The Role of Cyclic Photophosphorylation in Vivo

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ABSTRACT

When cyclic photophosphorylation is inhibited in Chlorella vulgaris cells by carbonylcyanide-trifluoromethoxy phenylhydrazone, photosynthetic CO2-fixation under anaerobic conditions exhibits a distinct lag. Under the same conditions, the light-dependent formation of ribulose diphosphate shows also this lag. It is concluded that cyclic photophosphorylation is required to fill up the pools of phosphorylated intermediates of the Calvin cycle at a time when noncyclic photophosphorylation cannot yet efficiently operate. Under aerobic conditions, the initial energy demand can be accommodated by respiratory ATP or cyclic photophosphorylation or both. Evidence for stoichiometric participation of cyclic photophosphorylation in photosynthesis is still lacking.

Whereas evidence for the existence of cyclic photophosphorylation in vitro and in vivo has accumulated from many laboratories, no agreement has been achieved up to now on the role of this photophosphorylation. Energy generated by cyclic photophosphorylation can be used under various experimental conditions by autotrophic cells for uptake and assimilation of organic molecules (5, 18, 20), uptake of ions (4, 9), and for protein synthesis (11, 14).

It has been questioned, however, for several reasons, whether ATP generated by cyclic photophosphorylation is stoichiometrically required for photosynthetic CO2-fixation (16, 17), as has been suggested by Arnon (1) and accepted by most textbooks of physiology and biochemistry. In addition, it has even been suggested that cyclic photophosphorylation only occurs under anaerobic conditions and that its physiological importance, therefore, seems doubtful (3).

Recently, Schürmann et al. (13) have published data which they claim to prove the participation of cyclic photophosphorylation in CO2-fixation of isolated chloroplasts. In reality, these data only show that cyclic photophosphorylation is required for the initial phase of photosynthetic CO2-fixation; e.g. for filling up pools of phosphorylated intermediates of the Calvin cycle. This fully agrees with previous suggestions from in vivo experiments (6).

In this paper, evidence is presented that cyclic photophosphorylation is obligatorily required in vivo under certain experimental conditions during the initial phase of CO2-fixation. It is used for example to increase the level of RuDP.

MATERIALS AND METHODS

Chlorella vulgaris (strain 211/11h, Göttingen) was grown as described previously (15). The rate of CO2-fixation was determined by adding NaH14CO3 at a final concentration of 756 µg/ml (= 0.272 µc/ml) to an algal suspension containing 165 µg chlorophyll/ml. At the times indicated, samples of 0.3 ml were added to 1 ml extraction-acetic acid v/v (95:5). Aliquots were counted on metal planchets at an efficiency of 40%. The determination of Pi and of RuDP was as described before (7).

RESULTS

Effect of CCP on Cyclic Photophosphorylation and on Photosynthetic CO2-Fixation. Anaerobic photoassimilation of glucose is a process definitely driven by cyclic photophosphorylation (10, 15–17). For instance, CCP at a concentration of 1 × 10–5 m inhibited this kind of glucose assimilation by 50%, but hardly affected photosynthetic O2 evolution (16). In the meantime these results have been confirmed by an independent measurement of cyclic photophosphorylation. The decrease of Pi due to light under anaerobic conditions has been shown to be caused only by cyclic photophosphorylation (6). CCP at 1 × 10–4 m fully inhibits this decrease in Pi when light is turned on (Fig. 1).

When 14CO2-fixation is measured under the same conditions, i.e. in N2, a severe lag is observed in the presence of CCP (Fig. 2a). That the lag is completely overcome after 15 min and the rate in the presence of CCP then equals the rate in the absence of uncoupler can be seen by the sample which had been preilluminated for 15 min in the presence of CCP in light before 14CO2 was added (Fig. 2b). CCP is not destroyed by light (16). Under aerobic conditions (Fig. 2a), the same fixation rate and no lag is observed in the presence or absence of CCP in agreement with previous results (16). Since CCP at a given concentration uncouples oxidative phosphorylation in vivo much less than cyclic photophosphorylation (16), a certain initial energy requirement for photosynthesis in air obviously can be met by respiration even in the presence of CCP; in N2 and in the absence of CCP, it is met by cyclic photophosphorylation.

