Effect of CO_2 on the energization of thylakoids in leaves of higher plants

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We investigated the effect of CO_2 on the flash-induced electrochromic absorbance change of chloroplasts in leaves of higher plants. In leaves depleted of CO_2 the initial electrochromic rise was followed by a fast ($t_{1/2}$; 10–20 ms) and a slow (100–200 ms) decay. These kinetic components could be correlated with a dissipative and an ATP-synthetizing current, respectively. In leaves supplied with CO_2 an additional slow electrochromic rise (5–10 ms) appeared in the absorbance change. This component could be tentatively correlated with the enhancement of the electron transport by CO_2 between the two photosystems. In leaves supplied with CO_2 the decay could be fitted with a single exponential with a $t_{1/2}$ ~200 ms. We conclude that both energization and ATP-synthesis are strongly regulated by CO_2 in chloroplasts in situ.

Photosynthesis *Chloroplast* *Electrochromism* *CO_2* *Energization*

1. INTRODUCTION

Bicarbonate or CO_2 is an important regulator of photosynthetic electron transport and ATP synthesis in isolated chloroplasts (review [1]). Here, we report the effect of CO_2 on the energization of the thylakoid membranes in chloroplasts in situ as studied by flash-induced electrochromic absorbance change in leaves of higher plants.

2. MATERIALS AND METHODS

The primary leaves of 2–3-weeks-old normal and chlorophyll *b*-less mutant seedlings of barley grown in a greenhouse (*Hordeum vulgare* L. cv. Donaria and strain 3613, Gatersleben, respectively) were used. The freshly harvested leaves were infiltrated by water under gentle vacuum (~1 kPa) for 5–30 min. After this simple treatment the leaves became optically transparent and depleted of CO_2 to a large extent. Depletion of the leaves of CO_2 is illustrated clearly by the effect of their consecutive treatment with CO_2 (e.g., fig. 2). The leaves were placed on a sample holder set diagonally in a 1 cm × 1 cm optical cuvette which then was closed hermetically to exclude O_2. Oxygen affects the decay of the kinetics of the electrochromic absorbance change especially in leaves preilluminated by intense light [2]. It must be noted that in our experimental conditions (infiltrated leaves adapted to and measured in dim light at low repetition rates of the exciting flashes) contamination of the atmosphere by air did not abolish the CO_2-effect. The kinetic traces recorded in CO_2-deficient and CO_2-enriched atmospheres were very similar to those obtained with leaves in N_2 or CO_2 atmospheres, respectively.

The measurements were carried out after a short (3–15 min incubation in air, N_2- or CO_2-atmospheres. The absorbance changes between 460 and 540 nm induced by saturating xenon flashes (>630 nm, 3 μs duration at half-peak emission) were measured in a set-up of [3]. The kinetical curves could be satisfactorily fitted by a linear combination of two exponentials and were analysed as in [4].

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1, 1-dimethylurea

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The time required for the depletion and for the supply of CO_2 varied from 5–30 min and ~3–15 min, respectively. The fastest response (1–2 min) to the CO_2 treatment could be observed in leaves which were not infiltrated with water. However, in this case, the effect of CO_2 was less marked since the control might have retained a considerable amount of CO_2. The slowest response (30–60 min) was observed in leaves which were simply allowed to stand in the air. The CO_2-effect also developed very rapidly (in 1–2 min) in *Chlorella* cells when the suspension was bubbled gently by CO_2 gas. The effect of CO_2 was reversible to a considerable extent also by 'washing' the leaves or cells in N_2 gas for several minutes. These observations imply that the development of the effect of CO_2 in leaves is limited in time mainly (and very likely is regulated) by its uptake through the stomatas.

