THE FUNCTION OF CYCLIC ELECTRON TRANSPORT IN PHOTOSYNTHESIS

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1. Introduction

The photosynthetic reduction of 1 mol CO_2 requires that coupled electron transport supply a minimum of 3 mol ATP and 2 mol NADPH [1]. While stoichiometries as high as 2 ATP/NADPH have been reported [2], more commonly obtained values indicate a ratio between 1.0 and 1.33 [3]: just short of the minimum required. Two light-dependent processes have been suggested to fulfill the ATP deficiency. One [4] is a ferredoxin-dependent cycle sensitized by photosystem I. The other is a Mehler reaction or pseudocyclic electron flow from H_2O to O_2 via a coupled portion of the electron transport sequence also involved in $NADP^+$ reduction [5].

In this paper, experiments are described which differentiate between cyclic and pseudocyclic phosphorylation, and assess their relative contribution to ATP synthesis in the intact chloroplast.

2. Materials and methods

Intact chloroplasts capable of sustaining HCO_3^--supported oxygen evolution at rates of 100–200 μmol O_2/mg chl-h were isolated from spinach (*Spinacia oleracea*) as in [6]. Measurements at 18–20°C were performed with samples composed of 20–22 μg chl·ml^{-1} in 3 ml reaction buffer containing: 0.36 M sorbitol; 50 mM tricine; 0.3 mM K_2HPO_4; adjusted to pH 8.15 with KOH. Near anaerobic samples (<20 μM O_2) were produced by flushing reaction buffer with N_2 prior to chloroplast addition. The 518 nm absorption change was recorded with a single beam spectrophotometer having vertical optics. The fluorescence of 9-amino-acridine (9-AA) at 460 nm was elicited by a weak 370 nm probe beam modulated at 270 Hz in samples containing 10 μM 9-AA. Chlorophyll fluorescence at 680 nm was measured in the same apparatus. ATP was assayed by the luciferin–luciferase method in samples extracted with $HClO_4$ and neutralized with MOPS–KOH. Total adenine nucleotide content was determined after enzymatic conversion of both ADP and AMP to ATP. Actinic illumination was 1000 W/m^2 of blue (corning 4-96) light for polarographic oxygen evolution determinations with HCO_3^- or PGA and 100 W/m^2 for the chlorophyll fluorescence measurements. Otherwise the samples were illuminated with 100 W/m^2 of red (corning 2-58) light.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MOPS, morpholinopropane sulfonate; OAA, oxaloacetate; PGA, 3-phosphoglycerate; 9-AA, 9-aminoacridine

3. Results and discussion

Experiments were conducted with intact chloroplasts to which no electron acceptor was added, in order to confine electron transport to cyclic and pseudocyclic pathways. Light-induced changes in the quenching of 9-aminoacridine (9-AA) fluorescence were recorded as a measure of the trans-thylakoid pH gradient generated by cyclic and pseudocyclic electron flow.

Figure 1A shows that in the absence of DCMU, quenching of 9-AA fluorescence is about 50% greater in air than N_2 (at an O_2 tension approx. 10% normal air saturation). This suggests that pseudocyclic electron flow to oxygen contributes to the establishment of ΔpH. To investigate this possibility further, we compared the sensitivity of both 9-AA fluorescence

