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Thermal dissipation during photosynthetic induction and subsequent dark recovery as measured by photoacoustic signals

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Abstract

The thermal photoacoustic signal (279 Hz) and the chlorophyll (Chl) fluorescence of radish cotyledons (*Raphanus sativus* L.) were measured simultaneously. The signals were recorded during a photosynthetic induction with actinic radiation of different quantum fluence rates [20, 200, and 1200 µmol(PAR-quantum) m^{-2} s^{-1}]. The rise of these signals upon irradiation saturating photosynthesis was followed in the steady state of the induction and during the subsequent dark-recovery (*i.e.*, in dark periods of 1, 5, 15, and 45 min after the induction). From these values various parameters (*e.g.*, quantum yield, photochemical loss, different types of quenching coefficients) were calculated. The results show that heat dissipation detected by photoacoustic measurements is neither low, constant, nor always parallel to Chl fluorescence. Therefore, the thermal signal should always be measured in order to fully understand the way leaves convert energy taken up by PAR absorption. This helps in the interpretation of photosynthesis under different natural and anthropogenic conditions (stress and damage effects).

Additional key words: chlorophyll fluorescence; energy balance; heat production; induction kinetics; photoacoustic spectroscopy.

Introduction

The energy of photosynthetically active radiation (PAR) absorbed by Chls and

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Abbreviations: AR = actinic radiation; Chl = chlorophyll; F = chlorophyll fluorescence; F_0 = ground fluorescence before the induction of photosynthesis; F_0' = ground fluorescence directly after turning off the actinic radiation or the induction of photosynthesis; F_M = maximum fluorescence upon saturating irradiation before the induction of photosynthesis; F_M' or P_M' = signal upon saturating irradiation in the final steady state of the photosynthetic induction; $F_M'1$, $F_M'5$, $F_M'15$, $F_M'45$ or $P_M'1$, $P_M'5$, $P_M'15$, $P_M'45$ = signal upon saturating irradiation during the dark-recovery 1, 5, 15, and 45 min, respectively, after photosynthetic induction with actinic radiation; F_P or P_P = signal at the onset (peak) of photosynthetic induction; F_T or P_T = signal in the terminal steady state of photosynthetic induction; MR = measuring radiation; PAR = photosynthetically active radiation; PAS = photoacoustic signal; SR = saturating radiation.

carotenoids is subsequently converted into three components: photosynthetic activity (photochemistry), Chl fluorescence (red radiation emission), and heat (non-radiative decay) (for a classical review see Butler 1977). The increase of one component in which the energy absorbed is converted must lead to the decrease of at least one of the other two components. In photosynthesis research, photosynthetic activity is often deduced from Chl fluorescence assuming an antiparallel behaviour of photosynthesis and Chl fluorescence (Kautsky effect, see review of Karukstis 1991). In some cases, explaining a lowered Chl fluorescence by an "increased thermal dissipation" (*e.g.*, in non-photochemical quenching, zeaxanthin formation, or photoinhibition) is generally accepted without further experimental proof. However, it implies that heat production is very low, constant, or parallel to one of the other two types of energy. In order to contribute to the understanding of energy balance in photosynthesis, this study simultaneously investigated Chl fluorescence and heat dissipation under adaptation to irradiance (photosynthetic induction) and dark adaptation (subsequent dark-recovery) of an intact leaf.

