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Dark reduction of Q_A in intact barley leaves as an effect of lowered CO_2 concentration monitored by chlorophyll *a* luminescence and chlorophyll *a* F_0 dark fluorescence

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When the CO_2 concentration in the atmosphere above an intact barley leaf was lowered in the dark after illumination, chlorophyll *a* luminescence and chlorophyll *a* dark fluorescence were stimulated. The stimulation was induced by lowered levels of CO_2 in a wide concentration range including concentrations well above that saturating photosynthesis. The stimulation of luminescence by lowered CO_2 concentrations was more pronounced after far-red excitation than after white light excitation. The difference in response to lowered CO_2 concentrations after white/far-red excitation was less pronounced for fluorescence than for luminescence. Stimulation of luminescence was more pronounced when the CO_2 concentration was lowered in an O_2-containing atmosphere than under anaerobic conditions. It is concluded that lowering of the CO_2 concentration in the dark after illumination causes a partial reduction of the primary Photosystem II acceptor Q_A.

Introduction

Chlorophyll *a* luminescence is a result of the recombination between electrons on the acceptor side of PS II with positive charges on the donor side [1]. The intensity, yield and decay kinetics of luminescence is affected by a number of factors such as temperature, pH, intensity and quality of actinic light, presence of ions, redox components, electron-transport inhibitors, uncouplers, etc [2]. These factors either affect the concentration of luminescence substrate (i.e., positive charges on the donor side of PS II and electrons on the acceptor side), or the activation energy for these charges to recombine. Luminescence is also quenched by the same nonphotochemical quenching mechanisms as chlorophyll *a* fluorescence.

In contrast to luminescence, fluorescence is light emission from the photosynthetic apparatus observed when the actinic light is on. However, the primary origin of the two phenomena is the same [2], i.e., deactivation of excited chlorophyll *a* molecules mainly associated with PS II. Fluorescence has been widely used as an intrinsic probe for research concerning the photosynthetic apparatus in different applications. In recent years the use of new techniques employing low-intensity modulated excitation light together with stronger photosynthetically active light and flashes of saturating light have made it possible to discriminate between different fluorescence quenching mechanisms, i.e., quenching due to the redox

Abbreviations: PS II, Photosystem II; PS I, Photosystem I; Q_A, the primary PS II electron acceptor.

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state of Q_A (q_Q quenching) and quenching due to nonphotochemical processes (q_E quenching) [3].

In this paper a low-intensity modulated light was used to monitor the redox state of Q_A 'in darkness'. The fluorescence being a result of this light is called 'dark fluorescence', because the light intensity was low enough not to cause accumulation of closed reaction centers.

The object of the present study was to characterize the effects of changes in CO_2 concentration on the redox state of Q_A, in darkness, following illumination, using luminescence and dark fluorescence as probes. The results presented provide a basis for interpretation of luminescence and fluorescence from CO_2 accumulating, photosynthetic organisms.

Material and Methods

Green-house-grown leaves of 7–10-day-old barley were used throughout the experiments. The leaves were dark adapted for at least 1 h prior to use in experiments.

Both luminescence and fluorescence measurements were carried out in a cylindrical plexiglass cuvette (volume, 25 cm^3). Luminescence excitation light was provided by metal halogen lamps Atlas 24 V 250 W. The light was guided to the cuvette by optical fibers. For far-red excitation the light was filtered through a Scott RG 715 filter. The photon flux density was 2 and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for far-red and white light, respectively. Luminescence emission was detected by a photomultiplier (selected Hamamatsu R 374) connected to the cuvette by an optical fiber. The signal from the photomultiplier was amplified and recorded on a chart strip recorder.