Effect of CCP on RuDP-formation under Aerobic and Anaerobic Conditions. The initial energy demand which is made obvious only in Chlorella under N2 plus CCP possibly reflects the filling up of some pools of Calvin cycle intermediates. To test this hypothesis the change in RuDP-concentration was followed under the conditions of the experiment of Figure 2. As can be seen in Figure 3, the amount of RuDP increases rapidly in Chlorella when light is turned on, and this occurs in N2 as well as in air (CO2 absent). The decrease in the maximum values reached could be due to CO2 produced by respiration; this would cause CO2-fixation and thus allow a reaction to proceed which uses up the newly formed RuDP. An important difference is, however, the way CCP affects RuDP forma-

1 Abbreviations: RuDP: ribulose diphosphate; CCP: carbonyl cyanide-trifluoromethoxy-phenylhydrazone; Calvin cycle: reductive pentose phosphate cycle.
phosphorylation. Thus, during this initial period there remains only respiratory ATP or that of cyclic photophosphorylation for any task. This photophosphorylation could certainly serve an important function, therefore, during the initial stage of photosynthesis, although a lack of ATP most likely is not the only reason for the lag (12).

This function of cyclic photophosphorylation, however, has to be clearly distinguished from that of supplying a stoichiometric amount of 1 ATP per CO₂ fixed as suggested by Arnon (1). If such a stoichiometric amount is required, an inhibition of cyclic photophosphorylation would be expected to affect severely photosynthesis at any time.

Schürmann et al. (13) claim to have presented evidence for a role of cyclic photophosphorylation in photosynthesis in this latter sense. They have shown first that a definite lag in ¹⁴C fixation with isolated chloroplasts can be shortened by the addition of ATP or by preillumination with far red light. Second, the authors found in 12-min fixations that all inhibitors known to affect cyclic photophosphorylation change the percentage of radioactivity found in glyceraldehyde-3-P as compared to that of sugar phosphates in favor of glyceraldehyde-3-P.

However, since the percentage of radioactivity in glyceraldehyde-3-P in any fixation will be a function of time—it will decrease with time (2)—all treatments which prolong a lag period, e.g. inhibitions of cyclic photophosphorylation, obviously will favor a relative amount of radioactivity in glyceraldehyde-3-P. It is most likely that such a prolongation of the lag period, rather than a decrease of the steady state rate of photosynthesis, occurred in the experiments of Schürmann et al. (13). Unfortu-

**DISCUSSION**

In order for the Calvin cycle to function and sufficient RuDP to be formed for a high rate of photosynthesis, reducing equivalents have to be produced, as has been shown previously (7, 8, 19), and also ATP, which is suggested by the data of Figure 3. Before the noncyclic electron pathway proceeds at a good rate, i.e. at a time of low CO₂-fixation, ATP cannot be produced in substrate amounts by noncyclic photosynthesis under anaerobiosis, as it is most likely that such a prolongation of the lag period, rather than a decrease of the steady state rate of photosynthesis, occurred in the experiments of Schürmann et al. (13). Unfortu-

**Fig. 1.** Effect of CCP on the anaerobic dark-light change of inorganic phosphate in Chlorella cells. Sixty milliliters of algal cells (165 µg chlorophyll/ml) in phosphate-free culture medium were gassed for 15 min with N₂ and then illuminated. One microgram P/ml algae corresponds to 18 µg PO₄/µg chlorophyll.

**Fig. 2.** Effect of CCP on photosynthetic ¹⁴CO₂-fixation in Chlorella. For experimental conditions see Figure 1 and "Materials and Methods." a: NaH ¹⁴CO₂ was added in the dark and the experiment started by turning on the light. b: The algae were preilluminated in the presence and absence of CCP for 15 min under N₂, then the experiment was started by the addition of NaH ¹⁴CO₂. CCP concentration: 1 x 10⁻⁴ M; light intensity: 1500 lux.

**Fig. 3.** Effect of CCP on the RuDP-formation after a dark-light change in Chlorella. Experimental conditions as in Figure 2. Five hundred cpm/ml algal suspension corresponds to a RuDP concentration in the cells of 0.1 µmol (≈ 0.2 µmol/10 mg chlorophyll). Light intensity: 8500 lux.
nately, these authors carried out one point experiments which do not allow one to distinguish between a lag period and a steady state rate of photosynthesis. Therefore, no real discrepancy between our data and those of Schürmann et al. (13) exists. The only difference is the mutual influence of respiratory energy supply and that of cyclic photophosphorylation, a phenomenon which of course cannot be observed with isolated chloroplasts. Thus evidence for a stoichiometric participation of cyclic photophosphorylation in steady state photosynthetic CO₂-fixation is still lacking.

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LITERATURE CITED