The Chl fluorescence of leaves, which has been studied for several decades now, can easily be detected as a red radiation even by the naked eye. The thermal signal, however, is much more difficult to measure, and it was not until the late 1970s that thermal signals of leaves were analysed *via* photoacoustic spectroscopy (for reviews see Malkin and Cahen 1979, Buschmann *et al.* 1984, Buschmann and Prehn 1990, Fork and Herbert 1993, Malkin and Canaani 1994). The following photosynthetic parameters were deduced from photoacoustic measurements: non-radiative decay (yield of energy: Malkin and Cahen 1979), induction kinetics (Buschmann 1987, Snel *et al.* 1990), time resolution (Nitsch *et al.* 1988), photosynthetic oxygen evolution (Bults *et al.* 1982, Poulet *et al.* 1983), oxygen consumption by Mehler-reaction (Kolbowski *et al.* 1990, Mauzerall 1990), CO_2-solubilisation by carbonic anhydrase (Reising and Schreiber 1994), molecular volume changes of the reaction centre (Ort and Parson 1979, Delosme *et al.* 1994), photosystem 1 cyclic electron transport (Herbert *et al.* 1990), excitation energy transfer (Frąckowiak *et al.* 1997), photosynthetic energy storage (Veeranjaneyulu *et al.* 1998, Veeranjaneyulu and Leblanc 1998), state 1-state 2 transition and Emerson enhancement (Canaani and Malkin 1984), *in vivo* pigment analysis (Buschmann and Prehn 1981), and depth profile analysis (Buschmann and Prehn 1983). In order to exclude non-thermal contribution to the photoacoustic signal (PAS), one measures at higher modulation frequencies (tobacco > 200 Hz: Bults *et al.* 1982; radish >130 Hz: Buschmann 1987). Thus the gradual increase of heat production during photoinhibition (Buschmann and Prehn 1988) and the higher heat production after a photoinhibitory treatment (Buschmann 1987, Havaux 1989) could be demonstrated as predicted from Chl fluorescence measurements. On the other hand, the induction kinetics of the non-photochemical quenching is not paralleled by the thermal PAS (Buschmann and Kocsányi 1989) as one would expect. Furthermore, in contrast to the current assumption, the increase of the thermal signal under conditions of zeaxanthin formation could also be found when the biosynthesis of zeaxanthin was inhibited by dithiothreitol (Havaux *et al.* 1991).

Here, the thermal PAS and the Chl fluorescence are studied simultaneously during the induction of photosynthesis by actinic radiation with different quantum fluence rates, and during the subsequent recovery of photosynthetic activity in the dark. The dark-recovery has been used to distinguish between different types of non-photochemical quenching (Horton and Hague 1988, Quick and Stitt 1989) depending on the proton gradient at the thylakoid (energy quenching q_E), on state transitions (state transitions quenching q_T), and on photoinhibition (photoinhibitory quenching q_I). In order to fully understand the energy balance of a photosynthetically active leaf it is necessary to determine not only the Chl fluorescence but also the thermal signal. This helps in an exact interpretation of photosynthesis under different natural and anthropogenic conditions (stress and damage effects).

Materials and methods

Plants: Radish seedlings (*Raphanus sativus* L. cv. Saxa Treib) were grown on peat in a climate chamber [22 ± 3 °C, 60 % relative humidity, 10 h "white light" per day: 100 μmol(PAR-quantum) m^{-2} s^{-1}]. Measurements were effected using the cotyledons of 14-d-old plants. All values present the mean of five independent experiments.

Photoacoustic instrument: The photoacoustic detection was done by a photoacoustic cell with a microphone as detector (*EG&G, D-81677* München, for details see Buschmann and Prehn 1990). A circular piece of a leaf (diameter 7 mm) was enclosed into the photoacoustic cell where it could be differently irradiated *via* the light guide of a *PAM 101-103* fluorometer (*Walz*, Effeltrich, Germany). The leaves were placed into the photoacoustic cell with the lower leaf side (spongy parenchyma) facing the light, since this resulted in the most pronounced variation of the PAS. The PAS was excited by "white light" (AR) from a projector modulated by a mechanical chopper (*EG&G*, München, Germany) at a frequency of 279 Hz (*i.e.*, *ca.* 1.8 ms irradiation followed by *ca.* 1.8 ms dark). This frequency was chosen in order to measure only the thermal signal (Buschmann 1987) and to exclude the photobaric contribution interpreted as oxygen evolution (Bults *et al.* 1982). By means of a lens and different grey filters (*Zeiss*, Oberkochen, Germany) the quantum fluence rate of the AR was adjusted to 20, 200, and 1200 μmol(PAR-quantum) m^{-2} s^{-1}, respectively. The detected signals were processed by a two-phase lock-in amplifier (type *5206, EG&G*, München, Germany) amplifying only those signals of the microphone which are detected *via* the modulation frequency of the chopper. Thus the PAS was detected and finally recorded independently of the fluorescence signal, and only with the AR turned on. A reasonable signal/noise-ratio (*ca.* 20 at 20 μmol m^{-2} s^{-1}, 150 at 200 and 1200 μmol m^{-2} s^{-1}) was achieved when the time constant was set to 300 ms. The parameters calculated from the photoacoustic values are defined in Table 1.

