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The Kok effect and the light-inhibition of chlororespiration in *Chlamydomonas reinhardtii*

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Measurements of O_2 exchange have been performed as a function of light intensity in the green alga *Chlamydomonas reinhardtii* by using $^{18}O_2$ and mass spectrometry. The Kok effect (i.e. a decrease in the slope of net O_2 exchange curve) was observed at a light intensity of about 15 $\mu E \cdot m^{-2} \cdot s^{-1}$. This effect was due to the inhibition by light of O_2 uptake. In conditions where mitochondrial respiration was inhibited by antimycin A and salicyl hydroxamic acid, illumination was found to reduce O_2 uptake to the same extent as in the control experiment. We conclude that the Kok effect in *Chlamydomonas* is not the consequence of the inhibition of mitochondrial respiration, but is due to the inhibition of chlororespiration induced by photosystem I activity.

Kok effect; Chlororespiration; Photosystem I; Photosynthesis; Antimycin A; (*Chlamydomonas reinhardtii*)

1. INTRODUCTION

In 1949, Kok observed in *Chlorella* cells a lack of linearity in the curve of net O_2 exchange measured as a function of light intensity [1]. This effect, now referred to as the 'Kok effect', was interpreted as the consequence of the suppression of dark respiration by photochemical reactions [1]. The Kok effect was observed in many unicellular green algae and in cyanobacteria (review [2]) and also in higher plants [3]. The initial interpretation of Kok was further confirmed by isotopic measurements using $^{18}O_2$ [4]. Because the Kok effect was insensitive to the PS II inhibitor DCMU, ATP produced by cyclic photophosphorylations around PS I was proposed as the mediator of the inhibition. This interpretation is however inconsistent with the insensitivity of the Kok effect to the uncoupler CCCP [5].

Using another method, recent studies on the eukaryotic green alga *Chlamydomonas* have shown the existence of a respiratory process located within the chloroplast (chlororespiration [6,7]). The chlororespiratory electron transport chain could share some carriers in common with the photosynthetic electron transport chain. In a previous study, we reported that flash-induced PS I activity, probably by oxidizing an electron carrier common to the two chains, inhibited this chlororespiration [7].

In the present study we reinvestigate, using $^{18}O_2$ and mass spectrometry techniques, the origin of the Kok effect. We confirm that this effect is due to the inhibition by light of a respiratory O_2-consuming process. On the basis of the action of respiratory inhibitors, we conclude that the Kok effect is mainly due to the inhibition of chlororespiration by PS I activity.

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Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCMU, 3-(3,4-dichlorophenyl)-1,1 dimethylurea; PS, photosystem; SHAM, salicyl hydroxamic acid

2. MATERIALS AND METHODS

Chlamydomonas reinhardtii wild-type 137c was grown

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phototrophically as described [8]. Algae were harvested by low speed centrifugation (1500 × g) and resuspended in fresh medium (pH 6.2) to obtain a chlorophyll concentration between 30 and 50 $\mu g \cdot ml^{-1}$. For O_2 exchange measurements, 5 ml of the cell suspension were transferred into a thermostated (25°C) reaction vessel and were continuously stirred with a magnetic bar. A polyethylene membrane at the bottom of the reaction vessel allowed dissolved gases to be introduced into the mass spectrometer (type 14-80, VG instruments, England). After bubbling the algal suspension with N_2 to remove the ambient $^{16}O_2$ initially dissolved, $^{18}O_2$ (98.1% ^{18}O from CEA, Saclay, France) was added to obtain an O_2 concentration of about 20%. Then the reaction vessel was closed and 2 mM bicarbonate were injected. The concentrations of the dissolved species $^{16}O_2$ and $^{18}O_2$ were measured by continuously and simultaneously recording masses 32 and 36, respectively. O_2 uptake, O_2 evolution and net O_2 evolution rates were calculated as reported in [9]. Illumination was provided by a projector (Oriel) equipped with a 1000 W quartz-halogen lamp. A water filter (7 cm) was used to reduce unwanted infrared radiations. Light intensity was measured using a Li-Cor (Lincoln, NE, USA) LI-85 B quantum sensor and was adjusted to the desired value by the use of Balzer neutral-density filters.

Chlorophyll content was determined after extraction with 90% (v/v) methanol as described in [8].

Antimycin A and SHAM were purchased from Sigma and were used dissolved in ethanol. At the concentration used, ethanol had no effect on both respiratory and photosynthetic activities.

