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Light-induced oxygen uptake in tobacco chloroplasts explained in terms of chlororespiratory activity

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Abstract

Chloroplasts from higher plants (*Nicotiana tabacum* var. John William's Broadleaf) exhibit a substantial oxygen uptake upon illumination with one or more short saturating light flashes. This uptake was detected and analyzed by means of mass spectrometry as $^{18}O_2$-gas exchange following the addition of 5 ml $^{18}O_2$ to the gas phase over the buffered reaction assay. Along with the light-induced oxygen uptake we measured photosynthetic water oxidation as oxygen evolution at $m/e = 32$. The oxygen uptake of $^{18}O_2$ measured at $m/e = 36$ can be completely inhibited by various electron acceptors and by silicomolybdate in particular. The same holds true for PS II-inhibitors like DBMIB. The effects of DCMU and of different light qualities (blue, red and far-red) on the light-induced oxygen uptake are discussed. We conclude from our results that the chlororespiratory activity within the thylakoid membranes is responsible for the observed oxygen uptake and that the plastoquinone pool is the component shared between both, photosynthetic and respiratory electron transport chains.

Keywords: Higher plant; Oxygen uptake; Chlororespiration; Photosynthesis; Respiration

1. Introduction

According to the generally accepted view in eukaryotic photosynthetic organisms, the processes of photosynthesis and respiration are strictly separated within different organelles, namely chloroplasts and mitochondria, respectively. Only in cyanobacteria, both the photosynthetic and the respiratory electron transport chains are located within the same thylakoid membrane system [1]. On the other hand, more than 30 years ago the effect of light not only on photosynthesis but also on the respiratory activity of algae was reported [2], suggesting a possible cooperation between the two processes. Only several years later this idea was substantiated and the respiratory activity in chloroplast membranes called chlororespiration was demonstrated in *Chlamydomonas* [3–5]. In these studies, the plastoquinone pool was proven to be the common element of both the photosynthetic and respiratory electron transport chains. Also, the respiratory enzyme NADH-plastoquinone-oxidoreductase was partially purified in the same species [6] and NADH was demonstrated to be able to donate electrons to the photosynthetic electron transfer chain [7]. Oxymetric measurements showed that the chlororespiratory activity in *Chlamydomonas* may be inhibited by light due to the Photosystem (PS) I-driven oxidation of the plastoquinone pool [8–11]. There are several indications that chlororespiratory activity is not only restricted to lower photosynthetic organisms. One of these is the presence of coding groups of genes in the liverwort chloroplast genome corresponding to the genes of human mitochondrial NADH-dehydrogenase components [12]. The existence of chlororespiratory activity in higher plants was recently postulated as an explanation for the non-photochemical reduction of plastoquinone in pea leaves [13]. Also, the light-dependent oxygen uptake in tobacco chloroplasts was reported to possess an action spectrum corresponding to that of the absorption spectrum of chlorophyll [14,15]. However, clear evidence

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromomethyl-6-isopropyl-*p*-benzoquinone; SiMo, silicomolybdate; PQ, plastoquinone; PS I, Photosystem I; PS II, Photosystem II; BL, blue light; RL, red light; FRL, far-red light.

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for the existence of respiratory activity in chloroplast membranes of *higher plants* has not been presented until now. In the present study we report on the light-dependent oxygen uptake in tobacco chloroplasts investigated by the application of mass spectrometry combined with an appropriate oxygen isotope labeling. This approach enabled us to discriminate between photosynthetic oxygen evolution from water ($H_2^{16}O$) and molecular oxygen consumption ($^{18}O_2$) processes [16–18].

2. Materials and methods

Chloroplasts from tobacco (*Nicotiana tabacum* var. John William's Broadleaf) were isolated as described elsewhere [19] and suspended in a 10 mM Tricine-NaOH buffer (pH 7.6) containing 0.3 M Mannitol as an osmoticum. The mass spectrometric set-up, sample chamber and vacuum system have been described in detail [20] as well as the experimental procedure and the protocol for oxygen uptake measurements [18]. Chloroplast samples subjected to the measurements contained 500 μg chlorophyll. Saturating light flashes (5 μs duration, spaced 300 ms apart) were provided by a Xenon stroboscope lamp (1539 A from General Radio) and the weak continuous light (blue, red and far-red) was provided by a projector lamp equipped with band-pass filters (690 nm interference filter in the case of far-red light).