Fluorescence instrumentation: The Chl fluorescence was measured by means of a *PAM 101-103* fluorometer (*Walz*, Effeltrich, Germany). The light fibre of the fluorometer was fixed closely above the window of the photoacoustic cell. The fluorescence was excited with a low-intensity red radiation (λ_{max} = 655 nm) of about

0.01 µmol(PAR-quantum) m^{-2} s^{-1}. This measuring radiation (MR) consists of 1 ms-pulses with a modulation frequency of 1.6 kHz. During the short times of additional saturating irradiation (SR) the frequency is automatically changed to 100 kHz. Using the selective amplifier of the PAM-fluorometer only the fluorescence excited by the MR is recorded as fluorescence signal independently of the PAS. As in photoacoustic measurements only those fluorescence signals, which are modulated with the pre-set frequency (1.6 or 100 kHz), are amplified. A continuous "white light" of 2200 µmol m^{-2} s^{-1} was used as SR (halogen source *KL1500, Schott*, Mainz, Germany). For inducing photosynthesis and thus influencing both the fluorescence and the thermal signal, the 279 Hz pulsed AR was applied. It was also used for exciting the PAS (see above). For the parameters calculated from the fluorescence values see Table 1.

Table 1. Parameters derived from fluorescence and photoacoustic signals (PAS) listed in chronological order. The definitions are given using the symbols of Fig. 1.

Parameter	Abbreviation	Definition	Reference
decrease ratio	Rd(F)	$(F_P - F_T)/F_T$ (vF) (Rfd)	Brown (1967) Lichtenthaler *et al.* (1983)
maximum quantum yield of photosynthesis	F_v/F_M	$(F_M - F_0)/F_M$	Kitajima and Butler (1975)
actual quantum yield of photosynthesis	$\Delta F/F_M'$	$(F_M' - F_T)/F_M'$	Paillotin (1976), Genty *et al.* (1989)
photochemical loss	PL(PAS)	P_T/P_M'	Malkin and Cahen (1979)
photochemical quenching	q_P	$(F_M' - F_T)/(F_M' - F_0')$	Bilger and Schreiber (1986)
non-photochemical quenching	q_N	$1 - (F_M' - F_0')/(F_M - F_0)$	Bilger and Schreiber (1986)
rate constant for non-radiative decay	k_D	$[F_M/(F_M' \cdot 0.074)] - 1$	Demmig-Adams *et al.* (1989)
energy quenching	q_E	$1 - (F_M' - F_0')/(F_M'5 - F_0)$	Quick and Stitt (1989)
transition state quenching	q_T	$1 - (F_M'5 - F_0)/(F_M'15 - F_0)$	Quick and Stitt (1989)
photoinhibitory quenching	q_I	$1 - (F_M'15 - F_0)/(F_M - F_0)$	Quick and Stitt (1989)
energy storage	ES(PAS)	$(P_M' - P_T)/P_M' \times 100$	Herbert *et al.* (1990)
relative photochemical quenching	$q_{(P)rel}$	$(F_M' - F_T)/(F_M - F_0')$	Buschmann (1995a)
relative non-photochemical quenching	$q_{(N)rel}$	$(F_M - F_M')/(F_M - F_0')$	Buschmann (1995a)

Sequence of a measurement (Fig. 1): The PAS is shown in the upper part and the fluorescence signal (F) measured simultaneously is shown in the lower part. First, the sample was only irradiated with the 1.6 kHz low-intensity MR of the PAM-fluorometer (F_0-signal). Shortly after the onset of irradiation with MR, a saturating "white" SR pulse of 1 s duration was applied (F_M-signal). After the decline of the fluorescence signal from the F_M-maximum to its initial F_0-height, induction kinetics were started by turning on the 279 Hz AR. When the induction kinetics had declined from the maximum F_P to its terminal steady state F_T (within 7 to 10 min), a saturating "white light" pulse of 5 s duration (SR) was given (F_M') and subsequently the AR was turned off (F_0'). During the following dark-recovery, the SR and the AR were turned on for 5 s, 1 min (F_M'1), 5 min (F_M'5), 15 min (F_M'15), and 45 min (F_M'45), respectively, after the end of the induction kinetics.