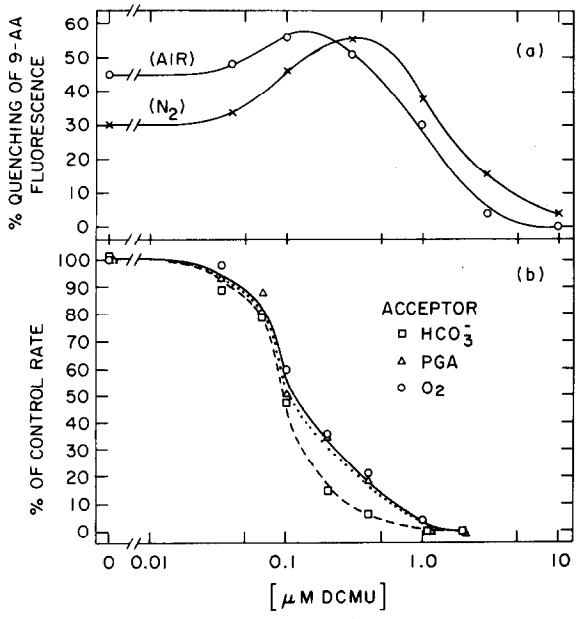

Fig.1. (A) DCMU concentration curves for 9-aminoacridine (9-AA) fluorescence quenching by illuminated chloroplasts equilibrated with N_2 (X–X) or air (o–o) in the absence of added acceptor. (B) DCMU concentration curves for linear electron transport function with 8.0 mM HCO_3 (□– –□), 5.0 mM PGA (△· · ·△) or 280 μM O_2 (o–o) alone. Samples for O_2 uptake contained in addition to 1.0 mM KCN and 3.3 mM NH_4Cl. Control rates at light saturation, for HCO_3^- and PGA reduction were 123 and 87 μmol/O_2/mg chl·h, respectively. The control rate of O_2 uptake was 12 μmol O_2/mg chl·h (about 1/3 saturated rate) in samples illuminated with 100 W/m² of red light for direct comparison with the results of fig.1A.

quenching and the Mehler reaction (measured as O_2 uptake) to DCMU, an inhibitor of noncyclic electron flow.

Addition of DCMU, under air or N_2, leads first to an increase, at concentrations above 0.1 μM, and then to a total inhibition of 9-AA fluorescence quenching. However, reference to fig.1B reveals that pseudocyclic electron flow (like linear flow to an added acceptor such as CO_2 or PGA) is 50% inhibited by about 0.1 μM DCMU and totally eliminated by 1 μM DCMU. It can be asserted with reasonable certainty then, that the quenching seen in the range 0.5–3 μM DCMU results entirely from cyclic electron flow. Furthermore, the enhancement of quenching by lower concentrations of DCMU may be explained on the basis of an increase in cyclic turnover through more perfect 'poising' of carriers in the pathway. A comparable stimulatory effect of DCMU on phosphorylation was noted [7] in reconstituted chloroplasts and [8] in intact chloroplasts fixing CO_2 in the presence of added dihydroxyacetone phosphate. The observed inhibition of 9-AA fluorescence quenching in air by the presence of >2 μM DCMU (fig.1A) presumably again reflects incorrect 'poising' and results from the inability of photosystem 2 to replace electrons bled from reduced cycle intermediates by a reaction with O_2.

The dependence of cyclic electron flow upon proper redox poising can be observed not only by 9-AA fluorescence quenching but by other energy-dependent parameters such as the rate of chlorophyll fluorescence quenching and the thylakoid conformational change indicated by a slow absorption rise at 518 nm [9]. Upon illumination of chloroplasts in the presence of a low oxygen tension, (fig.2) 9-AA fluorescence quenching is slow and suboptimal as is the quenching of fluorescence from chlorophyll itself. Similarly, there is little evidence for a slow component in the P518 absorption increase. The addition of 0.1 μM DCMU accelerates the formation rate and the

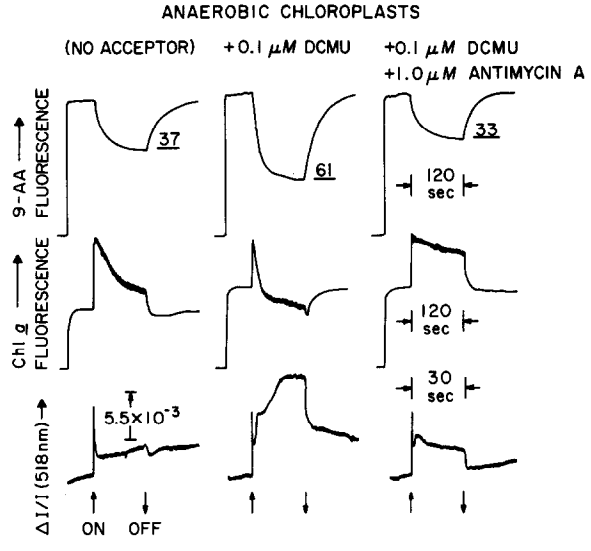

Fig.2. High energy state phenomena in near anaerobic chloroplast samples without added electron acceptors. Underlined numbers give % quenching of 9-AA fluorescence in the steady state. Measurement conditions as described in section 2.

extent of the pH gradient. Chlorophyll fluorescence is more rapidly quenched and a large slow component in the P518 response develops. Addition of the inhibitor of cyclic photophosphorylation, antimycin A [4], diminishes the pH gradient and the quenching of chlorophyll fluorescence, and abolishes the slow P518 response. Note that while antimycin A has been reported to inhibit [10] and uncouple [10,11] linear electron flow, we have shown [12] that these latter effects do not occur at the low concentration employed here.

