PROTONMOTIVE CYCLIC ELECTRON FLOW AROUND PHOTOSYSTEM I IN INTACT CHLOROPLASTS

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1. Introduction

Recent experiments have suggested that cyclic electron flow driven by photosystem I may function to supply a portion of the ATP needed for carbon assimilation in intact chloroplasts [1–4]. These studies further demonstrated the importance of electron flow from photosystem II in redox poising the photosystem I cycle: the inhibitor DCMU was used to vary the relative activity of the former and show that over-reduction or over-oxidation of the cycle could arise, respectively, from excessive or inadequate photosystem II activity [4]. Another variable shown to influence cycle turnover was the rate of electron flow from the reducing side of photosystem I to electron acceptors and oxygen [3].

In this work, photosystem II was completely inhibited by DCMU, no electron acceptors were provided and dithionite was employed to obtain a sufficiently negative redox poise to support cycling. Dithionite in the concentrations used does not over-reduce the cycle possibly due to a slow interaction with electron carriers within the thylakoid membrane. This system permits analysis of the energetic relationships of the photosystem I cycle without interference from linear or pseudocyclic electron transport, and provides the first direct evidence of electrogenic H^+-pumping driven by photosystem I alone in whole chloroplasts.

2. Materials and methods

Intact chloroplasts were isolated from spinach as in [2]. Integrity, as assayed by the ratio of oxygen evolution rates in the presence of ferricyanide before and after osmotic shock, was routinely around 70%. Fluorescence and absorption spectrophotometric measurements were performed at 18–22°C with chlorophyll at 20 μg/ml and 50 μg/ml, respectively. The buffer used contained 0.35 M sorbitol, 50 mM tricine, 0.3 mM orthophosphate and 3 mM $MgCl_2$, adjusted to pH 8.1 with NaOH and KOH. Catalase was added to all samples at 1500 units/ml. Fluorescence emission from 9-aminoacridine (9-AA) was measured as in [4] and measurements of flash-induced ΔA_{518} were made with the single beam spectrophotometer in [5]. The flashes, from two EG and G FX-201 Xe lamps, were filtered through red glass (Schott RG 665) and had durations at half-peak emission of 4 μs. The flashes gave > 80% saturation when used with 1 × 0.5 cm cuvettes. Averages of 128 traces were taken at a flash frequency of 1.5 Hz.

3. Results and discussion

Experiments were performed on intact chloroplasts without added electron acceptors. Similar results were obtained:

(i) After preillumination of the chloroplasts in the presence of 0.2 mM hydroxylamine to destroy the oxygen evolving system;
(ii) With far-red (Schott RG 695) illumination, making due allowance for the consequent lower actinic intensity;
(iii) With reaction medium deoxygenated either by prolonged bubbling with nitrogen or by glucose/glucose oxidase system.

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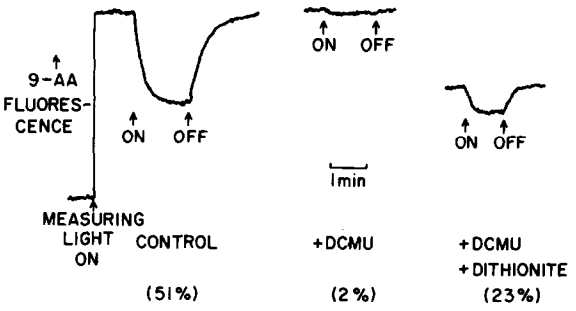

Fig.1. Light-induced changes in 9-AA fluorescence quenching in intact chloroplasts. DCMU (20 μM) and sodium dithionite (10 mM) were added to suspensions of intact chloroplasts as in section 2. The actinic light was passed through heat and Corning 2-58 filters to give red light with at 180 W/m^2. The sensititivity of the apparatus was the same for all three traces.

In all cases the additions described below produced results similar to those shown here. Hence it is unlikely that linear or pseudocyclic electron flows contributed to the following observations.