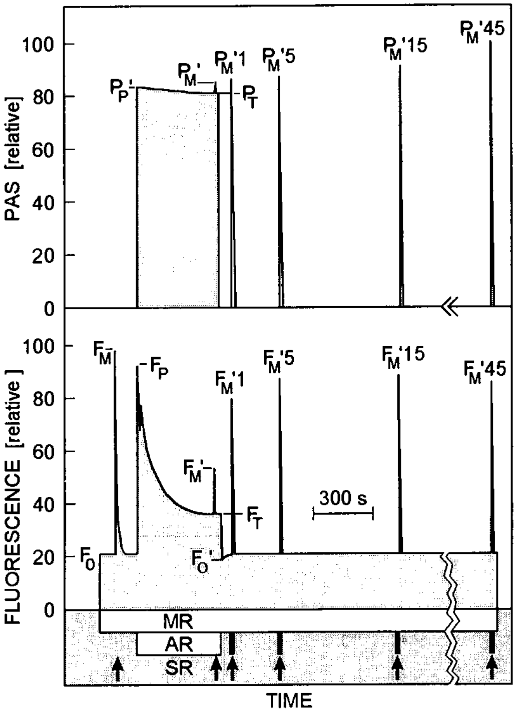

Fig. 1. Example of a simultaneous measurement recording of the thermal photoacoustic signal (PAS, *upper part*) and of the chlorophyll fluorescence (F, *lower part*) of a green radish cotyledon (*Raphanus sativus* L.). For details of the measuring procedure see Materials and methods. AR = actinic radiation: "white light" pulses of 200 μmol(PAR-quantum) m^{-2} s^{-1} at a modulation frequency of 279 Hz; MR = measuring radiation: 1 ms red radiation pulses of 0.01 μmol(PAR-quantum) m^{-2} s^{-1} at a modulation frequency of 1.6 kHz (without SR) or 100 kHz (with SR); SR = saturating radiation: continuous "white light" of 2200 μmol(PAR-quantum) m^{-2} s^{-1}. The abbreviations for different points of the measurement and the parameters derived from the signals are given in Table 1.

Layer of the leaves contributing to the signals: The modulation frequency of the radiation inducing the PAS determines the thickness of the sample from which a PAS is sensed. Assuming for a leaf the thermal properties of water (its main component), the PAS measured at a modulation frequency of 279 Hz should be detected from a thermally active layer of 80 μm (see Buschmann and Prehn 1990) which, for radish cotyledons irradiated from the lower side, includes the epidermis and about one third of the spongy parenchyma. As determined by Nagel (1988), radish cotyledons are about 400 μm thick (upper epidermis 20.7 μm, palisade parenchyma 150 μm, spongy parenchyma 215 μm, lower epidermis 21.6 μm). The fluorescence signal should also be detected from a thin layer close to the leaf surface, since the Chl fluorescence recorded by the PAM is exclusively excited by the red MR that is strongly absorbed by Chls and has a very low quantum fluence rate of 0.01 μmol m^{-2} s^{-1}. Thus it cannot penetrate deeply inside the leaf and only the Chls close to the leaf surface can contribute to the fluorescence signal.

Results

The thermal PAS and the Chl fluorescence signal were simultaneously measured during the induction of photosynthesis and during 45 min of subsequent dark-recovery. An example for one sequence of measurement is shown in Fig. 1 (details of the measuring procedure are given under Materials and methods).

F_0-signal and F_M-signal: The measurement was started with the detection of the F_0 and F_M signals of fluorescence. No PAS could be detected since the AR was turned off. Signals detected by the microphone are not amplified since the lock-in amplifier connected to the microphone amplifies only those signals detected by the modulation frequency of the chopper (in this case 279 Hz). For all samples the F_0 and F_M signals were constant resulting in the same value for the maximum quantum yield F_V/F_M under all conditions (Table 2).

Table 2. Parameters of the thermal photoacoustic signal (PAS) and of chlorophyll fluorescence (F) measured simultaneously with radish cotyledons (*Raphanus sativus* L.) using three different actinic irradiances (AR, $n = 5$, ± standard deviation). The values are based on those shown in Fig. 2. For a definition of the parameters see Table 1.