3. Results and discussion

Fig. 1 shows the typical response of mass spectrometrically monitored oxygen partial pressure for $^{18}O_2$ ($m/e = 36$) and $^{16}O_2$ ($m/e = 32$) upon illumination with a train of 10 saturating light flashes. Since only $H_2^{16}O$ is present in the assay, the photosynthetic water splitting mechanism results in the evolution of $^{16}O_2$. At the same time, light-induced oxygen uptake can be observed as $^{18}O_2$ which has been exogenously added to the assay in molecular form and brought into equilibrium between water and gas phase. This light-induced oxygen uptake is completely blocked by SiMo, which accepts electrons from the primary quinone electron acceptor in PS II (Q_A) and by DBMIB, an inhibitor of photosynthetic reoxidation of the PQ pool by the cytochrome b_6f complex (Fig. 2). The fact that the inhibition of reduction of the PQ pool via the direct flow of PS II electrons to SiMo or the efficient oxidation of the PQ pool by DBMIB both inhibit light-dependent oxygen consumption suggests that a respiratory process in which PQ is involved as an electron carrier might be responsible for the observed uptake phenomenon. A respiratory process in which the

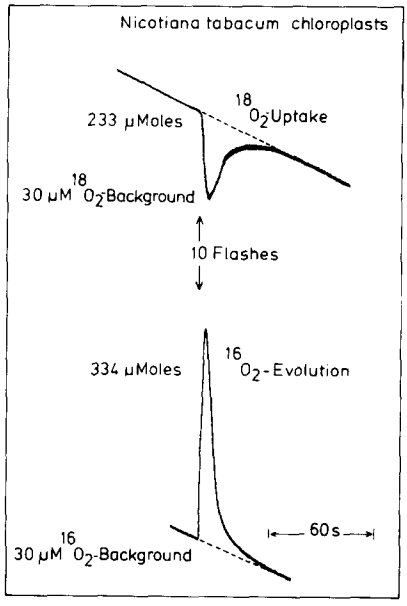

Fig. 1. Mass spectrometric recordings of oxygen gas exchange in thylakoids of *Nicotiana tabacum* var. John William's Broadleaf upon illumination with 10 short (5 μs) saturating light flashes spaced 300 ms apart. Photosynthetic oxygen evolution from $H_2^{16}O$ was detected at $m/e = 32$. In order to monitor oxygen uptake processes at $m/e = 36$ the assay was supplemented with 10 ml $^{18}O_2$ to the gas phase and brought to equilibrium between aqueous and gas phase. The arrow indicates the moment at which the train of 10 flashes was fired.

photosynthetic reduction of the PQ pool should enhance and photosynthetic oxidation of the PQ pool should inhibit oxygen consumption was postulated to be operative in *Chlamydomonas* [3–5,8–10]. According to this concept, the absence of a light-effect on the oxygen consumption process in chloroplast suspensions containing DBMIB and SiMo is directly related to the inability of substantially changing the redox state of PQ

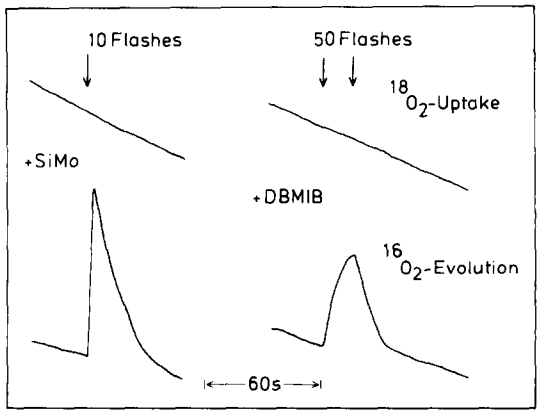

Fig. 2. Effect of silicomolybdate (10^{-4} M) and DBMIB (10^{-4} M) on the light-induced oxygen gas exchange of tobacco chloroplast. Note the difference in signal kinetics due to the different number of flashes (10 flashes in the case of silicomolybdate addition and 50 flashes for the DBMIB-containing assay). Other conditions as in Fig. 1.

by means of light reactions. Such an explanation is further supported by the effect of the electron acceptor ferricyanide $K_3[Fe(Cn)_6]$ which at a concentration of 10^{-4} M completely inhibited the light-dependent oxygen uptake (not shown).

