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Communication

Interaction between Chloroplasts and Mitochondria in Microalgae

ROLE OF GLYCOLYSIS

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ABSTRACT

In a mutant strain of *Chlamydomonas reinhardtii* devoid of active ribulose 1,5-bisphosphate carboxylase oxygenase, the addition of mitochondrial inhibitors in the dark resulted in a pronounced decrease in cellular ATP, a fall of the glucose 6-phosphate content, and a rise of the NADPH concentration. These biochemical changes were accompanied by an increase of the fluorescence level, showing changes in the redox state of the chloroplastic electron transport chain. Similar results were obtained in presence of an uncoupler. These data indicated that alterations in the mitochondrial electron transport chain in dark could affect the chloroplastic chain, probably through variations of the glycolysis activity. When mitochondrial oxidases were blocked, illumination of the algae reversed the effect of the inhibitors on the ATP and glucose 6-phosphate concentrations. This last result suggested that the chloroplastic photophosphorylations in these algae played a major role in the control of the glycolytic flux.

The question of how the mitochondrial respiration in green cells, and in particular oxidative phosphorylations, interacts with the photosynthetic activity in the chloroplast remains to be answered. Although direct evidence is lacking, it has been assumed that the phosphorylating power generated under light condition in the chloroplast, is transferred to the cytosol via shuttle systems (7, 15) and that mitochondrial respiration is inhibited by a high cytosolic ATP/ADP ratio (2). This was apparently the case in a mutant strain of *Chlamydomonas reinhardtii* devoid of active Rubisco[1] (3), where a 60 to 75% inhibition by light of the CO_2 evolution rate was recorded. However, an alternative proposal was to consider that NADPH produced at the chloroplast level was transferred to the cytosol and oxidized by the mitochondrial electron transport chain. In this connection it has been recently reported in barley leaf protoplasts (10) that selective inhibition of mitochondrial oxidative phosphorylation resulted in a 50% decrease of photosynthesis. The authors concluded that mitochondrial oxidative phosphorylation served an essential function for supplying the cytosol with ATP and postulated that synthesis of ATP in mitochondria could be sustained by oxidation of NAD(P)H generated at the chloroplast level. In the present paper we sought to further determine the effect of blocking mitochondrial oxidative phosphorylation on the chloroplastic electron transport chain and the metabolite pools of ATP, NADPH and NADH in *C. reinhardtii* rcl-u-1-10-6 C, a mutant devoid of active Rubisco.

[1] Abbreviations: Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; A.A., antimycin A; CCCP, carbonylcyanide *m*-chlorophenylhydrazone; SHAM, salicylhydroxamic acid; G_6P, glucose 6-phosphate.

MATERIALS AND METHODS

Chlamydomonas reinhardtii, mutant strain rcl-u-1-10-6 C (+), generously provided by Dr. P. Bennoun, was grown in 500 mL flasks on an acetate-supplemented medium (13) as previously described (3). Before each experiment, algae at the end of the exponential phase of growth were harvested by centrifugation (3000 rpm, 10 min), washed with an acetate-free medium and resuspended in the same acetate-free medium (3).

Net oxygen exchanges in solution were measured with a Clark-type electrode (Hansatech, King's Lynn, Norfolk, U.K.) at 25° C. For nucleotide and G_6P determinations, algae (3 mL suspension) were quickly dropped into liquid N_2 and the frozen suspensions were homogenized in the presence of perchloric acid (5% final) as previously described (3). After neutralization with saturated potassium bicarbonate, pyridine nucleotides were determined using an enzymatic cycling method (19) and adenine nucleotides as described by Strehler (20). G_6P was measured according to Lang and Michal (11). Chl content was determined according to Schmid (17).