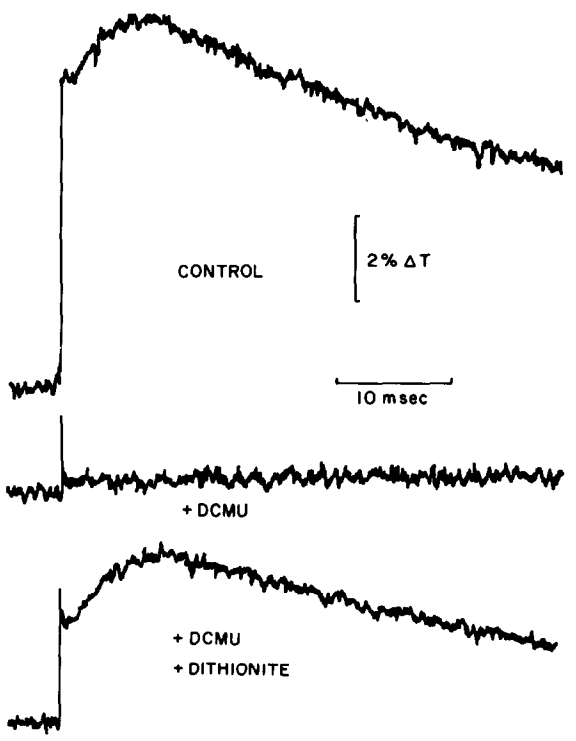

Fig.2. Flash-induced ΔA_{518} with additions as in fig.1.

Figure 1 shows the inhibition by 20 μM DCMU of light-induced quenching of 9-AA fluorescence. Upon subsequent addition of dithionite, a decline in fluorescence yield was registered by the modulated interrogating beam and the actinic light elicited a quenching of about 50% that seen in the control (the decline in fluorescence yield was seen with free solutions of 9-AA in buffer upon addition of dithionite; the extent of decline depended on the dithionite concentration). An inhibition and subsequent partial regeneration of the flash-induced ΔA_{518} was seen with the same additions (fig.2); the spectrum of the regenerated change was like that of the control before DCMU addition, although the absolute shape varied from preparation to preparation. Such additions of solid sodium dithionite caused pH changes of < 0.2 units.

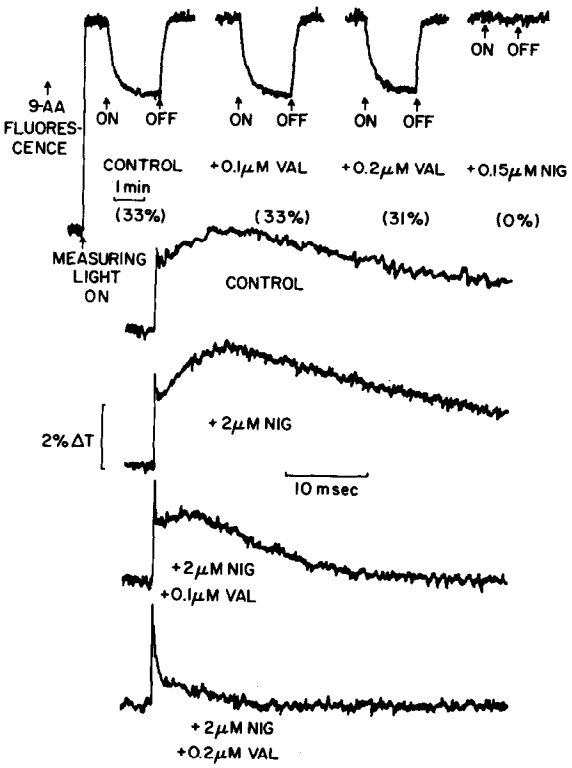

Fig.3. Light-induced 9-AA fluorescence quenching (upper) and flash-induced ΔA_{518} (lower) in intact chloroplasts. Control traces contained 20 μM DCMU and 10 mM sodium dithionite as in the final traces of fig.1,2. Valinomycin and nigericin were added as shown. ΔA_{518} in the absence of nigericin showed the same sensitivity to valinomycin.

Evidence that the regenerated responses of fig.1 and 2 demonstrate restoration by dithionite of transmembrane pH and electric potential gradients, respectively, is presented in fig.3. Nigericin completely inhibited the light-induced quenching of 9-AA fluorescence but did not lower the extent of the flash-induced ΔA_{518} (the rise time of the slow component of this change is reduced by nigericin, as would be expected if the pH gradient produced by the repetitive flashes retarded electron flow, according to chemiosmotic theory). Valinomycin, conversely, did not affect 9-AA fluorescence quenching at low concentrations but accelerated the decay of the ΔA_{518}. The mechanism of action of these ionophores is well documented [6,7]. Additional evidence that dithionite restores the ability of the thylakoid to develop a transmembrane pH gradient comes from data (not shown) demonstrating restoration of the slow change in chlorophyll-*a* fluorescence yield on illumination under the conditions in fig.1,2. This latter change has been associated with the production of a transthylakoid high energy state [8–10].