3. RESULTS

The rates of O_2 exchange have been measured as a function of light intensity in a suspension of *Chlamydomonas* cells (fig.1). Net photosynthesis was not a linear function of light intensity, a break (Kok effect) occurring at low light (about 15 $\mu E \cdot m^{-2} \cdot s^{-1}$). As shown by the O_2 uptake curve, this non-linearity is only due to the inhibition of respiration, since O_2 evolution linearily increased over the studied range of light intensities. This result is in agreement with the mass spectrometer data obtained by Hoch et al. [4] in *Anacystis* and also confirms the finding that the Kok effect in *Chlamydomonas* is due to the inhibition of a respiratory process [5].

In order to determine the nature of the respiratory O_2-consuming process inhibited by light, the same O_2 exchange measurements were carried out in the presence of inhibitors of mitochondrial respiration. As observed [9], addition of 2 μM antimycin A, an inhibitor of the cytochrome-*c* oxidase pathway, did not affect the rate of dark O_2 uptake (not shown). This lack of inhibition is due to the existence in

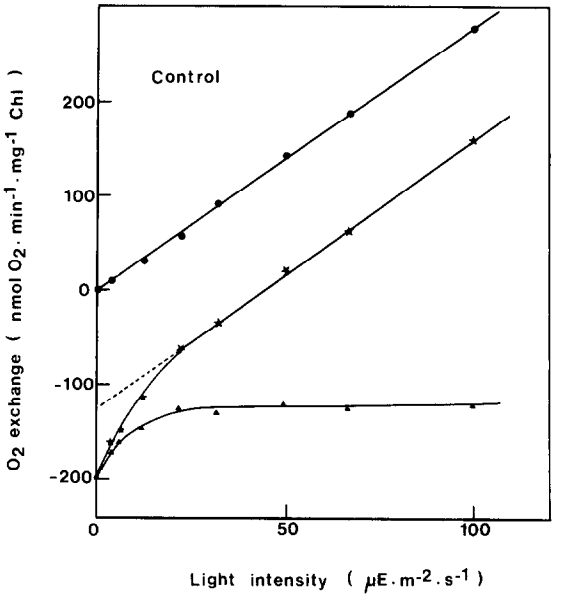

Fig.1. Oxygen evolution (●), oxygen uptake (▲) and net oxygen evolution (★) measured as a function of light intensity in the green alga *Chlamydomonas*. After adaptation of the cells in the dark (about 15 min), O_2 exchange was followed at different light intensities. At each light intensity, the measurement was carried out in a period of 3 min.

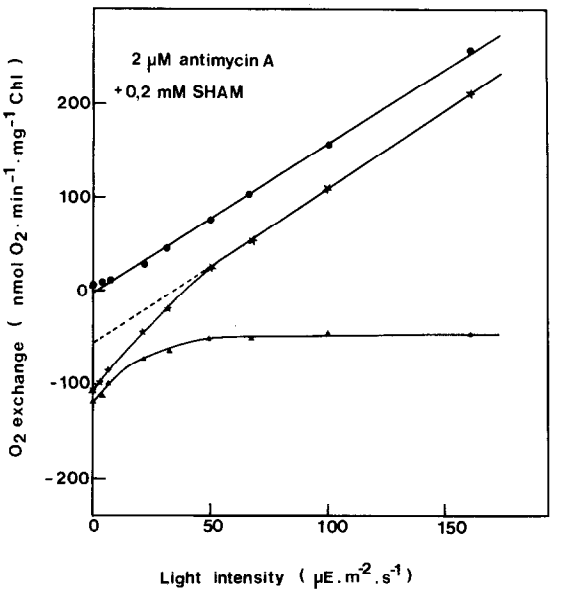

Fig.2. Same as in fig.1, but in the presence of 2 μM antimycin A and 0.2 mM SHAM.

Chlamydomonas cells of a very efficient alternative pathway [7,9,10]. The subsequent addition of SHAM, an inhibitor of the alternative oxidase pathway, inhibited the initial rate of O_2 uptake by about 45% (fig.2). In these conditions, the Kok effect was always observed, but the non-linear part of the curve was extended towards higher light intensities (up to about 45 $\mu E \cdot m^{-2} \cdot s^{-1}$) than in the control experiment. The part of dark O_2 uptake inhibited by light remained practically unchanged (about 68 nmol $O_2 \cdot min^{-1} \cdot mg\ Chl^{-1}$ compared to about 78 nmol $O_2 \cdot min^{-1} \cdot mg\ Chl^{-1}$ in the control experiment).