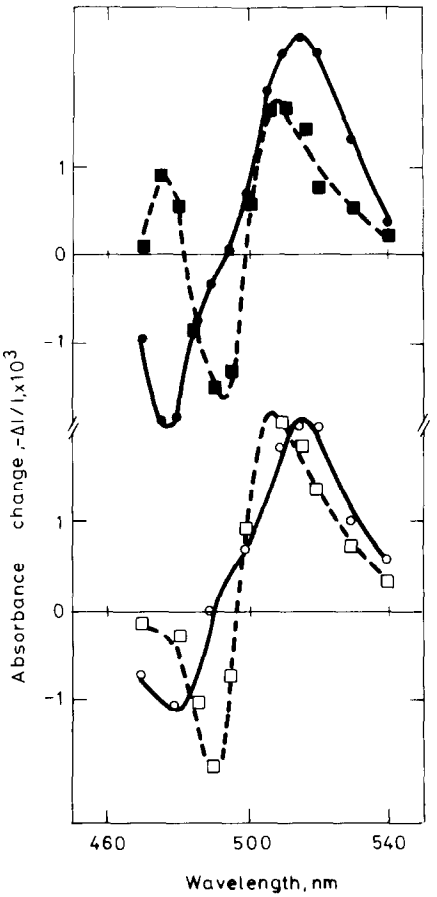

Fig. 1. Flash-induced transient absorbance spectra in isolated chloroplasts (upper curves) and in leaves (lower curves): (——) normal barley; (– – –) chlorophyll *b*-less mutant barley. With chloroplasts the valinomycin-sensitive electrochromic absorbance change is plotted.

Type B [5] chloroplasts were isolated from normal and mutant barley leaves which were the same age as the seedlings used in the measurements with leaves. The standard reaction mixture contained 0.35 M sorbitol, 20 mM tricine and 5 mM $MgCO_3$ and in certain measurements it was supplemented with 1.2 μM valinomycin and 5 mM KCl. The electrochromic absorbance change in chloroplasts was determined from the difference between the absorbance change in the absence and presence of valinomycin.

Fluorescence induction curves of the leaves were determined in a laboratory-built set-up in the same cuvette that was used in the absorption change measurements. Light from a 450 W high pressure xenon arc lamp was filtered through a Corning 4-96 glass filter and focused onto the lower surface of the leaf. The intensity of the homogeneous excitation beam was 16 $W.m^{-2}$. Fluorescence light from the upper surface of the leaf was detected by an EMI 9558 B photomultiplier perpendicular to the excitation beam. The scattered exciting light was blocked by a Corning 2-64 glass filter set in front of the photomultiplier. The fluorescence transients were collected in a multichannel analyser (ICA 70, KFKI). The time resolution with the optical shutter used (Wincent Assoc., type L 2175) was ~10 ms.

3. RESULTS AND DISCUSSION

Fig. 1 shows the flash-induced transient absorbance spectra determined in normal and mutant leaves and the corresponding electrochromic absorbance transients in isolated chloroplasts. From the close resemblance of the transient spectra it can be concluded that around 480 and 510 nm in the mutant leaves the absorbance changes originate mainly from an electrochromic response of the chloroplast pigments.

Fig. 2 shows typical absorbance changes in leaves depleted of CO_2 and after a short incubation of the leaves in CO_2 atmosphere. Supplying the leaves by CO_2 results in a deceleration of the decay and the appearance of a slow rise. (During the incubation of leaves in CO_2 deceleration of the decay