Fluorescence was excited and detected by a modified Heinz-Walz fluorometer PAM 101 (Heinz Walz, F.R.G.). The weak ($1 \mu mol \cdot m^2 \cdot s^{-1}$) red, modulated (100 kHz) exciting light was pulsed to the leaves in darkness (1 s pulse every 10 s) to minimize the risk of the measuring light giving rise to accumulation of Q_A^-. The modulated and pulsed fluorescence signal was detected and amplified by the selective amplifier of the fluorometer. The signal was thereafter recorded on a chart strip recorder. To ensure that the measuring light not caused accumulation of Q_A^- in pure N_2 atmosphere after CO_2 removal, two 1 s pulses of modulated light were given to a sample before and after CO_2 removal. The stimulation of fluorescence thus obtained was the same as during continuous pulsing of the modulated light. Preillumination with white and far red light were the same as for luminescence excitation. The preillumination light was superimposed on the weak modulated light.

The different gas mixtures were flushed through the cuvette at a rate of $60 \, l \cdot h^{-1}$. Changing of gas mixture in darkness was made either by switching between different gas mixtures or by removing CO_2 from the flushing gas.

Results and Discussion

When CO_2 was removed from barley leaves following exposure to a gas mixture 95% N_2/5% CO_2 for 2.5 min in white light, a transient stimulation of luminescence was observed in a subsequent dark period (Fig. 1a). The stimulation of luminescence was parallel with a stimulation of dark fluorescence, indicating partial Q_A reduction as the cause for stimulation (Fig. 1b). Since Q_A was reduced to a high extent during the period of white light preillumination, the partial reduction of Q_A, as indicated by the transient increase in dark fluorescence upon removal of CO_2, occurred as an interruption of the dark decay of $[Q_A^-]$.

In the experiments illustrated in Fig. 2a and 2b the excitation sequence included 2 min white light followed by 30 s far-red light. Far-red, (PS I) excitation resulted as expected in a rapid oxidation of Q_A (c.f. Figs. 1b and 2b). When CO_2 was removed from the gas mixture in the dark after far red illumination, Q_A was reduced in a way similar to that obtained after only white light excitation. The 'reducing effect' caused by removed CO_2 was, however, more pronounced on dark fluorescence after far-red excitation, since the reduction was superimposed on a low steady-state level of dark fluorescence (Fig. 2b) and not as in Fig. 1b on dark fluorescence, decaying as a result of decreasing $[Q_A^-]$

The difference in response to removed CO_2 after white and far-red excitation was even more pronounced on luminescence, with a more marked stimulation after far-red excitation (Fig. 2a). The

