Naturwissenschaften 75, 517 – 519 (1988)

Carbon Dioxide Affects Charge Accumulation in Leaves

Measurements by Thermoluminescence

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Warburg and Krippahl [1] discovered that CO_2 (or bicarbonate, HCO_3^-) was needed for the electron flow in the Hill reaction of plants and proposed (see discussion in [2]) that O_2 originated in CO_2, not H_2O. Although the phenomenon has been confirmed (see [3]) by many, the site of action of this CO_2 action has been shown to be not on the oxygen evolution, but on the electron acceptor site of the oxygen-evolving photosystem II (PS II) [4 – 6], where it participates in the reduction of plastoquinone by the bound quinone (Q_A). Blubaugh and Govindjee [7] have shown that it is HCO_3^-, rather than CO_2, that is the active species involved in stimulating the electron flow in thylakoids. Bicarbonate has also been shown to affect ATPase of chloroplast thylakoid membranes [8].

In contrast to the wealth of information in vitro, i.e., in isolated thylakoids, in vivo data are scarce. Effect of partial CO_2 depletion and readdition of CO_2 was studied in leaves by flash-induced electrochromic absorbance changes at 515 nm (A515) and chlorophyll *a* fluorescence induction [9]. CO_2, possibly due to its effect on the ATPase ([8]; cf. also [10]), has been shown to affect the rise and decay of the absorbance transient, A515, which is proportional to the transmembrane electric field [11]. Furthermore, chlorophyll *a* fluorescence induction data in leaves suggested that CO_2 has a regulatory role at the electron acceptor site of PS II in leaves [9]. Recently, detailed analyses of chlorophyll *a* fluorescence induction data in leaves have shown that indeed CO_2 has a direct role in electron transport regulation in leaves [12]. Bicarbonate effects in intact algal cells had been shown earlier (see e.g. [6, 13]).

Govindjee et al. [14] have used thermoluminescence (TL) measurements in thylakoids to show that CO_2 depletion blocks the cycling of the electron acceptor site of PS II. We have investigated TL in leaves which were partially depleted of intercellular gases, including CO_2, and were incubated in N_2 or CO_2-enriched atmosphere. TL is a measure of charge recombination around PS II. Our results, presented here, show that CO_2 affects the charge accumulation process around PS II in leaves: it is suggested to deplete these charges because it stimulates the forward electron flow from PS II to photosystem I (PS I).

Spinach leaves were obtained from the local market in Szeged. The TL of leaf segments was measured essentially as described earlier [15] following protocol 1 or 2: (1) Leaves were depleted of

their intercellular gas content by infiltrating them with water under gentle vacuum for 5 – 20 min [9]. A disc of 7 mm diameter was cut from a leaf, placed onto the TL sample holder equipped with a gas chamber, and incubated in CO_2-enriched or N_2 atmosphere for 3 min in the dark before measurement. (We found that CO_2 atmosphere and CO_2-enriched atmosphere, i.e., CO_2 plus N_2 or air gave equivalent results.) (2) The disc of 7 mm diameter was cut from noninfiltrated leaf, placed onto the sample holder and incubated in the gas chamber for various intervals in the dark or in the light. Samples were excited with white actinic light of 10 W m^{-2} during cooling from 20 °C to −60 °C at a rate of 1.38 °C s^{-1}. The glow curves were recorded between −60 and +60 °C.

CO_2-Depleted Leaves. Figure 1 shows that partial depletion of the intercellular gas content and readdition of CO_2 induces marked effects on the glow curves of infiltrated leaves. The major effect of the depletion process is the appearance of the 0 °C band, and the major effect of CO_2 addition is its diminution; in contrast, N_2 addition enhances this band further. These effects develop quite rapidly, within several minutes, in the dark.

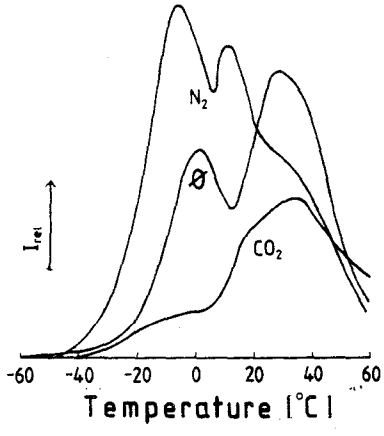

Fig. 1. Effect of gaseous environment (CO_2 or N_2) on the thermoluminescence (TL) of water-infiltrated spinach leaf discs; untreated leaf; N_2 and CO_2: leaves depleted of their intercellular gas content and incubated in the dark in N_2 or CO_2 atmosphere, respectively. Each TL curve was obtained by averaging three glow curves from independent experiments recorded under the same experimental conditions. (For other details, see description in the text, protocol 1)

Non-CO_2-Depleted Leaves. In samples where intercellular gases were not depleted (Fig. 2), incubation in CO_2 had apparently different effects: with 30 – 60-min preincubation in dark or just 1 min light and 5 min dark, the 0 °C band increased in intensity; the TL pattern was, however, close to the original sample, i.e., it had a high 40 °C and a low 0 °C band. Incubation under N_2, however, gave qualitatively the same results as shown in Fig. 1 except that the 0 °C band appeared at full intensity even after 1 min light and 5 min darkness. Preillumination apparently facilitated the rate of gas exchange between the leaf and its environment. In spite of the apparent difference in the CO_2 curves, a comparison with N_2 curves shows that the 0 °C band was more dramatically reduced under CO_2 than under N_2 atmosphere, confirming the data of Fig. 1.

