The role of respiration in the activation of photosynthesis upon illumination of dark adapted *Chlamydomonas reinhardtii*

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**A R T I C L E   I N F O**

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**A B S T R A C T**

It is reported that O2 is required for the activation of photosynthesis in dark adapted *Chlamydomonas reinhardtii* in State 1, under low light intensity. The concentration of dissolved O2 of ca. 9 μM is sufficient to saturate the requirement. When the concentration of O2 is 3 μM or below, the activation of photosynthesis is strongly inhibited by myxothiazol, a specific inhibitor of the mitochondrial cytochrome bc1. The effect of this inhibitor decreases as the O2 concentration is raised, to disappear completely above 50 μM. Low concentrations of uncouplers delay the activation of photosynthesis, but do not inhibit it when steady state is reached. It is concluded that in State 1 *C. reinhardtii* mitochondrial respiration is required for the activation of photosynthesis upon illumination of dark adapted cells only when the concentration of O2 is too low (less than 5 μM) to allow an appreciable activity of the Mehler reaction. The role of respiration does not seem to be due to the synthesis of ATP by oxidative phosphorylation, because photosynthesis activation is not sensitive to oligomycin.

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1. Introduction

The relationship between respiration and photosynthesis in plants has been a subject of controversy for long time, due to the statement by Warburg [1] that photosynthesis is strictly dependent on respiration. The experimental separation of the photosynthetic electron transport reactions, ATP formation and CO2 assimilation by the Calvin–Benson cycle from respiration ended that controversy. It was further established that isolated intact chloroplasts, uncontaminated by mitochondria, are capable of high rates of CO2 assimilation, comparable to the rates observed in vivo [2]. This established the complete autonomy of the isolated chloroplast as far as photosynthetic CO2 assimilation to the level of carbohydrates is concerned. However, if photosynthesis is considered to include nitrogen assimilation, the synthesis of the major cellular aminoacids and photorespiration, certainly the contribution of mitochondrial respiration and of other cytoplasmic activities is essential. This was emphasised by Noctor and Foyer [3], who proposed a model of the interactions of these different processes and their contributions to the dynamics of O2 evolution and uptake and of ATP/ADP changes in the green cells.

Even if we limit our discussion to the photosynthetic electron transport, photophosphorylation and O2 evolution, i.e. to the activities which can be observed in the isolated chloroplasts, one has nevertheless to recognise that the chloroplasts are endowed with oxidative activities, and that these may be part of the photosynthetic process. In 1951 Mehler discovered that the isolated chloroplasts can photoreduce O2 to H2O2 [4], and Forti and Jagendorf [5] have shown that photosynthetic electron transport from water to O2 (the Mehler reaction) is enhanced by ascorbate and coupled to photophosphorylation of ADP producing ATP, i.e. one of the essential reagents required for CO2 assimilation to sugars and starch. More recently, the Mehler reaction was shown to produce the radical O2 at the reducing side of PS I, which oxidises ascorbate to its free radical (AFR). The latter is also the product of ascorbate peroxidation by H2O2 catalysed by ascorbate peroxidase. AFR is an efficient electron acceptor [6] in thylakoids, acting at the reducing side of PSI alternative to NADP [7], at a rate of 50–60% of that of NADP reduction. The electron transport from H2O to AFR in thylakoids is coupled to photophosphorylation with the same stoichiometry of ATP/electrons observed in the NADP reduction system [8], and is understood to ensure the rate of ATP formation necessary to obtain the ratio ATP/NADPH of 1.5 required for CO2 assimilation in the Calvin–Benson cycle [9,10]. This interpretation of the role of Mehler-ascorbate reaction is in agreement with the observations by Egneus et al. [11] and by Forti and Gerola [12] that O2 is constantly consumed during steady state photosynthesis in isolated, intact chloroplasts. Dutileuil et al. [13] have reported that in a tobacco mutant lacking mitochondrial Complex I, photosynthesis is diminished under low CO2 and high O2 conditions, indicating a role for mitochondrial respiration in protecting photosynthesis under high photorespiration conditions.
A different mechanism of O₂ uptake by the chloroplasts is the "chloreshpiration" [14–16], a very slow O₂ uptake occurring only in the dark in the chloroplasts, which oxidises the plastoquinone pool. The very low rate and low affinity for O₂ of this system make it difficult to understand its physiological function. An inhibitory interaction of it with respiration has been reported [17].

