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IN VIVO STUDIES OF GROSS PHOTOSYNTHESIS IN ATTACHED LEAVES BY MEANS OF PHOTOTHERMAL RADIOMETRY

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In photothermal radiometry, heat radiation from an illuminated object, in synchronism with incident chopped light, is observed using an infrared detector with suitable electronics. By thus measuring the heat released during pulse-wise irradiation of leaves, conclusions can be drawn as to the gross efficiency of photosynthesis: More heat means less photochemically stored energy. Saturation of photosynthesis, by employing additional strong continuous-wave background light, affords an internal photothermal radiometry signal reference corresponding to the photochemical zero efficiency level, against which the signal in the absence of saturation can be compared. Through such means, gross energy storage efficiencies approaching 30% have been observed in *Caragana arborescens Lam.* at low light intensities. Several other examples are given, including measurements on dark-adapted leaves and leaves infiltrated with 3-(3,4-dichlorophenyl)-1,1-dimethylurea, to support our conclusion that photothermal radiometry provides a powerful new method for in vivo studies of photosynthesis in whole, attached leaves.

Introduction

Light energy absorbed in photosynthetic organisms becomes channeled into chemical storage, fluorescence (equal to or less than 5%) and (chiefly) heat release. Photochemical energy storage thus may be analysed by measurements on the competing heat dissipation, as was recently demonstrated using photoacoustic techniques [1–5]. In this communication we show that more direct studies of photochemical energy fixation can be made through photothermal radiometry, whereby temperature variations in the sample surface due to modulated illumination are monitored through the related oscillations in thermal (infrared) reradiation [6–8]. We have observed photothermal radiometry signals from Siberian pea bush (*Caragana arborescens Lam.*) irradiated at 687 nm. Signal magnitudes at all modulation frequencies (15–120 Hz) increased upon addition of saturating continuous-wave (i.e., non-modulated) background light. These studies give important information on the gross photochemical energy storage and its variation under different conditions. The photothermal radiometry technique also avoids interference from modulated gas evolution (as in low-frequency photoacoustic techniques), thereby resolving questions as to the interpretation of photoacoustic measurements.

Experimental Procedure

Our experimental setup shown in Fig. 1 essentially consisted of two light sources, a green leaf

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

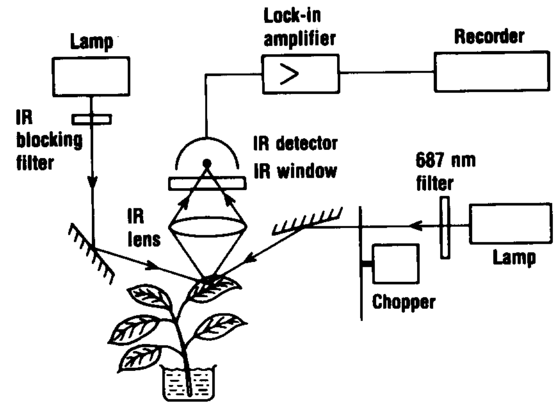

Fig. 1. Experimental setup for the investigation of *C. arboréscens Lam.* by photothermal radiometry. The infrared (IR) detector was a liquid N_2-cooled PbSnTe photovoltaic diode, with a preamplifier optimised for low $1/f$ noise, and conventional slide projectors were used for illumination. Lock-in amplifier time constant normally was $\tau = 3$ s.

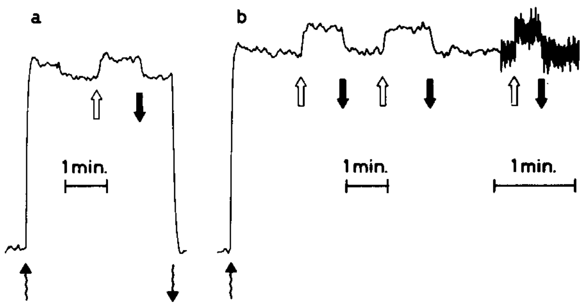

Fig. 2. Photothermal radiometry signals from two attached leaves with and without strong background illumination. Curved arrows indicate on/off of modulated red light, white and black arrows designate on/off of background white light. Signal differences between the two leaves typify the variations that were experienced from leaf to leaf. Trace a shows a leaf on a sprig picked at night shortly before measurement and dark adapted for 15 min in the laboratory, while the leaf in trace b, picked in daytime, had been kept under ambient laboratory lighting conditions before measurements; even this is seen to lead to a weak transient. In the final trace of b, the lock-in amplifier time constant was $\tau = 0.3$ s. Signal levels were approx. 110 μV.