We emphasize that the effect of CO_2 in TL was observed mostly under such experimental conditions where interference from the carbon (Calvin) cycle can be ruled out. Preillumination was not necessary to obtain the effect of the readdition of CO_2. Since CO_2 uptake at room temperature has a lag phase of about 5 s in intense light [16] and the leaves were excited only during the rapid cooling (at a rate of 1.38 °C s^{-1}) between 20 °C and −60 °C, the chloroplasts could not have much chance for carbon metabolism.

The most easily recognizable difference between the two types of gas treatment was the integrated TL area (Table 1). This parameter, at least in a semi-quan-

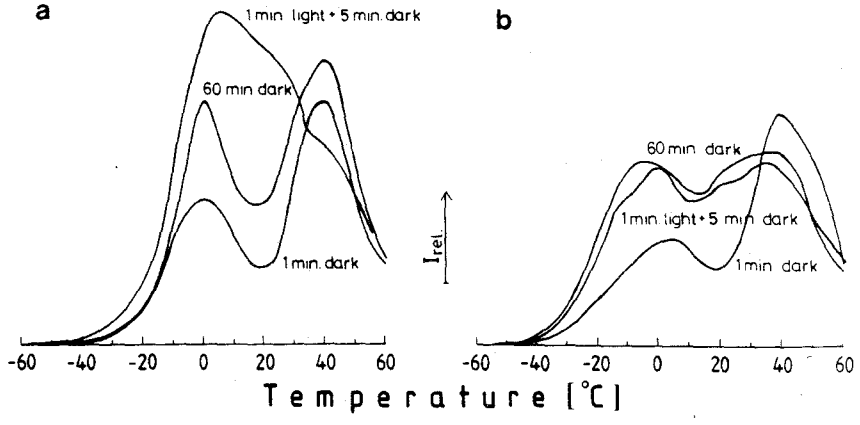

Fig. 2. Effect of incubation of noninfiltrated leaves a) in N_2 atmosphere and b) CO_2-enriched atmosphere under various experimental conditions as indicated. The glow curves were obtained by averaging three TL curves from independent experiments. (For details, see description in the text, protocol 2)

Table 1. Effect of CO_2 and N_2 on thermoluminescence in spinach leaves

	Integrated intensity[a] [%][b]		Ratio of peak intensity B band/D band	
Incubation	N_2	CO_2	N_2	CO_2
Untreated	100 ± 7		1.36 ± 0.21	
90 min dark	109 ± 6	73 ± 14	1.01 ± 0.08	1.99 ± 0.32
1 min preillumination; +5 min dark	118 ± 23	80 ± 6	1.02 ± 0.13	1.72 ± 0.29
Water-infiltrated; +3 min dark	184 ± 19	42 ± 14	0.71 ± 0.17	3.35 ± 0.94

[a] Integrated TL intensity between −60 and +60 °C

[b] Data represent mean values and standard errors calculated from 3 – 6 experiments

titative way, measures the concentration of the charges residing around PS II. Since the contribution of the reduced plastoquinol in PS II is insignificant when compared to that by the reduced bound plastoquinones Q_A and Q_B, the two secondary electron acceptors of PS II, a decrease in TL intensity in CO_2-enriched atmosphere suggests that CO_2 facilitates the flow of electrons from the vicinity of Q_A and Q_B towards photosystem I (PS I). In the presence of N_2 alone, the TL intensity is higher, suggesting the accumulation of Q_A^- and Q_B^- needed for charge recombination that produces TL.

Although the TL peak positions varied somewhat from sample to sample, the following three major peaks could be identified: a peak around $-15\,^\circ C$ (the so-called A peak); 0 to $10\,^\circ C$ (the so-called D or the Q peak); and 30 to $45\,^\circ C$ (the so-called B band, often made up of two peaks B_1 and B_2). The ratio of the intensity of the B band to the D (or Q) band, labeled in Table 1 as HT/LT, was elevated when CO_2 was present. The same ratio decreased when N_2 was present. The TL bands originate in recombination of Q_A^- or Q_B^- with the redox state of the oxygen-evolving complex (the electron donors of PS II), the so-called S states. Peak A is suggested to originate in $S_3\,Q_A^-$, D (or Q) in $S_2\,Q_A^-$, and B (B_1 and B_2) in $S_2/S_3\,Q_B^-$ [17]. Thus, our results show that the absence of CO_2 (presence of N_2; or depleted of all gases) increases the ratio of Q_A^- to Q_B^- in leaves and CO_2 has the opposite effect.

In conclusion, thermoluminescence of leaves has provided independent experimental evidence, without the use of inhibitory ions (such as formate or nitrite), often used in thylakoid studies, that CO_2 in leaves facilitates the flow of electrons from the reduced Q_A towards PS I. This places the role of CO_2 (HCO_3^-) in electron flow in vivo on a firm basis, a role totally different from that of the Calvin cycle, and explains the phenomenon discovered by Otto Warburg 30 years ago.

This work was supported by grants OKKFT (Tt) 222/1988 and 310/1988 from the National Foundation of Technical Development (Hungary). Govindjee was supported by an interdisciplinary McKnight Research Grant to the University of Illinois at Urbana, IL (USA).

Received June 20, 1988

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