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REVIEWS

THERMAL DISSIPATION RELATED TO CHLOROPHYLL FLUORESCENCE AND PHOTOSYNTHESIS*

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Summary. Energy taken up by light absorption of the leaf pigments is transformed in the process of the photosynthetic quantum conversion into photosynthesis, chlorophyll fluorescence and thermal dissipation. The mostly inverse correlation between chlorophyll fluorescence and photosynthetic activity has been extensively studied in the past, whereas only few studies dealt with the thermal dissipation. In many publications the intensity of thermal dissipation is a matter of speculation deduced from a lower chlorophyll fluorescence and expressed as non-photochemical quenching or rate constant for thermal decay. This review shows that by means of the photoacoustic technique one is able to really measure thermal dissipation. The principle of this method is outlined and examples are given for measurements of thermal photoacoustic signals of leaves related to chlorophyll fluorescence and photosynthesis. The various studies show that thermal dissipation is neither neglectibly low, nor constant or always parallel to chlorophyll fluorescence. Thus it is clearly demonstrated that thermal dissipation should always be measured in order to fully understand the energy balance in the photosynthetic quantum conversion.

Key words: non-photochemical quenching, photoacoustics, photothermics

Abbreviations: Chl – chlorophyll, PA – photoacoustic, PAM – pulse amplitude modulation

Introduction

The energy taken up by absorption of light by the leaf pigments is mainly used up for photosynthesis. A smaller part of this absorbed energy is, however, transformed

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into chlorophyll (Chl) fluorescence and thermal dissipation (i. e. heat). Light absorption by the leaf pigments can be assumed to be constant since within the minutes of measurement of photosynthesis, Chl fluorescence or heat biosynthesis or metabolism of pigments does not change their concentration in significant amounts. The efficiency of a photochemical process can be expressed as quantum yield (= photochemical effect per absorbed quantum) which is a value between 0 and 1 (for a classical review, see Butler 1977). In a photosynthetic system the quantum yields of photosynthesis (F_P), Chl fluorescence (F_F) and thermal dissipation (F_D) must result in a sum equal to 1. Under optimum conditions Kitajima and Butler (1975) gave: $F_P = 0.78$, $F_D = 0.20$, $F_F = 0.02$ which means that 78% of the absorbed energy is converted into photosynthesis, 20% into heat and 2% into Chl fluorescence. The increase in photosynthetic activity must be paralleled by a decrease of Chl fluorescence and heat and *vice versa*.

Most of the present studies on the induction changes of Chl fluorescence assume that Chl fluorescence alone can be taken as an inverse measure of photosynthetic activity assuming that thermal dissipation parallels Chl fluorescence. Many publications claim to give results on thermal dissipation without measuring it by simply deducing thermal dissipation from a lack of Chl fluorescence, e. g. by calculating the non-photochemical quenching (first defined by Bilger and Schreiber, 1986) or the rate constant for the thermal decay (Demmig-Adams et al., 1989). In this review it is outlined that it is really necessary to measure thermal dissipation in order to fully understand the energy balance of photosynthesis, Chl fluorescence and heat.

Measuring thermal dissipation

Thermal dissipation of a variety of samples has been measured by the photoacoustic (PA) technique which was applied again since the early 1970s after its first discovery by Alexander Graham Bell in 1881. The sample inside a tightly closed measuring chamber is illuminated by amplitude modulated (= pulsed) light via a transparent glass. In case that the light is absorbed by the sample thermal dissipation leads to the heating of the gas surrounding the sample. Heating the gas leads to an increased pressure inside the tightly closed measuring chamber (the “photoacoustic cell”). If the modulation frequency of the illuminating light is chosen within the audible range, pressure changes can be measured by a microphone (for more details see: Buschmann and Prehn, 1990). The PA signal is stored or recorded after specifically amplifying by means of a lock-in amplifier only those microphone signals which were detected with the frequency of the modulated light. This excludes (in a similar way as in PAM-measurements) the contribution of continuous, non-modulated signals or signals with other frequencies than the preset modulation frequency of the light illuminating the sample. There are other techniques (see reviews: Tam, 1986; Buschmann and Prehn, 1990) which enable to measure thermal dissipation e. g. by infrared detectors (for leaves: Bults et al., 1982b; Kanstad et al., 1983; Malkin et al., 1991), by detecting the beam deflec-

tion passing through the heated surrounding of the sample ("Mirage effect", for leaves: Havaux et al., 1990; Sinclair and Hall, 1995) or by "open cells" (de Paula et al., 1997). Compared to the other techniques applied for the detection of the thermal dissipation PA measurements have the highest sensitivity.