Though the Mehler-ascorbate reaction has been shown to be an essential part of steady state photosynthesis (see above), and to be required in Chlamydomonas reinhardtii for the transition from State 2 to State 1 [18], none of these phenomena can be attributed to an interaction of photosynthesis with the respiration occurring outside the chloroplasts. While the exchange of metabolites between chloroplasts, cytosol and mitochondria is a basic phenomenon in plant physiology and has been thoroughly studied, the mechanistic basis of a requirement of mitochondrial respiration for photosynthetic O₂ evolution has seldom been discussed. Le Maire et al. [19] described the inhibition of phototrophic growth and photosynthesis by an inhibitor of mitochondrial respiration in a C. reinhardtii mutant defective in the chloroplast ATPsynthase. Those observations were interpreted to indicate that ATP made in mitochondria utilizing reducing power photogenerated in the chloroplast could support the assimilation of CO₂ and the phototrophic growth of the mutant, though at a reduced rate. More recently, Cardol et al. [20] have reported the inhibition of photosynthetic O₂ evolution in C. reinhardtii mutants defective in different components of the mitochondrial electron transport chain, as compared with the wild type. They have shown that under light intensities below the compensation point, O₂ evolution curves have a positive relationship with the number of active proton-pumping sites in mitochondria. They have also demonstrated that the lack of activity of mitochondrial respiration induces the transition to State 2, probably caused by the non-photochemical reduction of the plastoquinone pool by cytosolic reducing power which accumulates in the mutants.

We report here studies on the requirement for mitochondrial respiration to activate photosynthesis during a dark to light transition in C. reinhardtii, under conditions where State transitions are not involved, because the cells are already and stay in State 1. In this organism the requirement for mitochondrial respiration to activate photosynthesis is only observed under O₂ concentrations low enough to prevent the activity of the Mehler reaction, and at low light intensity. It is shown that the requirement for mitochondrial respiration is not due to the formation of ATP by oxidative phosphorylation, because the specific inhibition of it by oligomycin has no influence on the requirement for mitochondrial respiration. When photosynthetic activity has reached the steady state, mitochondrial respiration is no more required and its specific inhibition has no effect on photosynthesis.

2. Materials and methods

2.1. Cultures and strains

C. reinhardtii WT (from strain 137 C was provided by the Laboratoire de Physiologie Cellulaire et Moléculaire du Chloroplaste, at the IBPC (Paris, France). Cells were grown at 24 °C in acetate-supplemented medium [21] under ca. 60 μM m⁻² s⁻¹ of continuous white light. They were harvested during exponential growth, and resuspended in minimal medium at pH 7.2 [21]. They were left in this medium and shaken for not less than 70 min before the experiments.

2.2. Measurements of oxygen evolution and uptake, and of fluorescence

Photosynthesis and respiration were measured as O₂ exchanges in the presence of 5 mM NaHCO₃ using a Clark type electrode (Radiometer, Copenhagen) in a home made cell, at 25 °C. Illumination was provided by a halogen lamp, which was filtered through a heat filter. Light intensity was measured in the cell and adjusted, as needed, by neutral density filters. Fluorescence emission was simultaneously measured in the same cell employed to measure oxygen exchanges using a PAM chlorophyll fluorimeter (Walz, Germany). Fm was measured by saturating flashes of 2 s, fired one /min. For measurements, algae were resuspended at 50 μg Chl ml⁻¹, or otherwise as indicated. Chlorophyll concentration was determined by measuring the absorbance of the cell culture at 680 nm in a spectrophotometer equipped with a scatter attachment, on the basis of a calibration curve constructed after extraction of the chlorophyll with 80% acetone [22].

2.3. Determination of ATP and ADP

An aliquot of the cell suspension used to measure oxygen exchanges and fluorescence emission was used to measure ATP and ADP as previously described [23].
3. Results

