes spaced 1 s apart (calculated assuming a first-order exponential relaxation of the transmembrane pH difference). In contrast to the frequency independence of the inhibition of ATP synthesis by valinomycin plus K^+, the uncoupling of ATP formation by nigericin plus K^+ is strongly frequency dependent. Although a change in flash frequency from 1 to 10 Hz had a minimal effect on the magnitude of ΔpH, it had a very large effect on the magnitude of $\Delta\psi$. The increase in the amplitude of the electrochromic absorption

change (518–540 nm) with successive flashes (Fig. 9) was virtually absent if the flashes were spaced 1 s apart. It follows that the Δp generated at 10 Hz would be larger than the Δp generated at 1 Hz, since $\Delta\psi$ is larger at 10 than 1 Hz (because of summation) and ΔpH is essentially unchanged. It should be pointed out that although the yield of ATP per flash in the presence of nigericin was restored at a flashing frequency of 10 Hz, the composition of the Δp was markedly different from that in the absence of the exchange carrier. In the presence of nigericin the membrane potential constitutes a much larger portion of the total Δp.

There are several differences between the effects which nigericin has on ATP formation from single-turnover flashes as compared to the effects it has on ATP formation [10] from multiturnover flashes. We found nigericin to be a potent uncoupler of initial phosphorylation resulting from continuous illumination at pH 8.0 (10). One might expect that increasing the frequency of single-turnover flashes would ultimately approach the condition of continuous illumination. Yet, the uncoupling of single-turnover flash ATP synthesis by nigericin was found to decrease as the frequency was increased. Nigericin (100 nM) was found to stimulate the electrochromic absorption change resulting from a 21 ms flash by only about 10% at pH 8.0 (Fig. 8 of Ref. 10). On the other hand, Fig. 9 shows 100 nM nigericin resulted in a 50% increase in the electrochromic absorption change after four flashes. There are evidently significant differences between the condition of multiple turnovers during a long flash and the condition of turning the electron-transport carriers over in unison with a series of xenon flashes. It is likely that synchronous turnover results in a higher proportion of the Δp in the form of a membrane potential, but we are presently unable to say what other differences might also exist.

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