sample and a PbSnTe infrared detector optimised for room temperature (10 μm wavelength) thermal radiation. In the basic measurement, red-filtered light ($\lambda = 687$ nm, half-width $\Delta\lambda = 20$ nm) was mechanically chopped at 32 Hz and 50% duty cycle and was focused onto Siberian pea bush leaves. Average light intensity was kept below 40 W/m^2 in order not to saturate the photochemical processes, unless otherwise stated. An $f/1$ BaF_2 lens doublet collected released heat radiation from the leaf onto the PbSnTe detector, whose Ge window ensured that scattered illuminating radiation did not interfere with the photothermal signal. The preamplified infrared detector output was further processed by a vector-mode lock-in amplifier and recorded vs. time. Whenever appropriate, additional strong continuous-wave 'white' light (cold filtered, $\lambda \leq 900$ nm) from a second light source was also used, and photothermal radiometry signal differences with and without such background illumination were recorded. The experiments were carried out with attached leaves on freshly picked sprigs placed in a glass of water. Prolonged measurements could be performed on the same leaf without noticeable changes in the results.

Results and Discussion

Fig. 2a shows the photothermal radiometry signals obtained from a dark-adapted leaf. The signal is seen initially to rise strongly; however, in the course of approx. 2 min it steadily decreases to settle at a constant level. In Fig. 2a and b is also shown the effect of the continuous-wave background illumination, viz., to increase the photothermal radiometry signal from the leaf. Such behavior contrasts with photoacoustic records at low frequencies, in which similar transient signals were observed to increase after dark adaption [1–5], whereas background illumination was found to reduce the photoacoustic signals [1–4]. Our photothermal radiometry records, however, are qualitatively similar to photoacoustic measurements at high pulse frequencies [1–4], and could be consistently reproduced within the region of modulation frequencies that gave useful signal-to-noise ratios (15–120 Hz). This provides direct evidence that, upon illumination, progressively less optical energy becomes thermalized until a steady state is reached. The photothermal radiometry transients from dark-adapted leaves thus reflect simply the induction period of photosynthesis, during which the photochemical processes become established and more light energy, initially wasted as heat, becomes consumed in photosynthesis. This interpretation supports the conclusions by Cahen and co-workers [1–4] that photochemically produced oxygen pulsations accompany thermal oscillations at low frequencies; in contrast to photoacoustic techniques, contact-free photothermal radiometry measurements remain undisturbed by such gas release. The conjecture by Inoue et al. [5] that transient photoacoustic signals at 8.3 Hz illumination could be ascribed to light-induced changes in thermal parameters of the leaf therefore appears untenable.

Our interpretation is further supported by observing that the photothermal radiometry signal at the start of the initial transient equals that obtained during additional white light illumination (Fig. 2a). Being unmodulated, the background illumination does not contribute directly to the photothermal radiometry signal. It serves, however, to saturate photosynthesis whereby the photochemical quantum yield tends to zero (cf. below); the corresponding photothermal radiometry signal due to the weak pulsating red light thereby provides an in situ photochemical zero level reference [1–4,9]. Immediately after switching on the modulated light, therefore, the photochemical processes are seen from Fig. 2a to be working at practically zero efficiency. It should be remarked here that the trace shown in Fig. 2a is typical of leaves picked at night. With 'daytime' leaves dark adapted for 10–15 min only, the initial peak of the photothermal radiometry transient might reach only halfway between the sample signal and the saturated reference level. This suggests that complete dark adaptation may take several hours, complementing similar long-term induction phenomena in illumination [10]. The final trace in Fig. 2b also demonstrates that the addition of strong background illumination instantly, i.e., within the time constant of the lock-in amplifier, saturates the photochemical processes, whereas recovery is seen to take a few seconds. That delay may be related to the pool size capacity of the particular bottleneck in the photosynthesis chain; however, no systematic measurements of recovery times were attempted.