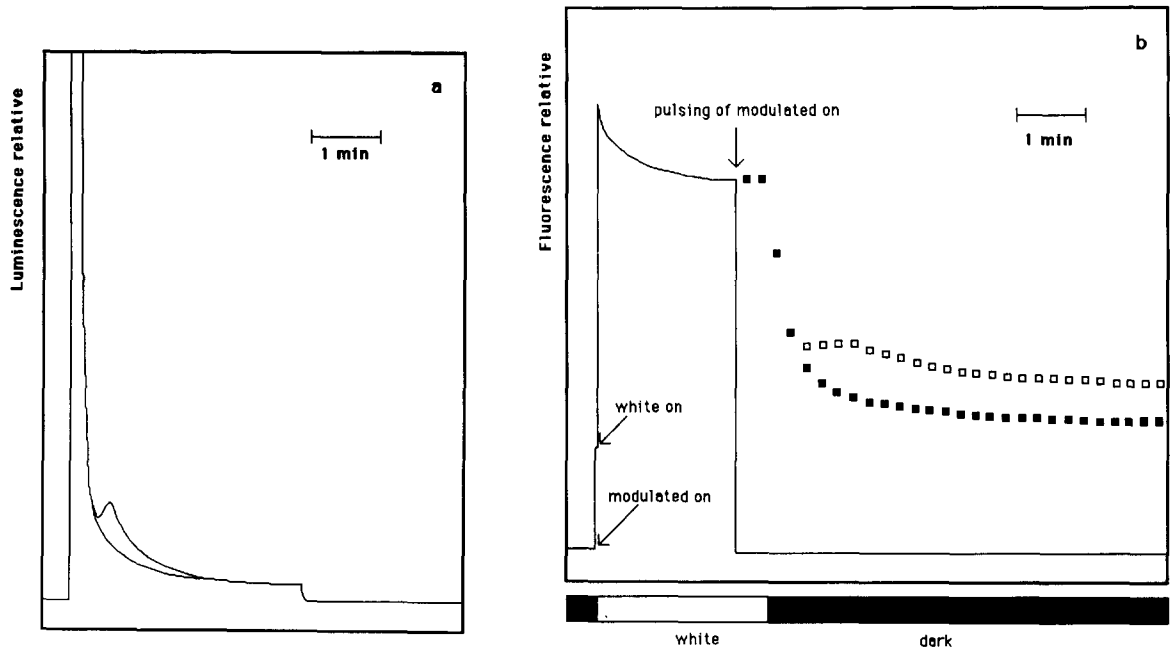

Fig. 1. (a) Luminescence decay from barley leaves excited with 2.5 min white light in a gas mixture of 95% N_2/5% CO_2. Upper curve (with the transient peak) indicates decay when CO_2 was removed from the gas mixture after 10 s in darkness. Lower curve (without the transient peak): control. Other experimental conditions given in the text. (b) Fluorescence and dark fluorescence from intact barley leaves illuminated with 2 min white light in a gas mixture of 95% N_2/5% CO_2. □, CO_2 removed from the gas mixture after 10 s in darkness; ■, control. The modulated fluorescence excitation light was first 'continous' but started pulsing from the time indicated by arrow. Other experimental conditions given in the text.

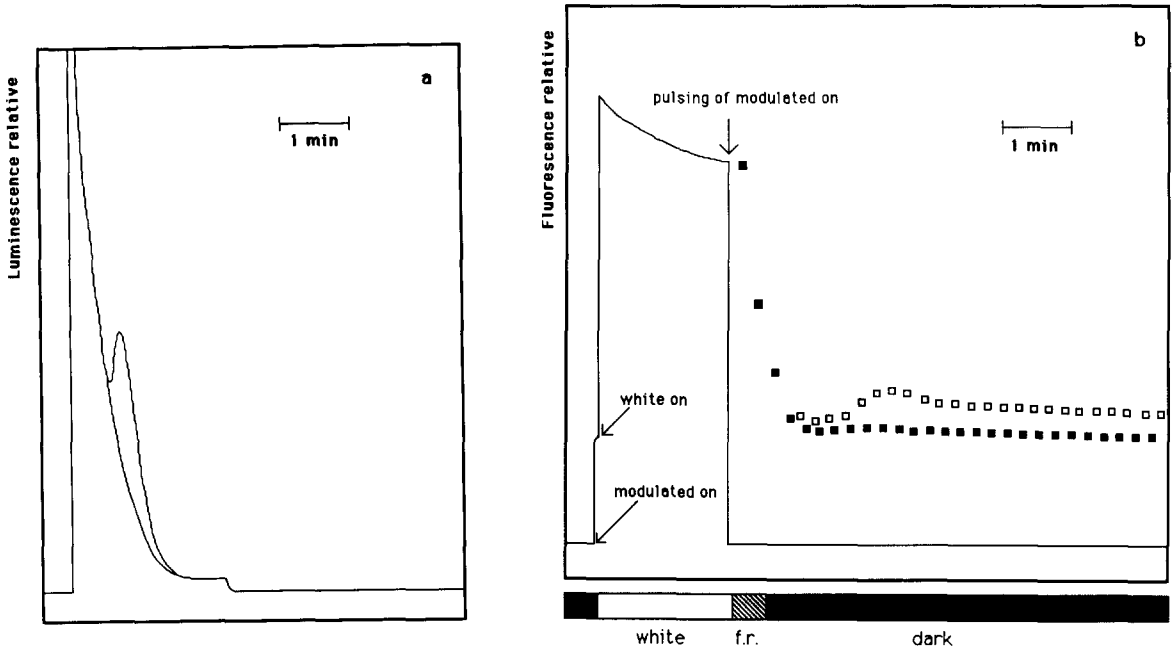

Fig. 2. (a) Luminescence decay from barley leaves excited with a sequence of 2 min white light and 0.5 min far-red light, in a gas mixture containing 95% N_2/5% CO_2. Upper curve (with the transient peak) indicates decay when CO_2 was removed from the gas mixture after 10 s in darkness; lower curve (without the transient peak), control. Other experimental conditions are given in the text. (b) Fluorescence and dark fluorescence from intact barley leaves illuminated with a sequence of 2 min white and 0.5 min far-red light in a gas mixture of 95% N_2/5% CO_2. □, CO_2 removed from the gas mixture after 10 s in darkness; □, control. The modulated fluorescence excitation light was first 'continous' but started pulsing from the time indicated by arrow. Other experimental conditions are given in the text.