When *C. reinhardtii* cells are allowed to consume O₂ in the dark, they remain in State 1, and their Fv/Fm ratio stays constant in the range from 0.72 to 0.75 until O₂ is exhausted. When anaerobiosis is reached, the transition to State 2 is signalled by an abrupt rise of Fo and a slower decrease of Fm [22]. We report here the time course of the activation of photosynthetic O₂ evolution in dark adapted *C. reinhardtii* cells in State 1 upon illumination at different initial concentrations of O₂. The fluorescence parameters F (actual fluorescence) and Fm (maximal fluorescence) and the changes of O₂ were measured simultaneously. If the light intensity is adjusted to give 15 to 25% of the light saturation rate, the time required to attain the steady state rate is around several minutes (Fig. 1C), and in the same time the ratio 1-F/Fm attains a constant value, indicating the steady state turnover of PS II. In *C. reinhardtii* no Fm quenching is observed at low light intensity (Fig. 1A; see also [22]), but rather a small increase occurs during illumination. When light is turned off, the ratio Fv/Fm is unchanged with respect to the initial. The time required for the activation of photosynthesis was unaffected upon decreasing O₂ concentration down to 8.9 μM, but it increased drastically when O₂ was further decreased below 2.0 μM (Figs. 1B and C), indicating that a high O₂ affinity respiration was required for the activation of photosynthesis of the State 1 cells.

The mitochondrial respiration through cytochrome oxidase (cyt aa₃) is the best candidate for such role, as it is well known [23] to have the highest affinity for O₂, and we have previously demonstrated that in *C. reinhardtii* the respiratory rate is, accordingly, saturated at 2–3 μM dissolved O₂ [18]. We therefore tested the hypothesis that mitochondrial respiration is required for photosynthesis activation under low intensity light after dark adaptation. For that purpose, we measured the effect of the specific inhibitor of mitochondrial electron transport myxothiazol, which inhibits the cytochrome bc₁, while not affecting the analogous photosynthetic electron carrier cyt b₅/₆ [24]. It should be noted that in *C. reinhardtii* myxothiazol has no effect on O₂ uptake, because in the presence of this inhibitor mitochondrial respiration is diverted to the Alternative Oxidase, which, however, has a lower O₂ affinity than cyt aa₃ [16] and bypasses two of the three Δψₐ₃ generating sites.

Fig. 2 shows that indeed myxothiazol inhibits strongly photosynthetic activation at the low dissolved O₂ initial concentration of 3 μM (Fig. 2a), but the inhibition decreases upon increasing O₂, and eventually disappears at 85 μM O₂ (Figs. 2b and c), indicating that cyt aa₃ respiration is not needed for photosynthesis activation when the initial O₂ concentration is high enough to support the Mehler reaction in the chloroplasts [18]. In all cases, the final steady state photosynthetic rate is unaffected by myxothiazol (see Fig. 2 and its legend).

The addition of oxaloacetate (OAA), which is an electron acceptor at the reducing end of PS I due to the presence in the chloroplast stroma of the NADP dependent malic dehydrogenase, stimulates the activation of photosynthetic O₂ evolution under low O₂ concentration (Fig. 2a). It is well known that chloroplasts [26], as well as the plant mitochondria [see the review [27]], are endowed with an exchange transport carrier for OAA and malate. Therefore these two acids can be exchanged rapidly between the two organelles, and this system acts as a shuttle transferring to the mitochondria reducing power (in the form of malate) photogenerated in the chloroplast, and OAA back to the chloroplast.

The question was then asked of the mechanism by which mitochondrial respiration influences the activation of photosynthesis in the dark–light transition in State 1 *C. reinhardtii* cells, under low light intensity. An obvious hypothesis was that mitochondrial respiration could contribute ATP, and that this contribution might be needed at higher O₂ concentrations, when the Mehler reaction, which has lower O₂ affinity than the cyt aa₃ chain [18], can provide the ATP needed to activate photosynthesis. The inhibitor of mitochondrial

### Table 1

<table>
<thead>
<tr>
<th>Conditions</th>
<th>ATP</th>
<th>ADP</th>
<th>Sum</th>
<th>ATP/ADP</th>
<th>Respiration</th>
<th>Photosynthesis</th>
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</thead>
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<tr>
<td>Dark, air, control</td>
<td>87±4</td>
<td>15.5±7</td>
<td>102</td>
<td>5.6</td>
<td>10±2</td>
<td>–</td>
</tr>
<tr>
<td>Dark, air, oligomycin</td>
<td>78±5</td>
<td>34.2±2</td>
<td>112</td>
<td>2.3</td>
<td>9.5±1</td>
<td>–</td>
</tr>
<tr>
<td>Light, air, control</td>
<td>88±4</td>
<td>9.9±1</td>
<td>98</td>
<td>8.9</td>
<td>–</td>
<td>17.9</td>
</tr>
<tr>
<td>Light, air, oligomycin</td>
<td>81±3</td>
<td>23.5±5</td>
<td>104</td>
<td>3.5</td>
<td>–</td>
<td>18.2</td>
</tr>
</tbody>
</table>