		AR [μmol(quantum) m^{-2} s^{-1}]		
		20	200	1200
thermal values	Rd (PAS)	0.08 ± 0.04	0.03 ± 0.02	0.04 ± 0.02
	PL (PAS)	0.82 ± 0.04	0.93 ± 0.03	0.99 ± 0.01
	ES (PAS)	18.10 ± 3.80	7.02 ± 2.52	1.36 ± 0.73
	k_D (F)	13.60 ± 0.40	23.90 ± 2.32	35.30 ± 5.24
fluorescence values	Rd (F)	1.22 ± 0.37	1.55 ± 0.44	1.81 ± 0.48
	F_V/F_M	0.79 ± 0.01	0.78 ± 0.01	0.76 ± 0.04
	$\Delta F/F_M'$	0.73 ± 0.03	0.31 ± 0.08	0.04 ± 0.02
	$q_{(P)rel}$	0.88 ± 0.07	0.21 ± 0.04	0.02 ± 0.01
	$q_{(N)rel}$	0.09 ± 0.03	0.57 ± 0.06	0.79 ± 0.10
	PL (F)	0.27 ± 0.03	0.69 ± 0.08	0.96 ± 0.02
	ES (F)	73.00 ± 2.90	31.40 ± 7.70	4.03 ± 2.18
	q_E	0.02 ± 0.02	0.49 ± 0.05	0.57 ± 0.21
	q_T	0.00 ± 0.02	0.02 ± 0.02	0.11 ± 0.04
	q_I	0.10 ± 0.03	0.13 ± 0.05	0.41 ± 0.08

Induction of photosynthesis followed by measuring the PAS and fluorescence signal during several minutes of AR irradiation showed a decrease from the maximum (fluorescence F_P and photoacoustic P_P) to the terminal steady-state maximum (fluorescence F_T and photoacoustic P_T) (Fig. 1). This decrease was much stronger for the fluorescence signal than for the PAS. With increasing quantum fluence rate of the AR, the decrease of the fluorescence became stronger whereas the decrease of the PAS became smaller (confer Fig. 2*A*,*B*,*C*). The dependence of the signals' decrease on the quantum fluence rate of the AR can also be seen by the changes of the Rd-values: increase of Rd(F), but decrease of Rd(PAS) [at least from 20 to 200 µmol(quantum) m^{-2} s^{-1}] with increasing quantum fluence rate (Table 2).

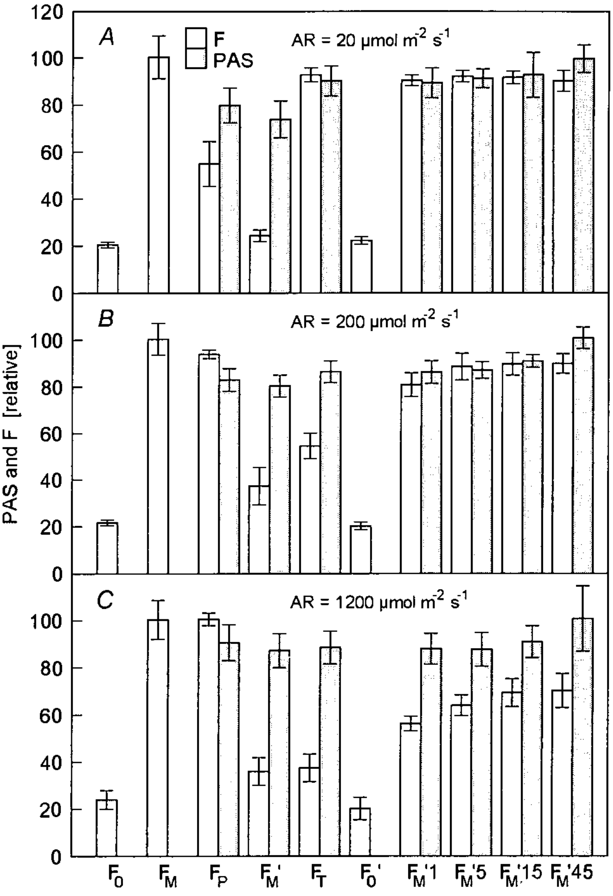

Fig. 2. Heights of the chlorophyll fluorescence (*white bars*) and of the thermal photoacoustic signal (PAS, *grey bars*) at different times of the measurement as indicated in Fig. 1. The values are normalised to 100 at the maximum value during a measuring sequence (photoacoustic values at P_M'45, fluorescence values at F_M). The quantum fluence rate of actinic radiation was adjusted to 20 (*A*), 200 (*B*), or 1200 (*C*) µmol(PAR-quantum) m^{-2} s^{-1} (n = 5, ± standard deviation). The ratios, coefficients, and constants calculated from the individual values are given in Table 2.