difference in luminescence response after white and far-red excitation was probably caused by different access to positive charges on the donor side of PS II at the time of removed CO_2. After some time in darkness following white light excitation the substrate-limiting luminescence is most likely positive charges [4]. After far-red excitation the positive charges of the PS II donor side has a higher probability to remain for some time in the dark due to limited access to electrons for recombination from the oxidized PS II acceptor side, as a consequence of the dominating PS I excitation. A reduction of Q_A (as a results of lowered CO_2) will therefore have a more pronounced effect on luminescence after far-red excitation, since electrons likely are more limiting as luminescence substrate than after white light excitation. From the control curves in Figs. 1a and 2a it is also evident that far-red illumination even without change in gas phase shifts the proportion of light emitted in early vs. late stages of the decay, towards more emission in the later phases.

In the experiments shown in Fig. 3 the shift in CO_2 concentration was made at the peak of the relative maximum during the decay of luminescence that is observed from most higher plants as

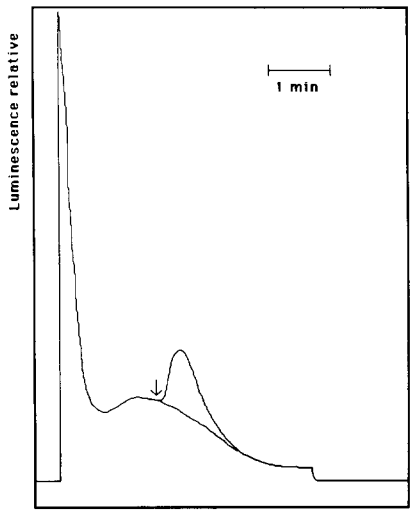

Fig. 3. Luminescence decay from barley leaves excited with a sequence of 2 min white and 0.5 min far-red light in air containing 2% CO_2. For the upper curve with two transient peaks) CO_2 concentration was lowered to 350 ppm as indicated by the arrow; lower curve (with one transient peak), control. Other experimental conditions given in the text.

well as from green algae when luminescence is excited with far red light in the presence of O_2 [5–9]. The biophysical and biochemical origin of this relative maximum is not known, although it has been shown to include reversed electron flow between the two photosystems and also to be sensitive to uncouplers [5,6,10]. As evident from the figure the stimulation caused by lowered CO_2 concentration was superimposed on the 'far-red induced relative maximum' as a separate peak. The far-red induced relative maximum as seen in the control curve of Fig. 3 was not related to reduction of Q_A (not shown). It is therefore concluded that the peak in luminescence observed after far-red excitation in the presence of O_2 has a different mechanistic origin compared to the peak caused by lowered CO_2 concentration. In this context it should be noted, however, that very small changes in the redox state of Q_A might be hard to detect with the present measuring system.

Several tentative explanations are possible in order to explain the mechanistic background to the stimulation of luminescence (and dark fluorescence) by lowered CO_2 concentrations. Here three possibilities are considered.

(1) Since luminescence was stimulated also when CO_2 concentration was lowered in a concentration range well above that for saturated photosynthesis (Fig. 4), it can be stated that the reduction of Q_A as a consequence of lowered CO_2 concentration is a phenomenon not directly related to photosynthesis by postillumination CO_2 uptake [11] and the role of CO_2 as a terminal electron acceptor in photosynthesis.