Chl fluorescence induction in microalgae was measured with a pulse modulation technique (basic system PAM 101 and saturation pulse unit 103, H. Walz, Effeltrich, West Germany) as described by Schreiber *et al.* (18). The fluorescence level was measured with a weak modulated light (integrated intensity, 0.8 $\mu E \cdot m^{-2} \cdot s^{-1}$) and the maximal fluorescence yield was determined with 500 ms pulses (light intensity 1600 $\mu E \cdot m^{-2} \cdot s^{-1}$) applied every minute.

RESULTS

Krause *et al.* (9) have shown that Chl fluorescence quenching has two major components. One is due to the primary acceptor of PSII, Q_A, and the other is energy dependent quenching, presumably related to intrathylakoid pH (9) or thylakoid membrane polypeptide phosphorylations (21). These two components can be differentiated with a pulse modulation fluorometer, in combination with a repetitive application of saturating flashes (18). From the fluorescence response curves obtained in these conditions, it is possible to estimate the relative extent of Q_A

reduction (18). As shown in Figure 1A, illumination of the algae induced a rapid rise of the reduction state of Q_A, due to PSII activity. In the same time, the O_2-uptake rate was strongly inhibited, as previously described (3). When added separately, inhibitor of the mitochondrial electron transport chain (A.A. for the cytochrome oxidase pathway, SHAM for the alternative pathway) (2) had no significant effect neither on the dark respiration, as previously reported (14) nor on the fluorescence level. However, the combination of both inhibitors caused in dark a 60 to 70% inhibition of the O_2-uptake rate, but also resulted in an increase of the Q_A reduction state (Fig. 1B). A successive illumination had practically no effect on the O_2-uptake rate but increased further the extent of Q_A reduction. Separate control experiments indicated that A.A. and SHAM had no significant action in light on the chloroplastic electron transport chain (14). Addition of 2μM CCCP in dark stimulated three fold the O_2-uptake rate and had a similar effect on the fluorescence response curves to A.A. plus SHAM, resulting in an increase of Q_A^- (Fig. 1C). Similar results were also obtained during an aerobic to anaerobic transition (result not shown). A common feature of these experiments was the suppression of mitochondrial ATP synthesis. This suggests that the variation of the redox state of Q_A observed in dark was related to changes in the cell energy charge.

As shown in Figure 2, the ATP pool in the dark was not affected by 1μM A. A. which indicated that activities of pathways such as alternative respiration and glycolysis were sufficient to maintain a high ATP level in these algae. Similarly, the ATP pool was not affected by SHAM alone (result not shown). However, in presence of A.A. plus SHAM, the ATP concentration dropped during the first minute, then increased back to a new but lower steady state value. It is now admitted that pyruvate kinase and fructose 6-phosphokinase, two key regulatory enzymes of the glycolysis, are tightly controlled by the cellular ATP concentration. Thus, it is likely that the decrease of the ATP pool, observed in presence of A.A. plus SHAM, induced an acceleration of the glycolysis rate (Pasteur effect), which in turn could contribute to the maintenance of this pool. Measurements of the G_6P pool (Fig. 2) indicated that this intermediate decreased markedly when the dark respiration was inhibited. The fluctuations of this metabolite may reflect the activities of either the glycolysis, or the oxidative pentose phosphate pathway initiated by the glucose 6-phosphate dehydrogenase. However, this last enzyme is known to be inactivated by reduced thioredoxin (16) and a high NADPH/NADP ratio (12). As the NADPH/NADP ratio in dark increased in presence of A.A. plus SHAM (see below Fig. 3), it is doubtful that the decrease in G_6P level might be entirely attributed to an increase in G_6P dehydrogenase activity. In these conditions, activation of the chloroplastic photophosphorylations by light resulted in a further increase of the ATP level and a concomitant rise of G_6P (Fig. 2). The G_6P variation could be the result of either a decrease of the glycolysis activity or a light inactivation of the glucose 6-phosphate dehydrogenase by the thioredoxin system (16), providing the fact that this enzyme was not already inhibited by the high NADPH/NADP ratio (12) recorded before illumination.