By keeping the average modulated red light intensity I_{mod} constant at 35.5 W/m^2 and varying the background light intensity I_{cw}, the approach to saturation could be studied in detail. Fig. 3a shows a plot against I_{cw} of $\Delta PTR = (PTR_{cw} - PTR_{mod})/PTR_{cw}$, i.e., the relative difference between photothermal (PTR) signal magnitudes with and without background illumination. Complete saturation is seen to require illumination in excess of 250 W/m^2; correspondingly, ΔPTR reaches a maximum of 12–13%. With $I_{cw} > 250$ W/m^2, the photochemically saturated leaf behaved as a passive absorber with respect to the modulated light, and a linear relation between PTR_{cw} and I_{mod} resulted, as shown in the lower trace of Fig. 3b. Recent experiments comparing photothermal radiometry measurements with photoacoustic results (Kanstad, Cahen and Malkin, unpublished observations) and, similarly, relating the photoacoustic response with fluorescence [4], show that the changes in photothermal radiometry signals due to background illumination are similar to the corresponding changes seen in the fluroescence yield; in both cases saturation of photosynthesis is observed

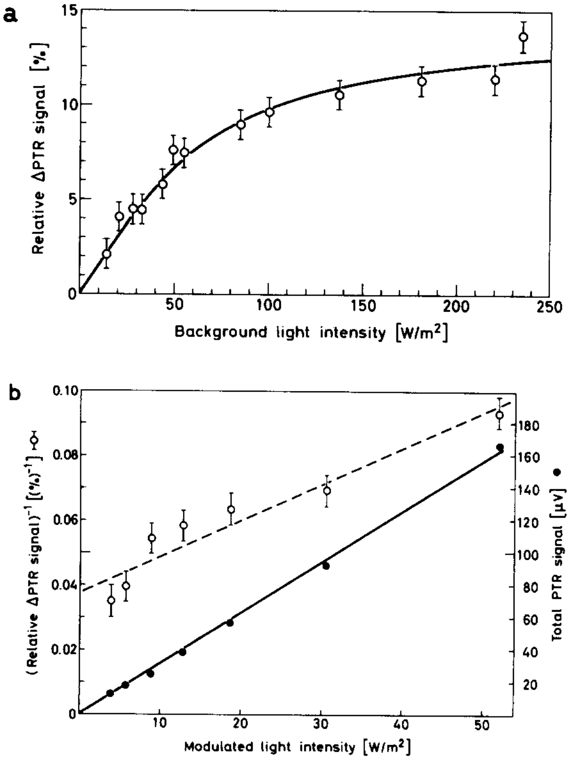

Fig. 3. Variation of photothermal radiometry signals with light intensities. Trace a shows the saturation of ΔPTR vs. I_{cw} at $I_{mod} = 35.5$ W/m^2. In b, the upper trace (○) shows the inverse of ΔPTR and the lower trace (●) the saturated photothermal radiometry signal PTR_{cw}, both as functions of I_{mod}.

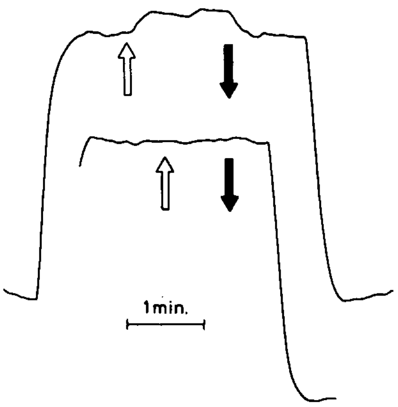

Fig. 4. Effect of background illumination on photothermal radiometry signals from two different leaves infiltrated under vacuum with water (upper trace) and with a 39 μM aqueous solution of DCMU (lower trace). Signal levels were approx. 100 μV, and the lock-in amplifier time constant was $\tau = 10$ s.

to increase the energy losses from the photosystem. Moreover, PTR_{cw} at saturation equals the afore mentioned photochemical zero level signal, whereas PTR_{mod} depends on the plant's photosynthetic efficiency at the particular I_{mod} and pulse frequency. Neglecting fluorescence, ΔPTR then equals the corresponding photosynthetic energy storage efficiency, i.e., that fraction of the absorbed optical energy that goes into photosynthesis in each cycle. Theory [11] predicts a hyperbolic dependence on I_{mod}; a plot of $(\Delta PTR)^{-1}$ vs. I_{mod} thus gave the straight line shown in the upper trace of Fig. 3b. The maximum energy storage efficiency at 32 Hz is seen to have been approx. 27% at zero light intensity, while for stronger illumination the photochemical processes become less efficient, since that brings them closer to saturation. With fixed intensitites in both beams, the energy storage efficiency was also measured as a function of pulse frequency. This produced an essentially flat curve in the range 15–120 Hz, indicating that rate constants for intermediates lie outside the interval 1.3–11 ms.

To make sure that the above results were not due to spurious effects, a leaf infiltrated with water (under vacuum) and another similarly infiltrated with a 39 μM aqueous DCMU solution were also investigated. The results presented in Fig. 4 clearly support the conclusion that photosynthetic action has been observed above: no effect of the background light was seen in DCMU-infiltrated leaves nor in any other ordinary passive samples.

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