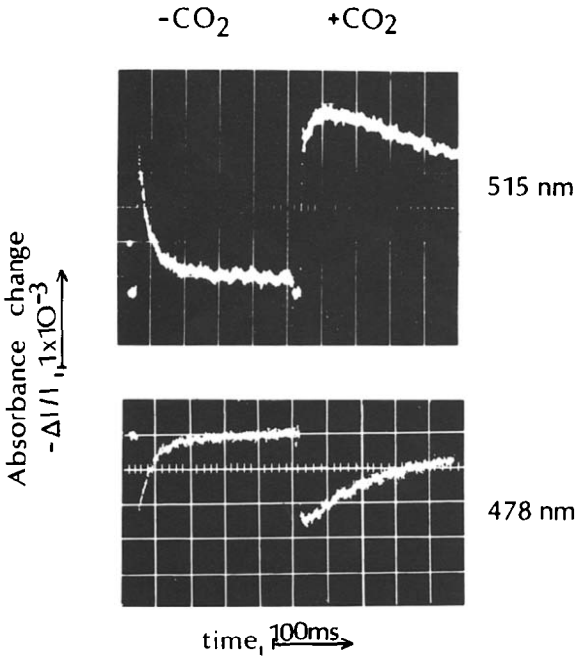

Fig. 2. Flash-induced absorbance changes in CO_2-depleted barley leaf in N_2-atmosphere ($-CO_2$) and in the same leaf after an incubation in CO_2-atmosphere for 5 min ($+CO_2$). 20 kinetic traces were collected in the multichannel analyzer with a dwell time of 200 μs. (Different samples from the same batch of leaves were used in the measurements at 478 and 515 nm.)

usually preceded the appearance of the slow rise around 10 ms.) The same phenomenon could be observed in mutant barley leaves around 510 and 490 nm. This demonstrates also that CO_2 affects the electrochromic absorbance change in chloroplasts in situ. We observed the same type of effect in various higher plants (spinach, tobacco, maize) and also in *Chlorella* cells. This suggests that the regulation of energization by CO_2 is general among green plants.

When the leaves were infiltrated with 15 μM DCMU the initial amplitudes of the absorbance change decreased to ~60% of the original values (fig. 3) (0.5% ethanol which had to be added did not cause any appreciable effect in the leaves). Upon supplying the leaves with CO_2 deceleration of the decay was similar to that in the control leaves. The apparent amplitude of the slow rise, however, was small in the DCMU-treated leaves. This shows that the two effects of CO_2, deceleration of the decay and enhancement of the slow rise, are rather independent.

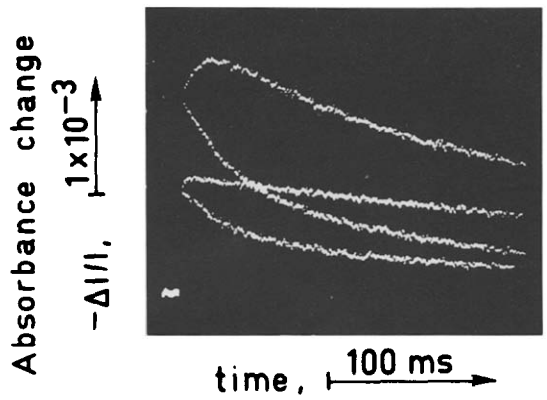

Fig. 3. Flash-induced absorbance changes at 515 nm in barley leaf. From the top to the bottom: $+CO_2$, $-CO_2$ (cf. fig. 2) in the absence of DCMU and $+CO_2$ and $-CO_2$ in the presence of 15 μM DCMU, respectively.

Deceleration of the decay could be explained with the effect of CO_2 on the membrane permeability, an effect observed in isolated chloroplasts [6]. The results of a kinetic analysis of our data are in line with this explanation. In the absence of CO_2 the decay could be fitted by the sum of a fast ($k_1 = 57 \pm 13\ s^{-1}$) and a slow ($k_2 = 5.1 \pm 0.8\ s^{-1}$) exponential with 72% and 28% relative amplitudes, respectively (20°C, mean values and standard errors calculated from 7 independent expt). When the leaves were supplied with CO_2 only the slow decay could be observed. The rate constant ($k_2' = 3.2 \pm 0.9\ s^{-1}$) of this kinetic component was about the same as the corresponding rate constant in the CO_2-depleted leaves. This kinetic component can be correlated with the ATP synthesis (cf. [4,7,8]). The fast decay, with a $t_{1/2}$ ~10–15 ms, can be associated with a dissipative current due to the increased membrane permeability [6]. Based on this interpretation it can be concluded that in the absence of CO_2, ATP-synthesis is suppressed to a considerable extent. A similar conclusion was reached from experiments in chloroplasts in the absence and presence of bicarbonate [1]. Binding of CO_2 to the binding site close to the secondary electron acceptor B [1] could be shown also in our experiments by investigating the fluorescence induction of the leaves [9]. When the leaves were supplied with CO_2, unlike in the CO_2-depleted leaves, the fluorescence was not saturated during the induction period and the yield dropped considerably