Addition of antimycin A and SHAM not only inhibited O_2 uptake, but also caused a partial inhibition of O_2 evolution. This inhibition of photosynthesis may reflect either a direct effect of the added compounds on photosynthetic electron transport or a dependence of photosynthesis towards respiratory activity as already observed in *Chlamydomonas* wild type [9] and more recently in a mutant lacking chloroplast ATP synthase [11]. This decrease of photosynthetic activity might explain the requirement for higher light intensities to observe the Kok effect in the presence of the inhibitors. We have plotted in fig.3 the rates of O_2 uptake and of net O_2 evolution as a function of O_2 evolution as a direct measurement of photosynthetic activity. It clearly appears that the Kok effect and the inhibition by light of O_2 uptake occur at the same photosynthetic activity in the presence or absence of mitochondrial electron transport inhibitors.

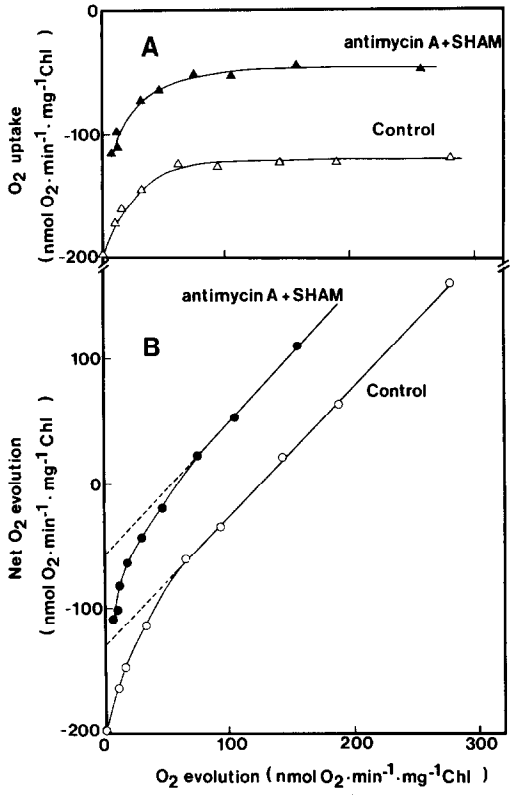

Fig.3. Oxygen uptake (A) and net oxygen evolution (B) plotted as a function of oxygen evolution. (○, △) Control experiment; (●, ▲) in the presence of 2 μM antimycin A and 0.2 mM SHAM. Oxygen exchange data are those of the experiments shown in figs 1 and 2.

Finally, one may distinguish between two components in the respiration of *Chlamydomonas* cells. (i) A light-inhibited component (78 nmol $O_2 \cdot min^{-1} \cdot mg\ Chl^{-1}$ in the control experiment) only slightly inhibited (about 12%) by antimycin A and SHAM. (ii) A light-insensitive component (about 130 nmol $O_2 \cdot min^{-1} \cdot mg\ Chl^{-1}$ in the control experiment) which is inhibited by more than 60% by antimycin A and SHAM and is therefore probably related to the activity of mitochondrial respiration.

4. DISCUSSION

Recent investigations on eukaryotic algae have shown the existence of respiratory activity (referred to as chlororespiration) within the chloroplast [6,7]. The chlororespiratory electron transport chain could be located between the two photosystems and could share some electron carriers in common with the photosynthetic electron transport chain. An NAD(P)H-plastoquinone oxidoreductase [12] and a cyanide-sensitive oxidase [6,7] could also be involved in the chlororespiratory activity. In a previous study we have reported that chlororespiration was inhibited by PS I activity, probably due to the oxidation by PS I of one of the common electron carriers [7]. Moreover, we observed that in contrast to mitochondrial respiration, chlororespiration was insensitive to antimycin A and SHAM. Hence, we conclude that the light-inhibited component of O_2 uptake, which is insensitive to antimycin A and SHAM and is consequently unrelated to the mitochondrial activity, is mainly due to the activity of chlororespira-

tion. In other words, the Kok effect observed in *Chlamydomonas* cells would be due to the inhibition of chlororespiration by PS I activity.

This interpretation is consistent with the observations that the Kok effect is greater at 695 nm than at 645 nm and that it is not inhibited by DCMU [5]. Moreover, contrary to the initial interpretation in which the inhibition of respiration would be mediated by ATP produced by photophosphorylation [4,13], this conclusion takes into account the insensitivity of the Kok effect to the uncoupler CCCP [5]. Indeed, as inferred from flash excitation experiments [7], the inhibition could be due to a diversion of electrons from the chlororespiratory electron transport chain towards PS I.

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