The decrease of the ATP and G_6P pools in dark shown in Figure 2, was accompanied by a rise of the NADPH concentration which slightly increased further during illumination (Fig. 3). In contrast the NADH pool remained almost undetectable throughout the experiment.

The observed increase of NADPH in dark cannot be simply explained by an inhibition of the NADPH reoxidation through mitochondrial oxidases, as shown by the effect of CCCP (Table I). Indeed, in presence of the uncoupler, *i.e.* in cases where mitochondrial oxidases were fully operational, the NADPH concentration varied in the same direction (although in a lower extent) as in presence of A.A. plus SHAM.

DISCUSSION

The similar effects of an uncoupler (CCCP) and inhibitors of mitochondrial oxidases (A.A. plus SHAM) suggest that altering the mitochondrial oxidative phosphorylations in dark triggered the following cascade of events: (a) The mitochondrial ATP synthesis was inhibited and the cellular ATP concentration dropped; (b) as ATP exerts a tight control on key enzymes of the glycolytic pathway—pyruvate kinase and phosphofructokinase (2)—it may be assumed that the glycolytic rate increased. This hypothesis may account for the simultaneous decrease of G_6P, rise of NADPH and partial recovery of ATP. In Chlamydomonas, the initial part of glycolysis from starch to 3-P-glycerate is mainly chloroplastic (8), and part of the NADPH production was probably located in this compartment; and (c) the rise of the chloroplastic NADPH level would be responsible for the observed increase of the reduction state of the primary acceptor of PSII, Q_A, as indicated by the variations of the fluorescence levels. Possibly, NADPH could be reoxidized in dark by a chloroplastic respiratory pathway, presumably at the plastoquinone level (1, 4, 14). Although the precise nature of this process remains to be elucidated, the existence of a NAD(P)H-plastoquinone oxidoreductase bound to the thylakoid membrane of *Chlamydomonas*

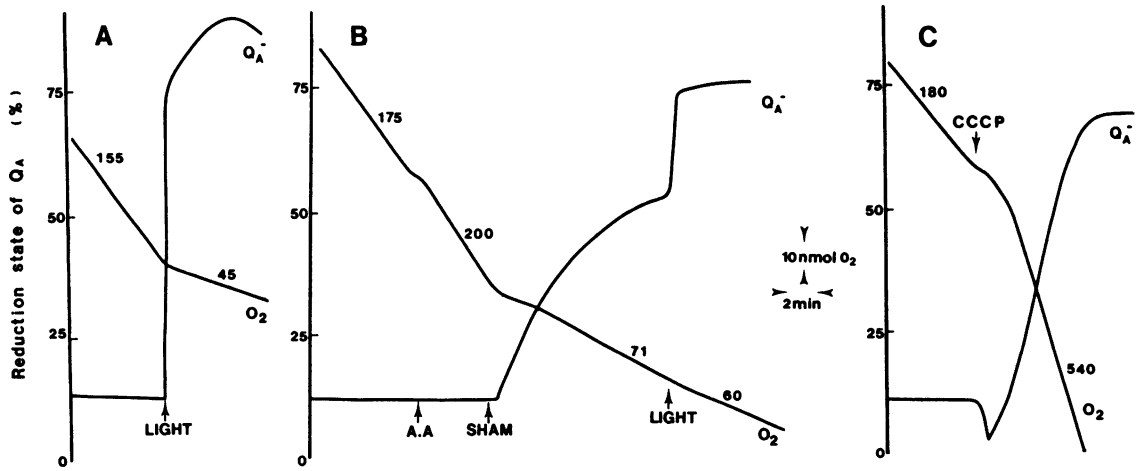

FIG. 1. O_2 electrode traces and Q_A reduction state of *C. reinhardtii* mutant strain rcl-u-1-10-6 C. A) Effect of a dark to light transition; B) Effect of A.A. 1 μM and SHAM 1 mM; C) Effect of CCCP 2 μM. Light intensity: 400 $\mu E \cdot m^{-2} \cdot s^{-1}$. Numbers along the lines are nmol $O_2 \cdot min^{-1} \cdot mg^{-1}$Chl.