Figure 3 shows that virtually identical results to fig.2 are obtained in air with the notable exception that the initial control rates and levels are higher. This suggests that oxygen brings about a closer approximation to optimal poising of cyclic activity, without the need for DCMU. The addition of antimycin A, with or without DCMU present, significantly inhibits all three manifestations of the high energy state in chloroplasts. Clearly a predominant portion of the high energy state must originate from cyclic rather than pseudocyclic electron flow, in the absence of an added electron acceptor.

Table 1, shows that the light-induced change in the endogenous ATP pool, measured with two separate chloroplast preparations, is well correlated with the results in fig.2,3. Optimal production of ATP by cyclic electron flow clearly requires proper poising by O_2 or DCMU or preferably by both. When the endogenous acceptors such as HCO_3^-, PGA and OAA are omitted from intact chloroplasts and poising is optimized, the addition of antimycin A reduces the amount of ATP formed by 35–40%. These results are supported [13], where low concentrations of

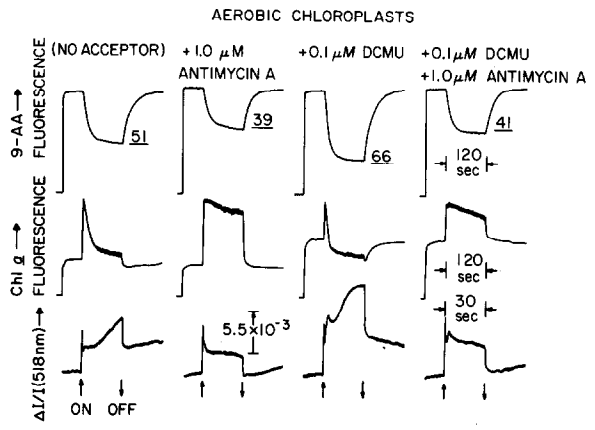

Fig.3. High energy state phenomena in aerobic chloroplast samples without added electron acceptors. Measurement conditions as described in section 2.

DCMU enhanced the rate and antimycin A decreased the rate of CO_2 fixation in a chloroplast system dependent only on the supply of ATP.

4. Conclusions

These results are important observations in several respects:

(i) They provide clear evidence that cyclic photophosphorylation can occur in intact chloroplasts under aerobic conditions. Even in the absence of added DCMU, cyclic electron transport is apparently well 'poised', and antimycin-sensitive phos-

Table 1
Light-induced change in the endogenous ATP levels of intact chloroplasts without added electron acceptors

Exp.	Anaerobic (nmol ATP/mg chl)[a]				Aerobic (nmol ATP/mg chl)			
	Light	Control	+DCMU	+DCMU +AntiA	Control	+AntiA	+DCMU	+DCMU +AntiA
1	15 s	5.2	8.1	4.8	7.2	5.7	9.8	6.5
2	30 s	4.2	7.0	4.3	5.9	4.2	7.5	4.5

[a] Values represent the increases in ATP produced by the illumination time indicated and under the conditions given in fig.2,3. The initial dark distributions of ATP, ADP and AMP in nmol/mg chl for preparation 1 were 3.6, 15.4 and 4.5, respectively. For preparation 2 the values were 2.7, 11.6 and 10.2

phorylation of the stromal ADP pool can occur rapidly. This result suggests that the cyclic contribution to ATP production is an integral requirement for optimal aerobic CO_2 fixation as proposed [4,6,12,13].

(ii) Since optimal cyclic turnover requires the presence of O_2, it seems that pseudocyclic electron flow may function to poise correctly the redox carriers in the cyclic pathway. The fact that energy-dependent processes such as chlorophyll *a* fluorescence quenching are inhibited under anaerobic conditions [14,15] may be due to improper poising of the cyclic system rather than inhibition of proton pumping associated with pseudocyclic electron flow.

(iii) The extreme sensitivity to antimycin A of energy-dependent membrane structural changes which are monitored by chlorophyll *a* fluorescence and light scattering (P518) suggests that coupled cyclic electron transport activity is critical for these processes. Other experiments suggest that cyclic electron transport also contributes to slow chlorophyll *a* fluorescence quenching in isolated intact chloroplasts which are reducing either CO_2 or oxaloacetate.

It is clear that cyclic electron flow contributes greatly to the energetic requirements of the chloroplast under several different conditions and in the presence of oxygen. This conclusion conflicts with [2,16,17] which have suggested that cyclic electron flow is unnecessary for ongoing photosynthesis in vivo.

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