The reduction/oxidation state of the PQ-pool in thylakoid membranes can be influenced by different light qualities, knowing that blue and red light is absorbed by both photosystems, and that far-red light is preferentially absorbed by PS I. This circumstance is often used to accelerate PQ-reoxidation by applying far-red light (FRL). Fig. 3 presents traces of mass spectrometric measurements monitoring the oxygen level in an assay and the effect of the onset of weak continuous light of different quality. As may be seen from the $^{16}O_2$ traces, the light intensity is not high enough to yield net photosynthetic oxygen evolution. On the other hand, light-driven photosynthetic electron transport even at a low rate may have an effect on the studied respiratory process. The onset of photosynthetic processes has, as discussed above, an effect on oxygen consumption. This may be seen as a drop of the oxygen level at the moment of switching on the light. Afterwards, the oxygen consumption stabilizes at a different rate, depending on the light quality. As is clear from the traces presented in Fig. 3, the rate of oxygen consumption in the presence of FRL is lower compared with the initial one. The presence of BL or RL results in an increase in the rate of oxygen consumption. This finding is consistent with the idea that respiratory processes are active in chloroplast membranes of tobacco, that the PQ pool is shared between the respiratory and photosynthetic electron transport chain, and that the reduction state of PQ, which is essential for respiratory oxygen consumption, is influenced by photosynthetic processes. This concept was substantiated by the application of DCMU known to

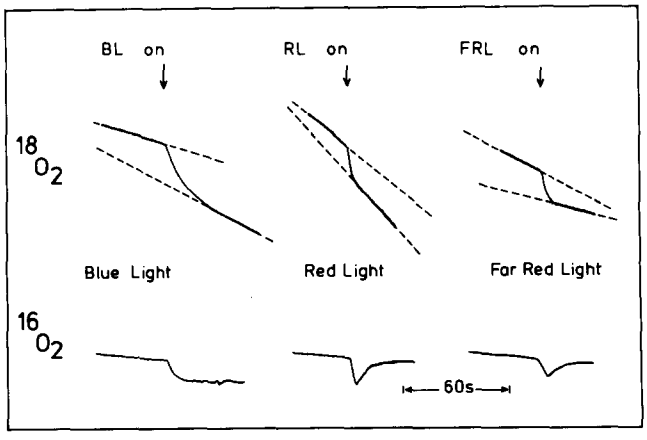

Fig. 3. Effect of different light qualities: blue light (BL), red light (RL) and far-red light (FRL) on the oxygen gas exchange in tobacco chloroplasts. The arrows indicate the onset of illumination. Equilibration after addition of $^{18}O_2$ and sensitivity as in Fig. 1.

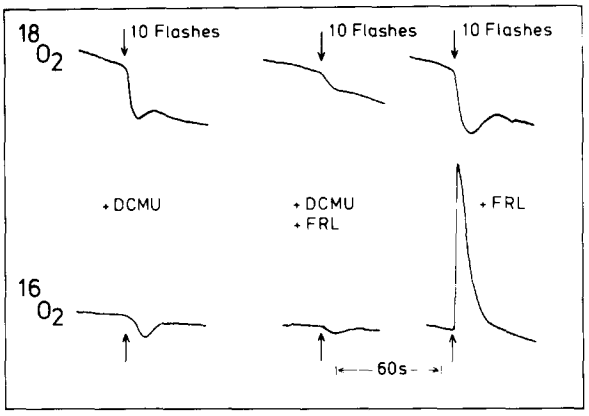

Fig. 4. Effect of DCMU ($5 \cdot 10^{-5}$ M) and FRL on oxygen gas exchange in tobacco chloroplasts induced by a train of 10 flashes. Conditions as in Fig. 1.