Excitation spectra

As in fluorescence, the PA signals can be taken as an excitation spectra by illuminating the sample with monochromatic light scanned within a given spectral range. In this way the absorption characteristics of the samples can be obtained with the unique possibility of "depth profiling" the sample (see below). Taking PA spectra of leaves has the advantage that the PA signal (in contrast to fluorescence signal) is not influenced by re-absorption or light scattering. Thus PA spectra are particularly suitable to characterize the pigment composition of leaves (Buschmann and Prehn, 1981, 1983; Szigeti et al., 1989) or even needles (Nagel et al., 1987) and fruits (Kocsányi et al., 1988).

Depth profiling

The PA signal decreases with increasing modulation frequency of the light illuminating the sample (Fig. 1). By changing the modulation frequency one is able to assess the distribution of absorbing substances in different depths of the sample ("depth profiling", for leaves: e. g. Buschmann and Prehn, 1983). When using a low modulation frequency the PA signal is sensed also from deeper below the sample surface than with high modulation light. The exact depth from where a signal can be calculated as thermally active layer which depends on the thermal properties of the sample. In water (which can be assumed to have thermal properties similar to leaves) at 10 Hz the PA signal is detected from a depth of about 500 µm, whereas at 400 Hz the depth is restricted to ca. 70 µm (Fig. 1).

Components of the photoacoustic signal of a leaf

The PA signal measured at a modulation frequency above ca. 150 Hz reflects only the thermal dissipation. An induction kinetic can be observed which resembles that of the Chl fluorescence (Fig. 2). But, when measuring intact leaves with lower modulation frequencies the induction kinetic of the PA signal is different, as was first shown by Inoue et al. (1979). Under these conditions the pressure changes detected by the microphone of the PA cell are dominated by a "photobaric" component which today is mostly explained as photosynthetic oxygen evolution (Bults et al., 1982a) although there are other possible explanations (personal communication: U. Haas, University

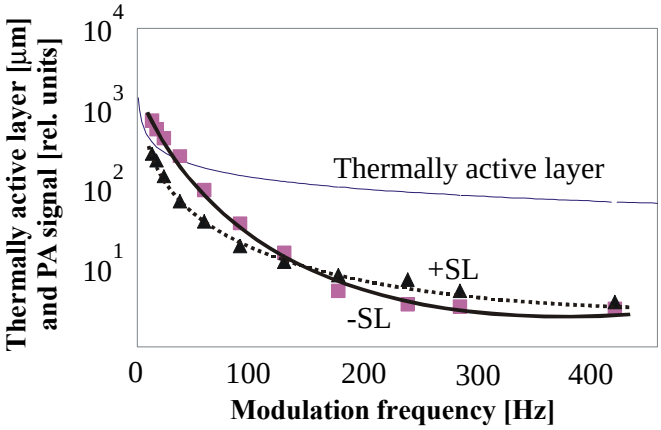

Fig. 1. Dependence on the modulation frequency of the light illuminating the sample during a photoacoustic (PA) measurement: Thermally active layer of water in µm (thin continuous line) calculated with the data of the thermal properties of water which should be close to those of an intact leaf (usually 60 to 80% water content, see Buschmann and Prehn, 1990), Photoacoustic signal of a radish cotyledo (*Raphanus sativus* L.) in relative units without (-SL, squares with broad continuous line) and with (+SL, triangles with broad dotted line) continuous, non-modulated white light saturating photosynthesis (SL, "background light": 2000 µmol.m^{-2}.s^{-1}) (data taken from Buschmann, 1987).