The chlorophyll concentration was 150 μg/mL. Oligomycin was 10μg/mL. Other conditions as in Fig. 1. O₂ evolution and uptake are expressed as μmol of O₂/mg Chl h. ATP and ADP are indicated as nmol/mg Chl. Light intensity was 24 μmol m⁻² s⁻¹. At saturating light intensity, the photosynthetic rate was, respectively, 127 and 134 in the control and in the presence of oligomycin. The data are the average of 4 experiments, ±S.E.
Wild type C. reinhardtii cells in State 1 were incubated at the concentration of 25 μg of Chl/mL and illuminated with 24 μmol m$^{-2}$ s$^{-1}$. Other conditions as in Fig. 1. The first line indicates the time after starting illumination, in seconds. The figures indicate: the fraction of open PS II centres, 1-F/Fm; the photosynthetic O$_2$ evolution rate, Pa; in μmol O$_2$/mg Chl h; the maximal fluorescence Fm, in arbitrary units. Oligomycin was 12 μg/mL (where added). At light of saturating intensity Pa was, respectively, 201±11 and 197±8.5 in the control and the oligomycin treated sample. The initial Fv/Fm ratios were 0.75 in both cases. The data are the average of 4 experiments, ±SE. Temperature was 24 °C, the same as during cell growth.

### 4. Discussion

The observations reported here show that dark adapted C. reinhardtii cells in State 1 initiate photosynthetic O$_2$ evolution after a lag of several minutes when illuminated with low intensity light. This behaviour corresponds to what is observed in most higher plants, where it is generally interpreted as the consequence of the time needed for activation of the Calvin–Benson cycle, requiring electron transport to generate ΔpH across the thylakoid membrane, alkalization of the stroma, reduction of S–S bridges in key enzymes of the carbon cycle, and regulation of the concentration of Mg$^{2+}$ in the stroma. During the lag of O$_2$ evolution, synthesis of the ATP needed to activate CO$_2$ assimilation must also occur, and this could in principle be coupled either to cyclic electron transport around PS I, or to the Mehler reaction. However, in C. reinhardtii in State 1 there is no cyclic electron transport [22,29] therefore either the Mehler reaction or the cooperation of mitochondrial respiration must be responsible for initiation of photosynthetic O$_2$ evolution as far as ATP synthesis is concerned. The results with C. reinhardtii reported here show (Fig. 1) that O$_2$ is required for the activation of photosynthesis (measured as the turnover of PSII 1-F/Fm and as O$_2$ evolution) in dark adapted cells in State 1. The requirement is clearly apparent at 1.5 μM, and is saturated at an O$_2$ concentration of 8.9 μM (see Figs. 1B and C). At the former concentration cytochrome aa$_3$ respiration is active in C. reinhardtii, but not the Mehler reaction [18]. Furthermore, the activation of photosynthesis is inhibited by myxothiazol (Fig. 2) but not by oligomycin (Tables 1 and 2). The former is an inhibitor of mitochondrial respiration at the level of cyt b$_6$ and the latter specifically inhibits mitochondrial ATP synthesis, while not affecting the chloroplasts ATP synthase. Therefore, these observations strongly indicate that mitochondrial respiration is required for activation of photosynthesis after dark adaptation, but this requirement is not related to the contribution of ATP by oxidative phosphorylation. It must be emphasised that no State transition was required in our experiments, as the Chlamydomonas cells were always in State 1, therefore they had no cyclic electron transport [22,29].

The lack of effect of myxothiazol at the O$_2$ concentrations above 20 μM can be explained thinking that under these conditions