be a competitor to plastoquinone molecules in the Q_B binding niche of PS II. As may be seen from Fig. 4, DCMU, if present in a chloroplast suspension in concentrations of 5×10^{-5} M, suppresses photosynthetic oxygen evolution detected by our technique. Consequently, light-induced oxygen consumption can be seen at $m/e = 32$, i.e., the trace for $^{16}O_2$. This effect is usually overlapped by a pronounced photosynthetic oxygen evolution. The light-induced oxygen uptake in DCMU-poisoned chloroplasts is not completely suppressed but inhibited in relation to the control (Fig. 1). This might indicate that in a competitive PQ/DCMU action some electrons are leaking out of PS II and reduce the PQ pool. The effect of light on oxygen uptake can be almost completely suppressed in DCMU-treated chloroplasts by illumination with weak FRL, thus increasing the rate of PQ reoxidation by PS I (Fig. 4, middle). This is, however, not the case in the assay with control chloroplasts illuminated additionally with FRL (Fig. 4, right). The findings obtained by the combination of DCMU and FRL indicate that a very low rate of photosynthetic electron flow already has a pronounced effect on chlororespiratory activity. Fig. 5 shows the dependence of light-dependent oxygen consumption on photosynthetic oxygen evolution, produced with different doses of light quanta realized by increasing numbers of flashes in the train (from 1 to 50). The fact that this dependence shows saturation indicates that in fact cooperation of chlororespiratory and photosynthetic pathways is essential at low light intensities when the rate of photosynthetic PQ reduction/oxidation is not maximal.

Treatment of chloroplast suspensions with Antimycin A (5 μM), a known inhibitor of mitochondrial respiration, has no effect on light-induced oxygen-uptake (not shown) indicating that this process is not involved. Other inhibitors of respiration, namely salicyl hydroxamic acid (SHAM) and KCN at concentrations

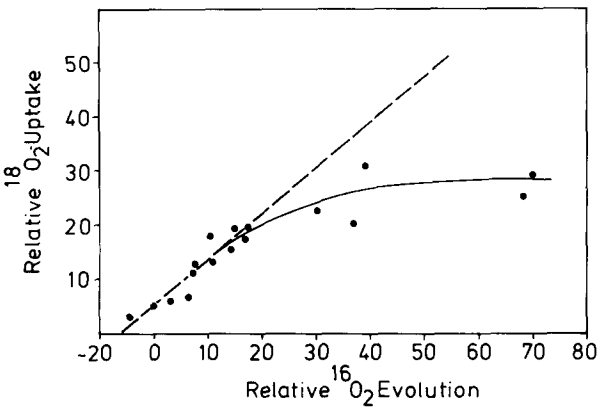

Fig. 5. Correlation between photosynthetic oxygen evolution (m/e = 32) and light-dependent oxygen consumption (m/e = 36) in tobacco chloroplasts illuminated with increasing numbers of flashes (1–50). Relative values correspond to the integrals of the respective recorder tracings at identical sensitivity.

of 1 mM and 0.5 mM respectively, increased the effect of light on oxygen consumption (not shown). In the case of KCN treatment, photosynthesis was inhibited by about 20% as concluded from the light-driven oxygen evolution. The enhancement of a light effect on O_2-uptake in the presence of SHAM and KCN can be explained by an inhibition of electron transfer processes between cytochrome b_6f complex and Photosystem II. The existence of such a KCN-sensitive process was recently reported in the cyanobacterium *Synechocystis* [22].

In conclusion, the described light-dependent oxygen uptake in tobacco chloroplast is dependent on the photosynthetic reduction/oxidation rate of the PQ pool and can be regarded as the demonstration of chlororespiratory activity in higher plants. Such a concept is coherent with the model of chlororespiration in algae [1–11]. On the other hand, on the basis of the findings presented in our paper one cannot exclude the possibility that in tobacco chloroplasts, further elements of photosynthetic electron transport are shared with respiratory processes, namely cytochrome b_6f and/or plastocyanin. This possibility might be supported by the light-induced oxygen-uptake measurements in chloroplasts treated with DBMIB. Reduction of the PQ pool by DBMIB at the concentration applied is not sufficient to maintain uninhibited linear electron transport through PS II, monitored by oxygen evolution (see Fig. 2). Under these conditions a process of light-induced oxygen-uptake is blocked, suggesting the possible involvement of some other than PQ electron carriers located between PS II and PS I in respiratory processes. This interpretation is supported by the results of our experiments in which weak light of different qualities was applied (see Fig. 3). In this context it should be noted that a chlororespiratory activity in which the cytochrome b_6f complex and plastocyanin are involved was postulated for the cyanobacterium *Aphanocapsa* [22].

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