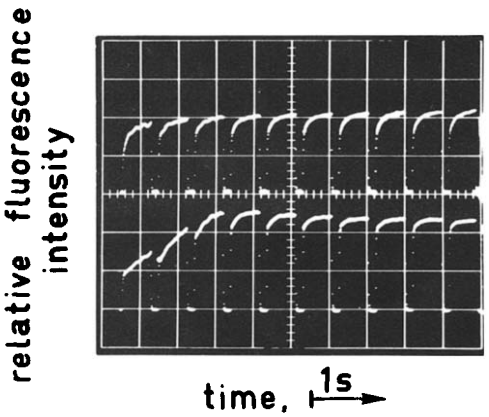

Fig. 4. Fluorescence induction in barley leaf exposed to a multiple rectangular excitation (400 ms light/100 ms dark). The leaf was dark adapted for 5 min before measurements. Upper and lower trace, $+CO_2$ and $-CO_2$, respectively (cf. fig. 2).

in the dark intervals (fig. 4). This indicates that CO_2, like bicarbonate in isolated chloroplasts restores the electron transport between the two photosystems. This difference between the induction curves appeared only after 3–5 induction periods while no characteristic difference could be revealed from experiments with single rectangular excitations. Also in the absorbance change at 515 nm the typical symptoms of CO_2-depletion of leaves, fast decay and lack of the slow rise, could be observed only after a few exciting flashes. Very likely these phenomena indicate the presence of some 'residual' CO_2 bound to the thylakoids.

Our data provide evidence that the slow rise in leaves is regulated by CO_2. No slow rise could be resolved in CO_2-depleted leaves and it could be restored by supply of leaves with CO_2. It must be noted that these changes cannot be accounted for by a possible lowering of the intercellular pH as it should decrease the relative amplitude of the slow rise [3]. We have also shown that the amplitude of the slow rise in leaves was diminished considerably, or its rate dramatically changed, in the presence of high [DCMU]. These facts may give an insight into the origin of the slow electrochromic rise. The most plausible supposition, in the light of the above experiments, is that the slow rise originates from the electron transport between the two photosystems. Kinetically, if this electron transport is inhibited or decelerated considerably, the slow rise may also be inhibited or it may apparently disappear (masked by the decay) due to the deceleration. The physical basis of an interpretation that correlates the electron transport and the slow rise has been outlined [10]. Translocation of charges from the di-electric membrane into the conductive phases could give rise to an increase of the transmembrane field which could be detected by the field-sensitive pigments. The translocation processes (both the proton uptake at the acceptor side of photosystem 2 and the consecutive proton release at the donor side of photosystem 1) are affected by bicarbonate in chloroplasts [6].

The slow rising kinetic component is regulated by various factors and its origin has been explained in various models [11–15]. Our interpretation, that establishes a correlation between the slow rise and the electron transport between the two photosystems, is supported by other experimental results. Proton uptake is associated with the slow rise of the electrochromic absorbance change [16]. Binary oscillation of the amplitude of the slow rise in algal cells indicated that the secondary electron acceptor B of photosystem 2 is involved in the slow electrogenic rise [17].

Nevertheless, from our data it can be concluded that CO_2 regulates both the energization of thylakoids and the formation of ATP in chloroplasts in situ.

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