(2) Since carbonic anhydrase is present in chloroplasts of barley cells, as well as in chloroplasts from a large variety of photosynthetic organisms [12], a shift in CO_2 concentration will rapidly affect the concentration of HCO_3 and H^+ in the chloroplasts according to

$$CO_2 + H_2O \leftarrow^{CA} \rightarrow HCO_3^- + H^+$$

A decrease in external CO_2 concentration might therefore increase the pH of the stroma and give rise to a transthylakoid ΔpH. On the membrane level a lowered CO_2 concentrations would according to this hypothesis stimulate luminescence in the same way as an acid/base transition, i.e.,

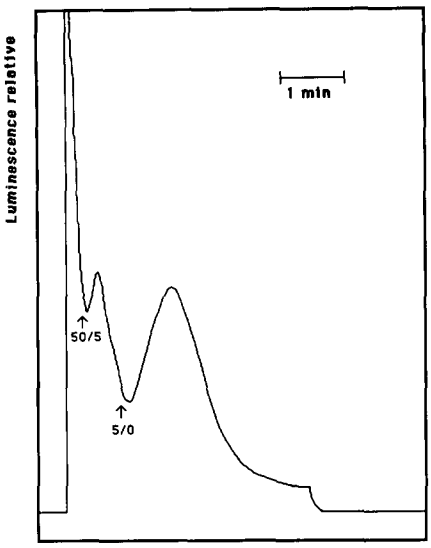

Fig. 4. Luminescence decay from barley leaves excited with a sequence of 2 min white and 0.5 min far-red light in a gas mixture initially containing 50% N_2/50% CO_2. As indicated by arrows CO_2 concentration was first lowered to 5% and there after to 0%. Other experimental conditions were as given in the text.

through lowered activation energy for PS II recombinations by an imposed ΔpH.

(3) Another possibility is that CO_2 concentration affects the equilibrium in a redox reaction

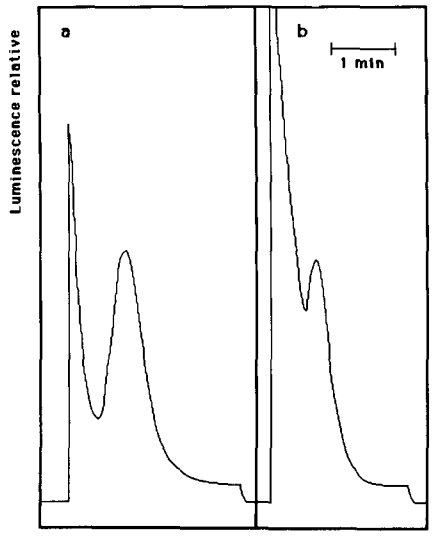

Fig. 5. Luminescence decay from barley leaves excited with a sequence of 2 min white and 0.5 min far red light in a gas mixture containing (a) 95% O_2, 5% CO_2, (b) 95% N_2, 5% CO_2. CO_2 were removed from the gas mixture after 10 s in darkness. Other experimental conditions given in the text.

(directly or through pH), and in this way the redox state of the cell and/or chloroplast, thereby providing electrons for reversed electron flow in the chloroplast. One candidate for such a reaction is the HCO_3^--sensitive decarboxylation of glycine to serine with the concomitant reduction of NAD^+ to NADH [13]. Support for this explanation comes from the result shown in Fig. 5 where luminescence was stimulated by lowering of CO_2 concentration in aerobic and anaerobic atmospheres. Considering the role of glycine in photorespiration, the more pronounced response in luminescence upon lowered CO_2 concentration in O_2 could be interpreted as more NADH being produced at lowered CO_2 concentration under photorespiratory conditions. However, it should also be taken into account that the presence/absence of O_2 might affect the redox state of both the donor and the acceptor side of PS II, making interpretation of luminescence difficult.

At present there is no conclusive evidence favouring either of the last two mechanisms suggested.

Whatever the mechanism for Q_A reduction by lowered CO_2 is, the results presented in this paper contain basic information that provides a basis for the understanding of fluorescence and luminescence kinetics from CO_2-accumulating, photosynthetic organisms. Correctly interpreted such kinetics should contribute to the understanding of the mechanisms of adaptation to low CO_2 conditions, but potentially also be useful in more applied research concerning CO_2 accumulation.

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