Figure 4 shows the effects of the electron-transport inhibitors antimycin A and DBMIB on the 9-AA and

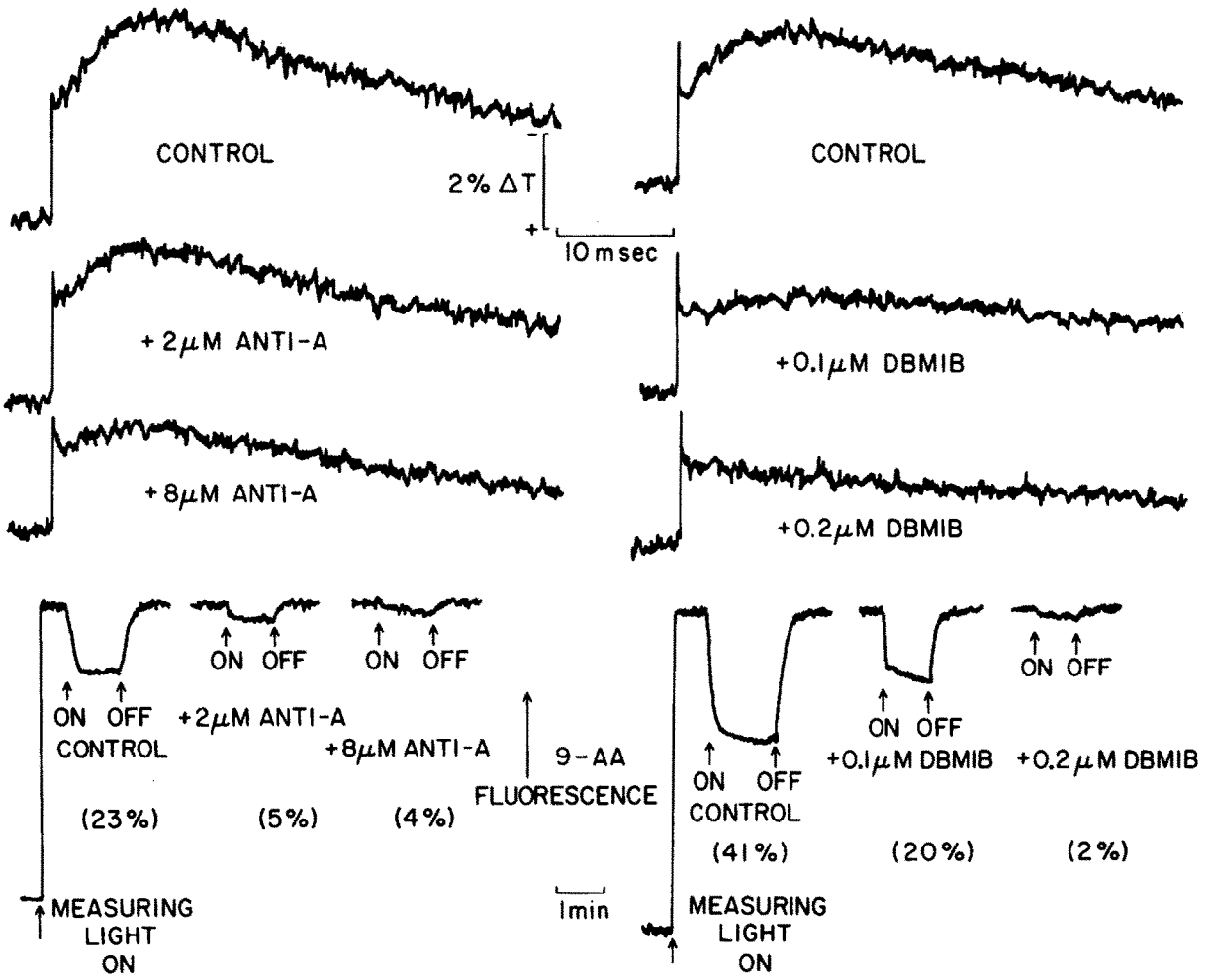

Fig.4. Sensitivity of flash-induced ΔA_{518} (upper) and of light-induced 9-AA fluorescence quenching (lower) in intact chloroplasts to antimycin A and DBMIB. DCMU and dithionite were present in the control samples, as in fig.3. Antimycin A and DBMIB were added as shown.