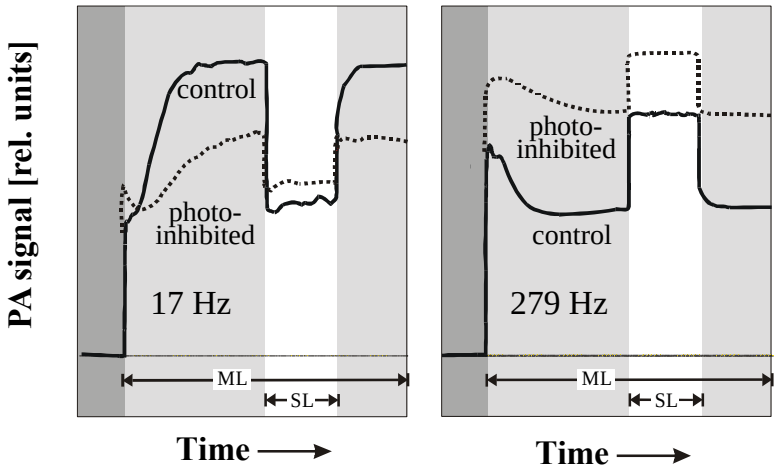

Fig. 2. Induction kinetic of the photoacoustic (PA) signal of a radish cotyledo (*Raphanus sativus* L.) taken with a red measuring light at a modulation frequency of 17 Hz (left) and 279 Hz (right) before (control) and after a photoinhibitory treatment (15 min white light of 600 W.m^{-2}). Before the measurement the sample was dark-adapted for 15 min. 5 min after onset of illumination with modulated measuring light (ML) continuous, non-modulated white light saturating photosynthesis (SL, "background light": 2000 µmol.m^{-2}.s^{-1}) was applied during 2 min (data taken from Buschmann, 1987).

of Hohenheim). The photobaric component appears only at low modulation frequencies because one assumes that at higher frequencies the oxygen evolution becomes continuous. Since the lock-in amplifier does not amplify non-modulated signals, this continuous oxygen evolution then can not contribute to the PA signal measured with high frequencies. PA signals under certain particular conditions have also been interpreted in terms of oxygen uptake, e. g. by Mehler-reaction (Kolbowski et al., 1990; Mauzerall 1990), CO_2-solubilisation by carbonic anhydrase (Reising and Schreiber, 1994) and molecular volume changes of the reaction centre (Ort and Parson 1979; Delosme et al., 1994).

Measuring exclusively the thermal dissipation of a leaf

The high contribution of the photobaric component to the PA signal measured at low frequencies can be suppressed when measuring during additional illumination with

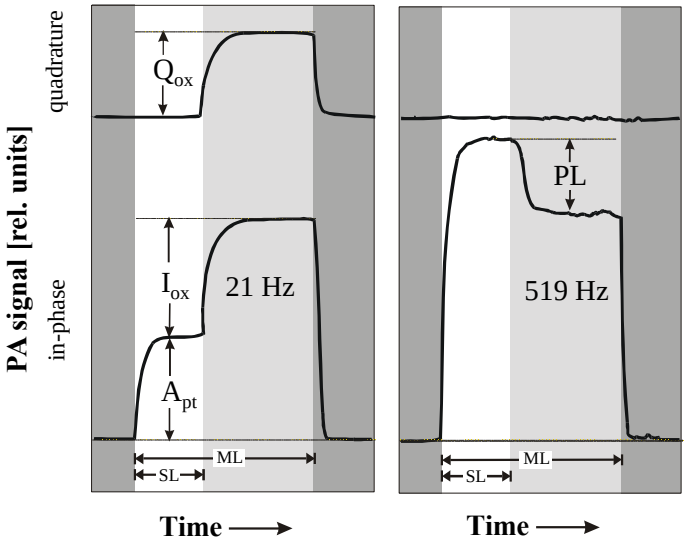

Fig. 3. Photoacoustic (PA) signal of a leaf of *Senecio vulgaris* L. illuminated by a blue-green measuring light (ML) modulated with a frequency of 21 Hz (left) and 519 Hz (right) (taken from Havaux, 1989b). Continuous, non-modulated light saturating photosynthesis (SL, „background light“: 2700 µmol.m^{-2}.s^{-1}) was applied from the onset of illumination and turned off after about one minute. Upper part: PA signal with the lock-in amplifier out-of-phase (“quadrature” component), Lower part: PA signal with the lock-in amplifier in-phase (“in-phase” component). Q_{ox} – quadrature “oxygen” PA signal, I_{ox} – in-phase “oxygen” PA signal, A_{pt} – in-phase photothermal PA signal, PL – photochemical loss (for definition see Table 1).