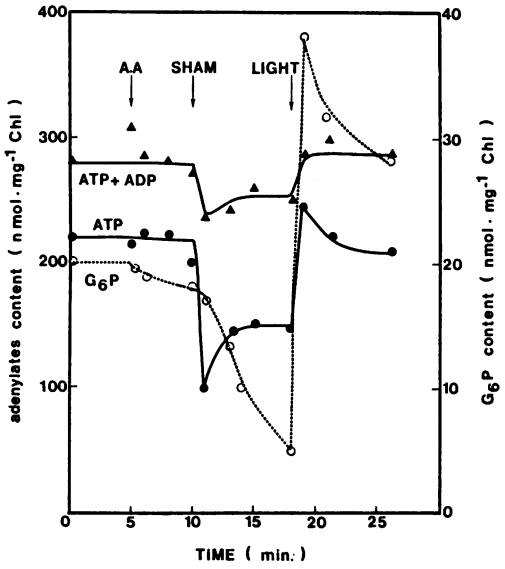

FIG. 2. Effect of A.A. (1 μM) and SHAM (1 mM) on the ATP, ATP + ADP and G_6P content. Light intensity: 400 $\mu E \cdot m^{-2} \cdot s^{-1}$.

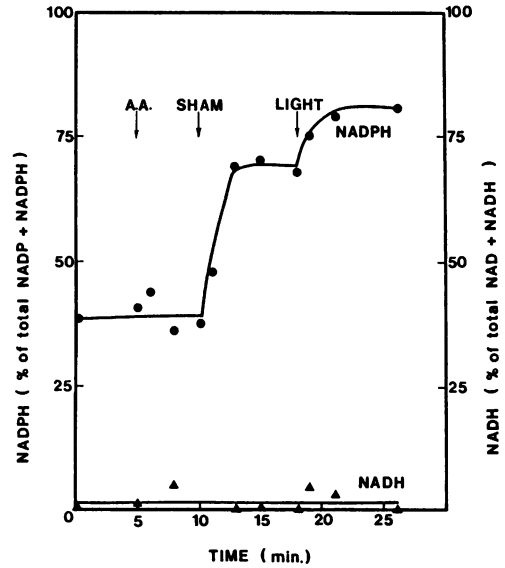

FIG. 3. Effect of A.A. (1 μM) and SHAM (1 mM) on the NADPH and NADH content. The total amount of NADP + NADPH and NAD + NADH were, respectively, 12.5 and 33 nmol/mg Chl. Light intensity 400 $\mu E \cdot m^{-2} \cdot s^{-1}$.

Table I. *Effect of CCCP on the ATP, ATP + ADP, NADPH and NADH Content of Chlamydomonas reinhardtii in Dark*

The reaction was stopped 5 min after the addition of CCCP. The total amount of NADP + NADPH and NAD + NADH were, respectively, 11 and 50 nmol/mg Chl.

CCCP	Nucleotides			
	ATP	ATP + ADP	NADPH	NADH
μM			*nmol/ mg Chl*	
0	250	280	2.6	4<
2	145	250	5	4<

reinhardtii has been reported (5). This point is currently under investigation.

Chloroplasts were shown to have a high capacity for transferring redox equivalents from the stroma to the cytosol (6) and it has been proposed that oxidation of chloroplastic redox equivalents by mitochondrial oxidases might serve as a respiratory control in the light (10). However, in these algae, the effect of illumination on the ATP and G_6P pools when mitochondrial oxidative phosphorylations were blocked was in good agreement with the hypothesis of a tight control of the glycolytic flux by the chloroplastic ATP synthesis. If this hypothesis holds true the light-inhibition of respiration observed in the mutant strain devoid of active Rubisco (Fig. 1A) (3) could be due, at least in part, to this last regulatory control mechanism.

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