In general, the values for the PAS increased strongly with increasing quantum fluence rate of the AR, whereas the fluorescence signals first rose and then levelled off. Fig. 3 shows the heights of the PAS and fluorescence signal in the steady state (F_T and P_T) of the induction kinetics for different quantum fluence rates of the AR.

Saturation pulse in the steady state of photosynthesis: When the steady state of the photosynthetic induction had been reached after several minutes of irradiation with AR, an intense radiation pulse saturating photosynthesis induced a rise of both the PAS and fluorescence signal (Fig. 1). The increase was most pronounced with the AR of 20 μmol(quantum) m^{-2} s^{-1} and became lower towards 1200 μmol(quantum) m^{-2} s^{-1} (Fig. 2). The variation with increasing quantum fluence rate of the AR was also reflected in the increase of the values for PL(PAS), k_D, $q_{(N)rel}$, and PL(F) as well as in the decrease of the values for ES(P), $\Delta F/F_M'$, $q_{(P)rel}$, and ES(F) (Table 2).

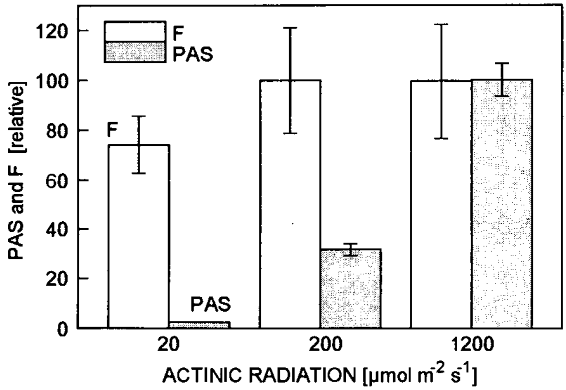

Fig. 3. Heights of the chlorophyll fluorescence (*white bars*) and of the thermal photoacoustic signal (PAS, *grey bars*) and in the steady state (F_T or P_T in Fig. 1) of the photosynthetic induction upon irradiation with different quantum fluence rates of the actinic radiation [20, 200, and 1200 μmol(PAR-quantum) m^{-2} s^{-1}]. The values are normalised to 100 at the maximum obtained *via* the three quantum fluence rates (*n* = 5, ± standard deviation).