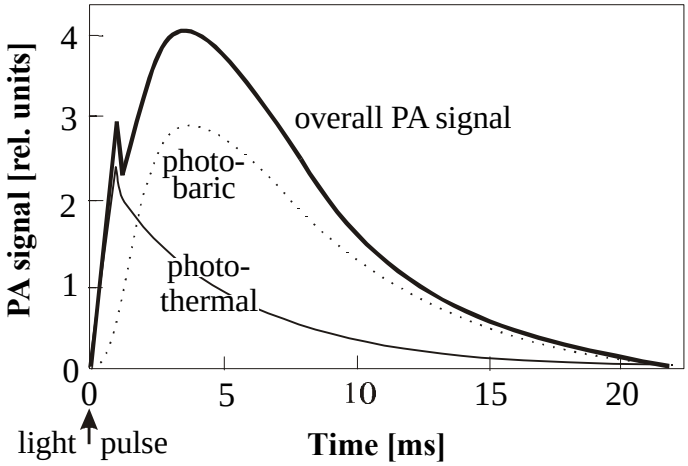

Fig. 4. Photoacoustic (PA) signal of a tobacco leaf (*Nicotiana tabacum* L.) in the millisecond time domain after a 0.72 ms light pulse (light-emitting-diode: peak wavelength 650 nm) applied at time = 0. The photothermal component (thin continuous line) was deduced from a separate measurement with continuous, non-modulated light saturating photosynthesis and normalized to fit the overall PA signal (thick continuous line). The photobaric component (thin dotted line) was calculated by subtracting the deduced photothermal signal from the overall PA signal (taken from Kolbowski et al., 1990).

a continuous (non-modulated) strong light saturating photosynthesis ("background light"). In this case, one assumes that the oxygen evolution is continuous and does no longer contribute to the amplified PA signal. Saturating light applied during the measurement at low modulation frequencies leads to a decrease of the PA signal due to the damped photobaric component (Figs. 1 and 2). During the measurement at high modulation frequency, saturating light induces an increase of the PA signal due to the fact that energy of the modulated light is then transferred only into heat and Chl fluorescence since the continuous strong light saturates the photochemical route (Figs. 1 and 2). Another possibility to exclude the photobaric component is to measure "out of phase" ("quadrature" signal with a phase shift of 90°), i. e. by keeping the time of signal amplification between the pulses (Fig. 3). The extraction of the thermal dissipation from the PA signal has been carried out by Kolbowski et al. (1990) by a mathematical treatment of the PA signal kinetic measured in the time domain. The kinetic of pressure changes in the millisecond range after a short light pulse (overall PA signal) are subtracted by the same kinetics measured with continuous saturating light (photothermal component) and thus the photobaric component can be deduced from the overall signal (Fig. 4).

Thermal dissipation and photosynthesis

Several reviews on the theory and application of the PA technique in photosynthesis research have been given in the past (Malkin and Cahen, 1979; Braslawsky, 1986; Buschmann, 1989, 1990; Buschmann and Prehn, 1990; Fork and Herbert, 1993; Malkin and Canaani, 1994; Malkin, 1996). PA signals mostly have been measured with leaves, but in some cases also with isolated chloroplasts (Dienstbier et al., 1984), isolated thylakoid particles (Vacek et al., 1979; Lasser-Ross et al., 1980; Dienstbier et al., 1984; Carpentier et al., 1985, 1987, 1991; Herbert et al., 1990; Lapointe et al., 1993; Allakhverdiev et al., 1994; Velitchkova and Carpentier, 1994) and artificial model systems (Frackowiak and Ptak, 1994).