A_{518} responses restored by dithionite after DCMU inhibition. Both substances clearly inhibit the establishment of a pH gradient and a slow component of the flash-induced A_{518} response, with DBMIB being the more effective. This result agrees with the reported effect of these inhibitors on cyclic electron flow in unpoisoned intact chloroplasts [5]. Slow chlorophyll-*a* fluorescence yield changes on illumination showed the same sensitivities.

Restoration by dithionite of light-induced membrane potential generation and proton pumping does not occur to a significant extent in chloroplasts subjected to osmotic shock (fig.5 and slow chlorophyll-*a* changes – not shown). This denotes participation in the restored system of at least one soluble stromal factor (recent experiments by J. D. Mills suggest that ferredoxin alone may not be sufficient to restore full activity in broken chloroplast preparations) and explains why the slow rise in the A_{518} signal was not observed in [11].

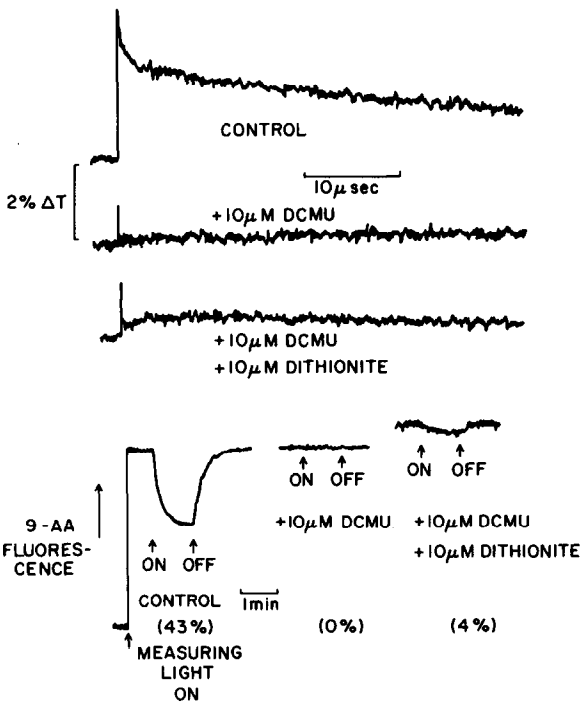

Fig.5. Flash-induced ΔA_{518} (upper) and light-induced 9-AA fluorescence quenching (lower) in osmotically-shocked chloroplasts. Conditions as for fig.1,2 with additions as shown. Sensitivity of apparatus increased for lower trace with DCMU and dithionite.

The results described here demonstrate that cyclic electron flow driven by photosystem I alone may produce an H^+ electrochemical gradient across the thylakoid membranes of intact chloroplasts. An interesting comparison can be drawn with the energy-transducing electron transfer chain of the purple non-sulfur photosynthetic bacteria *Rhodopseudomonas capsulata* and *spheroides*. These organisms utilize light-driven cyclic electron flow to generate a transmembrane H^+ gradient and produce ATP. Their electron transfer chains are inhibited both by antimycin A and by DBMIB [12–15] and they require an added reductant to poise the redox potential for optimal operation. However while a potential of $< +150$ mV at pH 7.0 is adequate for the bacterial cycle, the thylakoid cycle seems to need much lower potentials: for example only the bacterial system can be suitably poised by ascorbate.

The transmembrane electric field generated by a light flash in chromatophores from the above organisms rises in three phases, two occurring in $< 100\ \mu s$ and the third having a half-time in the ms range that depends on the state of reduction of a component 'Z' [12,13,16,17]. Under optimal conditions, the extent of this slow phase is equal to the summed extents of the fast phases and has been proposed [12,17] to reflect a second transmembrane electrogenic step. The results presented here suggest that a similar process may operate around photosystem I in intact chloroplasts. In this case, the simple schemes so far proposed for cyclic electron transport in the chloroplast will have to be amended to accomodate a dark transmembrane electrogenic reaction. For discussions of possible arrangements of the equivalent system in photosynthetic bacteria see [18].

Further studies on the composition, arrangement and function of the cycle are under way.

Acknowledgements

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