Dark-recovery: During the dark-recovery following the light-induced induction of photosynthesis, the leaf returns from the light-adapted state to the dark-adapted state. When turning off the AR, no PAS can be detected and the fluorescence signal goes down to the value F_0'. For the induction at 20 μmol(quantum) m^{-2} s^{-1}, the F_0'-signal was similar to the F_0-signal before the onset of the induction. But F_0' decreased with the increase of the quantum fluence rate of the AR given during the induction (Fig. 2). When SR and AR were applied for 5 s during the first 15 min of dark-recovery ($P_M'1$, $P_M'5$, $P_M'15$), the PAS was similar to that induced by the SR in the steady state of the induction kinetics (P_M'). Only between 15 ($P_M'15$) and 45 min ($P_M'45$) of dark adaptation the PAS increased slightly (in the order of 10 %) and reached a maximum which also represented the maximum of the whole measuring sequence (normalised to 100 in Fig. 2). During dark-recovery the PAS did not depend on the quantum fluence rate of the AR given before, whereas the fluorescence signal was strongly reduced with increasing AR. When the leaf had been irradiated during the induction period with 20 μmol(quantum) m^{-2} s^{-1}, the fluorescence signal upon irradiation with SR after 1 min of dark-recovery ($F_M'1$) was somewhat smaller than upon SR irradiation in the steady state of the induction period (F_M'). Then the fluorescence signal increased only slightly between 1 and 5 min of dark adaptation and finally levelled off (Fig. 2*A*). After the induction with AR of 200 or 1200 µmol m^{-2} s^{-1}, the fluorescence induced by SR was higher after 1 min of dark-recovery (F_M'1) than in the steady state of induction (F_M'). After irradiation with the two higher quantum fluence rates during the induction of photosynthesis, the fluorescence increased clearly upon SR irradiation, especially during the first 5 min from F_M'1 to F_M'5 (Fig. 2*B,C*). After the induction with 1200 µmol(quantum) m^{-2} s^{-1} the fluorescence excited by SR during dark-recovery remained much lower than after the induction with AR of the lower quantum fluence rates (Fig. 2*C*). In all cases, the SR applied after 45 min of dark-recovery (F_M'45) lead to a lower fluorescence signal than before the onset of the induction (F_M) (Fig. 2). The changes in the fluorescence signals during dark-recovery can also be seen as changes in the coefficients for q_E, q_T, and q_I (Table 2). Each coefficient increased with increasing quantum fluence rate of the AR applied before the dark-recovery. After an induction period with 20 µmol(quantum) m^{-2} s^{-1} of AR the value for q_I was the highest among these three coefficients, whereas at higher quantum fluence rates q_E became predominant.

Discussion

Dependence of the signals on the quantum fluence rate of the actinic radiation: The height of the thermal signal parallels the quantum fluence rate of the AR (Fig. 3). The increase of the thermal signal with increasing quantum fluence rate does not show up in Fig. 2, since here the signals are normalised to the maximum value. It is mainly the AR which induces the finally recorded PAS, since signals with no modulation and/or modulation frequencies other than 279 Hz are excluded by the lock-in amplifier. Radiation which is not modulated with 279 Hz may only indirectly influence the PAS by changing the thermal properties of the leaf. In contrast to the thermal signal, the fluorescence signal is only indirectly affected by the AR *via* the changes induced to the photosynthetic activity of the leaf. Thus there is only a slight change with increasing quantum fluence rate (Fig. 3). At 20 µmol m^{-2} s^{-1} quantum fluence rate of the AR part of the MR is also transferred into photosynthesis and thus less fluorescence is emitted than at a higher quantum fluence rate. With an increasing quantum fluence rate of AR photosynthesis becomes saturated by radiant energy, and the energy of the MR transferred into fluorescence reaches higher values, since the fluorescence induced by AR is excluded from the fluorescence signal by the specific amplifier of the PAM.

Effect of saturating radiation in the steady state of photosynthesis: When the SR is added to the AR and MR, the modulated radiation types (AR and MR) are fully converted into fluorescence and heat, and the maximum F_M' or P_M' is reached (Fig. 1). The rise from P_T to P_M' of the thermal signal and of the fluorescence F_T to F_M' reflects the quanta needed for saturating photosynthesis under steady state conditions. It is mainly this increase which determines the "photochemical loss" (PL), the energy storage (ES), the actual quantum yield $\Delta F/F_M$', and the "photochemical quenching" q_P or $q_{(P)rel}$ of a plant. The height of F_M' determines indirectly and inversely also the "non-photochemical quenching" q_N or $q_{(N)rel}$ and the "rate constant for non-radiative decay" k_D which, in principle, are deduced from the distance between F_M' and F_M. Since q_P and q_N (Bilger and Schreiber 1986) are related to different references, in this study the relative quenching coefficients $q_{(P)rel}$ and $q_{(N)rel}$ (Buschmann 1995a) were determined which both refer to F_M - F_0' and thus can be compared with the same scaling (the sum of both values never exceeding 1). The value k_D (Demmig-Adams *et al.* 1989) is determined only from fluorescence measurements and thus represents an indirect approach to the thermal signal which is only valid if the assumption leading to the equation for k_D holds true.

The height of F_M' in the steady state of the induction kinetics and the distance between F_M' and the steady state signal F_T decrease with increasing quantum fluence rate of the AR, both for the thermal signal and fluorescence (Fig. 2). This is due to the drop of photosynthetic quantum efficiency with increasing quantum fluence rate.