### Table 2

<table>
<thead>
<tr>
<th>Oligomycin</th>
<th>50 s</th>
<th>100 s</th>
<th>200 s</th>
<th>300 s</th>
<th>400 s</th>
<th>500 s</th>
<th>600 s</th>
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<tbody>
<tr>
<td>Control</td>
<td>Pa</td>
<td>29.8±0.82</td>
<td>33.1±2.47</td>
<td>33.1±2.47</td>
<td>33.1±2.47</td>
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<tr>
<td>1-F/Fm</td>
<td></td>
<td>0.50±0.01</td>
<td>0.53±0.04</td>
<td>0.53±0.03</td>
<td>0.56±0.04</td>
<td>0.59±0.03</td>
<td>0.61±0.02</td>
</tr>
<tr>
<td>Fm</td>
<td>252±13.5</td>
<td>251±16</td>
<td>262±18.5</td>
<td>255±11</td>
<td>256±17.5</td>
<td>257±20.5</td>
<td>256±19.5</td>
</tr>
<tr>
<td>Oligomycin</td>
<td>Pa</td>
<td>32.7±2.45</td>
<td>32.7±2.45</td>
<td>32.7±2.45</td>
<td>32.7±2.45</td>
<td>32.7±2.45</td>
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</tr>
<tr>
<td>1-F/Fm</td>
<td>0.57±0.01</td>
<td>0.58±0.0</td>
<td>0.59±0.01</td>
<td>0.59±0.005</td>
<td>0.62±0.005</td>
<td>0.62±0.005</td>
<td>0.62±0.0005</td>
</tr>
<tr>
<td>Fm</td>
<td>200±15</td>
<td>220±23</td>
<td>228±25</td>
<td>231±19.5</td>
<td>227±24</td>
<td>222±24.5</td>
<td>213±15.5</td>
</tr>
</tbody>
</table>

### Table 3

<table>
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<tr>
<th>Initial O$_2$, μM</th>
<th>Sample</th>
<th>Illumination time, minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Control</td>
<td>19.7±2.6</td>
</tr>
<tr>
<td></td>
<td>FCCP</td>
<td>22±2</td>
</tr>
<tr>
<td>2.5</td>
<td>Control</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>FCCP</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Wild type C. reinhardtii cells were incubated and illuminated in the same conditions as in Fig. 1. The figures indicate photosynthetic O$_2$ evolution rates in % of the final steady state attained, which was, respectively, 151±0.72 and 15.7±1 μmol O$_2$/mg Chl h in the control and the FCCP treated sample. The respiration rate was 7.72±0.33 and 16.33±2.23, respectively, in the control and FCCP treated samples. FCCP was 1.25 μM. At saturating light intensity the photosynthetic rates were, respectively, 85.4±14 and 99.5±5 in the control and FCCP treated sample. Initially, Fv/Fm was 0.75±0.018. The samples treated with FCCP at low O$_2$ recovered the same activity as the controls after a 30 s. illumination with higher light intensity of 77 μmol m$^{-2}$ s$^{-1}$, which allowed them to start photosynthesis.
photosynthetic electron transport through the Mehler reaction (the linear electron transport from H₂O to O₂ through both photosystems) can replace mitochondrial respiration because it performs the two roles of: (a) the oxidation of the intersystem chain of chloroplasts, a necessary step to activate photosynthetic electron transport. (b) It provides ATP, which is needed to activate and to run photosynthesis. The experiments reported here therefore indicate that the role of O₂ in the activation of photosynthesis after dark adaptation is concerned in the first place with the reoxidation of the intersystem electron transport chain. At the very low O₂ concentrations, insufficient to allow an adequate rate of the Mehler reaction, mitochondrial respiration fulfills this role, which also requires the transport of reducing equivalents from the chloroplast to the mitochondria, a well-established phenomenon [26,27]. The exchange carriers of malate/oxaloacetate transfer the photogenerated reducing power from the chloroplasts to mitochondria, and transfer back to chloroplasts the electron acceptor OAA. The stimulation by OAA of photosynthesis initiation is indeed shown in Fig. 2a, and is interpreted thinking that OAA is used as an electron acceptor from NADPH through the catalysis of the stromal NADP malate dehydrogenase. This system can perform the reoxidation of NADPH and O₂ evolution before the activation of the Calvin–Benson cycle, because it requires no ATP, but rather it is linked to ATP generation coupled to the non-cyclic electron transport from water to NADP. Therefore only when O₂ concentration is too low to support an active Mehler reaction is the reoxidation of the intersystem chain performed by the oxidative activity of mitochondria and inhibited by myxothiazol. On the other hand, the requirement for ATP is fulfilled by the photosynthetic electron transport to the endogenous NADP, and then to O₂ as soon as the Mehler reaction is activated by the O₂ produced by PS II. The production of ATP activates the carbon cycle, and photosynthesis then proceeds at the steady state rate, only dependent on light intensity and CO₂ concentration, fully performed by the chloroplasts.