Thermal signal parallel to Chl fluorescence

The signal of thermal dissipation may be, at least in tendency, parallel to the signal of Chl fluorescence and antiparallel to the photosynthetic activity, e. g. in the induction kinetics (Fig. 2) (Buschmann, 1987; Gruszecki et al., 1994; Buschmann, 1999). Both for the Chl fluorescence and the thermal signal there is (a) a decrease during the induction of photosynthesis, (b) an increase upon addition of saturating light, (c) a reduced increase upon saturating light when increasing the quantum fluence rate of the actinic light, (d) an increase of the signal achieved with saturating light during dark-recovery subsequent to an induction of photosynthesis. Under special conditions parallel oscillation of the Chl fluorescence and of the thermal PA signal were found (Buschmann, 1995). The antiparallel behaviour of the thermal dissipation and the photobaric photosynthetic oxygen evolution could be demonstrated by measuring the thermal dissipation via the high-frequency PA signal and the oxygen evolution via the low-frequency PA signal (Snel et al., 1990).

Photothermal signal not parallel to Chl fluorescence

In some cases an increase of thermal dissipation goes along with a decrease of Chl fluorescence and photosynthetic activity, e. g. during photoinhibition (Buschmann, 1987; Havaux, 1989a). The increase of the thermal signal during photoinhibition was expected from earlier studies of Chl fluorescence, but the (still widely used) prediction that non-photochemical quenching deduced from Chl fluorescence measurements and that zeaxanthin formation during high light treatment would clearly result in an increase of thermal signal could not be fulfilled (Buschmann and Kocsányi, 1989; Dau and Hansen, 1990; Havaux, 1990; Gruszecki et al., 1996; Havaux and Tardy,

Table 1. Parameters of photoacoustic signals of leaves characterizing the photosynthetic activity. SL = (non-modulated) saturating light

Name of parameter	Abbre-viation	Definition	Reference
A) PA measurement at low modulation frequency			
photothermal signal	A_{PT}	in-phase signal with SL	Poulet et al., 1983
in-phase O_2 signal	I_{OX}	in-phase signal, difference between + and - SL	Poulet et al., 1983
out-of-phase O_2 signal ("quadrature" O_2 signal)	Q_{OX}	out-of-phase signal with SL	Poulet et al., 1983
A) PA measurement at high modulation frequency			
photochemical loss	PL	in-phase signal, difference between + and - SL	Malkin and Cahen, 1979
energy storage	ES	in-phase signal, difference between + and - SL divided by signal without SL times 100	Herbert et al., 1990
quenching coefficient photothermal emission	qPH	in-phase signal, difference between + and - SL divided by signal with SL	Carpentier et al., 1985
decrease ratio	Rd(P)	in-phase signal, difference between maximum and steady state of an induction kinetic divided by the steady state signal	Buschmann, 1999

1997). An increase of the PA thermal signal parallel to a constant Chl fluorescence has been observed at the end of the dark-recovery phase subsequent to a photosynthetic induction (Buschmann, 1999).

Photoacoustic parameters characterizing photosynthetic activity

Several parameters have been proposed to deduce photosynthetic activity from the PA signal (Tabl. 1). The amplitude of the pure thermal PA signal A_{PT} is detected by measuring with saturating light at low modulation frequency with the phase of the lock-in amplifier adjusted to a maximum amplitude (in phase signal, Fig. 3). When switching off the saturating light the photobaric oxygen signal appears as the in phase signal I_{ox} and also with a phase shift of 90° as the quadrature signal Q_{ox} (Fig. 3). The photochemical loss PL has been deduced from the difference of the PA signal at high modulation frequency without and with saturating light (Fig. 3). In a similar way the

energy storage ES was defined as being 100 times the photochemical loss PL divided by the signal without saturating light. By analogy with the photochemical quenching of Chl fluorescence a photochemical quenching of the thermal emission qPH has been defined as the difference between the PA signal without and with saturating light divided by the signal with saturating light. By comparing the qPH with the corresponding value for the Chl fluorescence one concluded that the qPH value contains a contribution of a cyclic electron transport (Markovic and Carpentier, 1995). By analogy with the Rfd-value for Chl fluorescence a decrease ratio Rd(P) has been defined for the thermal PA signal as the difference between the maximum and the steady state of an induction kinetic divided by the steady state signal.

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