Changes during the induction of photosynthesis: During the induction of photosynthesis the rise of photosynthetic activity is paralleled by a decline of the Chl fluorescence signal (Kautsky effect). The decline of fluorescence during the induction period reflects the increase of the photosynthetic activity of photosystem 2; it is stronger at higher irradiances than at the low ones (Fig. 2). This can also be seen by the decrease of the photochemical quenching and the concomitant increase of the non-photochemical quenching and the rate constant k_D (Table 2). The thermal PAS declines like the fluorescence but to a much smaller degree (Fig. 1). Judging from Fig. 2, which gives the mean of five measurements with standard deviation, one could assume that the thermal signal shows no significant change during the photosynthetic induction, but for one single measurement there is always a small decrease of the thermal signal. The small kinetics of the thermal PAS in radish cotyledons are more pronounced than in other leaf types (Buschmann 1990). In fact, most of the photoacoustic measurements at high modulation frequencies show no decline after the onset of irradiation (*e.g.*, tobacco: Bults *et al.* 1982, pea: Havaux 1989). One explanation for the small kinetics of the thermal PAS is the fact that the thermal signal increases proportionally to the radiant energy taken up by all substances of the leaf absorbing "white light" (not only Chl). Thus the variation due to the induction of photosynthesis becomes less pronounced with increasing the AR. On the other hand, the small thermal kinetics could also be explained by the better signal propagation of Chl fluorescence inside the leaf tissue and/or to the insufficient photoacoustic detection systems/techniques available at present. Compared to other leaves, the rather thick radish cotyledons with small cells obviously have, particularly in the spongy parenchyma, a good thermal coupling to the outer gas phase which transmits the PAS to the microphone. In any case, the rise of the thermal PAS upon irradiation with SR clearly demonstrates that under the measuring conditions applied in this study the PAS is influenced by the photosynthetic activity of the leaf.

Changes during the dark-recovery: The different phases of the rise of the fluorescence signal induced by SR during dark-recovery are used for distinguishing several types of the non-photochemical quenching (Horton and Hague 1988, Quick and Stitt 1989). The fact that, after 45 min of dark-recovery (F_M'45), the F_M-value before the onset of the induction could not be reached is an indicator of photoinhibition which can also be expressed by the coefficient q_I. This photoinhibition is most pronounced at a quantum fluence rate of the AR of 1200 µmol m^{-2} s^{-1} (highest value of q_I, Table 2). The rise of the thermal signal may be due to photoinhibition, as has been demonstrated earlier (for the first time: Buschmann 1987, Buschmann and Prehn 1988). However, the strong increase of the thermal signal in the darkness between 15 and 45 min of dark-recovery may also be due to a change in the leaf tissue structure which possibly alters the thermal propagation properties of the leaf leading to a more efficient way of thermal dissipation.

Energy balance between fluorescence and heat: The thermal signal and the fluorescence are, in most cases, changing in the same sense: (*a*) decrease during the induction of photosynthesis, (*b*) increase upon addition of SR, (*c*) reduced increase upon SR when increasing the quantum fluence rate of the AR, (*d*) increase of the signal achieved with SR during dark-recovery. Close, parallel changes of the fluorescence and of the thermal PAS were also found in a parallel oscillation of both photoacoustic and fluorescence signals (Buschmann 1995b). Under these conditions the energy conversion into photochemistry is expected to be inverse to that of Chl fluorescence and heat dissipation. This has also been demonstrated in the correlation of the photochemical loss calculated from the high-frequency PAS and the photosynthetic oxygen evolution deduced from the low-frequency PAS (Snel *et al.* 1990).

No parallel change was detected (*a*) in the rise of the thermal signal at the end of the dark-recovery (increase of the thermal signal but constant fluorescence) (Fig. 2), and (*b*) in the changes of the decrease during the photosynthetic induction with increasing quantum fluence rate of the AR (smaller values of the thermal signal but higher values for the fluorescence) (Fig. 2). Under these conditions the amount of energy converted into photochemistry cannot only be predicted by measuring Chl fluorescence.

Conclusion: The results show that heat dissipation detected by photoacoustic measurements is neither low, constant, nor always parallel to Chl fluorescence. Therefore, the thermal signal should always be measured in order to fully understand the way leaves convert energy taken up by light absorption.

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