This conclusion is consistent with that of Krömer and Heldt [28], who reported that oligomycin, causes some inhibition of photosynthesis in barley leaves and in protoplasts prepared from the leaves, but the inhibition disappears if the protoplasts are disrupted mechanically and intact chloroplasts are liberated from them. These authors interpret their results in terms of inhibition by oligomycin of the further metabolism, in leaves and in protoplasts, of the products of photosynthesis due to the decrease of ATP.

A different situation was described by Le Maire et al. [19] who studied the slow phototrophic growth of a Chlamydomonas mutant deficient in the chloroplast ATP synthase. In their case, no ATP production was possible in the chloroplasts, and photosynthesis was made possible by the cooperation of mitochondria, which provided ATP, with chloroplasts producing reduced substrates utilized by the mitochondria in the tricarboxylic acids cycle. The phototrophic growth was possible, though at slower rate, because the Krebs cycle and the electron transport to O₂ produces 5ATP/CO₂, while the assimilation of CO₂ in the Calvin cycle requires only 3ATP/CO₂.

The inhibition by low concentrations of uncouplers (such as FCCP) of photosynthesis initiation under low light intensity is observed (Table 3) only at very low concentrations of O₂, as is the case of myxothiazol inhibition. It was observed that at concentrations inhibiting only partially ATP formation, the uncouplers delayed the activation of photosynthesis, as expected because of the lack of ATP. However, the samples treated with 1.25 μM FCCP at low O₂ recovered the same activity as the controls after a 30 s illumination with higher light intensity of 77 μmol m⁻² s⁻¹, which allowed them to start photosynthesis (Table 3). Once initiated, photosynthesis does proceed at the same rate as the control. This could also be expected on the basis of the previous observation that, under low light intensity, photosynthesis in C. reinhardtii is limited by the rate of NADP reduction more than by that of ATP formation [25]. The lack of inhibition by oligomycin (Table 2) is in agreement with this concept, because it shows that no inhibition of photosynthesis is observed even when the ATP/ADP ratio in the cells is decreased by a factor of 2.5 at low light intensity (see Table 1).

Our observations on C. reinhardtii are in no disagreement with the results of Cardol and collaborators [20], who have reported that in different C. reinhardtii mutants defective in the mitochondrial respiratory chain, photosynthesis is impaired. The most severe limitation of photosynthesis was reported in the mutants at light intensities below the compensation point (see Figs. 1 and 2 of [20]). This is in agreement with our observations on the effect of myxothiazol on the wild type. The minor inhibition of photosynthesis they observed at saturating light intensity, can be attributed to the fact that a consistent fraction of the cells are permanently in State 2 in the respiratory mutants, and it is well known that in C. reinhardtii in State 2 there is no photosynthetic O₂ evolution [22,29]. This is interpreted by Cardol et al. [20] as a consequence of the reduction of the plastoquinone pool by cytosolic reductants accumulated because of the impaired respiration. In our conditions this is not the case, because all the cells are in State 1, as shown also by the fluorescence parameters Fv/Fm and Fm (see Fig. 1).

We conclude that mitochondrial respiration is needed to initiate photosynthesis after a dark period in C. reinhardtii, and probably in other plants, when the concentration of O₂ is very low. So, the cooperation of mitochondria to start photosynthesis at sun rise might be a general phenomenon of basic importance in the physiology of plants in all cases where the O₂ concentration is low in the chloroplasts during the night, which is certainly the case in C. reinhardtii and possibly in most aquatic organisms.

The experiments with C. reinhardtii discussed here indicate, in my opinion, that the function of mitochondrial respiration in the initiation of photosynthesis is due to its very high affinity for O₂, which allows the oxidation of the interchain electron carriers under conditions where the Mehler reaction rate is severely limited by the concentration of O₂. As soon as some O₂ is produced by PS II, the Mehler reaction takes over that function.

These results confirm the concept that the chloroplast is completely autonomous in the performance of steady state photosynthesis, thanks to the continuous and relevant activity of the Mehler reaction [11,12,7,28]. The latter provides ATP synthesis at rates adequate to complement that coupled to NADP reduction, so maintaining the ATP/NADPH ratio of 1.5 required for CO₂ assimilation. The process is performed utilizing the two photosystems in series and works in the absence of cyclic electron transport around PSI in State 1 Chlamydomonas, [22,29].

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References
