Bull. Environ. Contam. Toxicol. (2005) 74:126–132

DOI: 10.1007/s00128-004-0558-y

Bulletin of Environmental Contamination and Toxicology

Influence of Nonphotochemical Quenching in Methylviologen Treated Dandelion Leaves (*Taraxacum officinale* Weber) on Energy Storage Measured by Photoacoustic Spectroscopy

J. Szurkowski, W. Dobrowolska

Institute of Experimental Physics, University of Gdansk, Wita Stwosza 57, 80-952 Gdansk, Poland

Received: 18 June 2003/Accepted: 6 October 2004

Photoacoustic (PA) spectroscopy has been employed successfully to study the effect of environmental stress on photosynthetic energy storage (ES) (Szurkowski and Tukaj 1994, Szurkowski and Tukaj 1995, Szurkowski 2001, Szurkowski 2002). When a photosynthetic sample is exposed to a modulated light beam, a part of the absorbed light energy is emitted in the form of modulated heat (photothermal signal) resulting from the thermal deactivation of pigments. The photothermal part of the PA signal is reduced by a fraction equal to that amount of absorbed energy that is stored (by the photosynthetic process) as chemical energy. The remaining energy dissipates in photochemical processes leading to a modulated O_2 emission and appears as a photobaric signal. Both contributions originate in the chloroplasts, where heat and oxygen diffuse to the cell envelope and generate acoustic waves in the PA cell. Measurements performed by Buschmann (1999), for radish cotyledons (*Raphanus sativus*), indicated a dependence the photothermal signal decrease kinetics on the light intensity. A decrease of the illuminating light intensity leads to a drop of the signal amplitude. Such changes in the photochemical signal amplitude must lead to the corresponding changes of ES during the course of the measurement. That was already observed in studies on water infiltrated pea and sugar maple leaves by Malkin et al. (1992).

In the study described here, we have used methylviologen in dandelion (*Taraxacum officinale* Weber) leaves to create conditions which allow one to directly monitor the relation between the photothermal signal decreasing time and the energy storage (ES). Methylviologen was used many times in ES measurements to inhibit the photobaric component at low modulation frequencies (Bukhov et al. 1996, Bukhov et al. 2000, Szurkowski 2002).

MATERIALS AND METHODS

The measurements of selected dandelion leaves were conducted within two hours after collection. Two sampling station were situated in the municipal Tri-city (Gdynia, Sopot) area having permanent contamination monitoring systems while another, a distant one, was situated about 100 km from the agglomeration where air pollution is very low. At least 15 leaves of comparable dimension (15 cm long)

Correspondence to: J. Szurkowski

were studied from each location. Methylviologen (MV) which inhibits the photobaric component was introduced into leaves through the transpiration stream by placing the lower part of leaf blades into 1 mM water solution of MV (Bukhov et al. 1996, Bukhov et al 2000, Szurkowski 2002).

A laboratory-built PA spectrometer described in detail elsewhere (Szurkowski and Tukaj 1995) was used for the measurements. The intensity of modulated light (wavelength of 680±10 nm) was 25 $\mu Em^{-2}s^{-1}$. Background nonmodulated white light (2500 $\mu Em^{-2}s^{-1}$) from an illuminator (PL-800, Dolan & Jenner) was focused on the sample through a fibre light guide. The signal was selected and amplified with a lock-in amplifier (Type 232 B, Unipan, Warsaw, Poland). The measurement was done at a 20 Hz modulation frequency and the time constant was 3s. The output of lock-in was connected to a computer (IBM PC) for further data processing.

The energy storage was calculated as $(a - b)/a \times 100$, where a is the PA signal produced by the modulated light in presence of the nonmodulated background light, and b represents the PA signal resulting from the modulated light alone. Addition of strong background light saturates photochemistry in the sample, increasing the absorbed modulated light to heat conversion to nearly 100% and producing a maximal PA signal proportional to absorption of the modulated light by the sample.

The decrease ratio (*Rd*), defined for fluorescence studies by Brown (1967) and adapted to PAS measurements by Buschmann (1999), was determined from the following relation: $Rd=(P_P - P_T)/P_T$, where P_P is the PA signal at the onset of photosynthetic induction, and P_T is the terminal steady state amplitude.

RESULTS AND DISCUSSION

We discovered that *T. officinale* leaves are a good indicator for the level of contaminations as a whole (Szurkowski 2002). There is a correlation between the measured energy storage value (using the action of methylviologen on leaves) and the level of environmental contamination. However, for measurements lasting many minutes and leaves treated with methylviologen, the effect of photochemical and nonphotochemical quenching on the measured photoacoustic signal amplitude must be considered.

The mean values of *Rd* resulting from measurements performed on a set of at least 15 leaves collected at each measuring site are summarized in Table 1. These values almost do not depend on the sampling site location and subsequently on the environment contamination level. It means that a decrease of the PA signal amplitude does not affect the ES difference in leaves originating from different sampling stations. For all the cases studied, the *Rd* values are significantly higher than these reported by others (see Table 2 Buschmann 1999). Similarly, as

observed by Buschmann (1999), a value of *Rd* is lower when the light beam illuminating the sample is of higher intensity.

Table 1. Decrease ratio (*Rd*) of photoacoustic signal in *Taraxacum officinale* leaves at different intensity of light.

Place of sample collection	Decrease ratio(*Rd*)	
	AR	AR + SR
SOMINY	0.74 ± 0.04	0.18 ± 0.01
SOPOT	0.89 ± 0.01	0.16 ± 0.02
GDYNIA	0.79 ± 0.03	0.18 ± 0.02

AR (25 $\mu Em^{-2}s^{-1}$) – actinic radiation,

SR (2500 $\mu Em^{-2}s^{-1}$) – saturating radiation

Table 2 summarizes *Rd* values obtained by Buschmann (1999) at different intensities of the illuminating light. The reported intensities are lower than those measured in our studies with MV treated leaves samples. It could be an evidence that the small thermal kinetics, found in his measurements, could not be explained by the low thermal signal propagation of Chlorophyll inside the leaf tissue and/or related to the insufficient photoacoustic detection systems/techniques available at present (Buschmann 1999).

Table 2. Decrease ratio (*Rd*) of photoacoustic signal in *Raphanus sativus* leaves for three different actinic irradiances (from Buschmann 1999).

Actinic radiation [$\mu Em^{-2}s^{-1}$]	Decrease ratio (*Rd*)
20	0.08 ± 0.04
200	0.03 ± 0.02
1200	0.04 ± 0.02

The slow stage of photosynthetic induction observed in our studies is attributed both to the photochemical and nonphotochemical quenching effects on the signal

amplitude. Photochemical quenching may be related to the charges separation present in the photosynthetic reaction centres. Nonphotochemical quenching covers different phenomena. At modulation frequencies (20 Hz) used in our measurements, both the mentioned mechanisms of quenching contribute to a certain extent in the observed changes of the photothermal signal amplitude. The methylviologen treatment sets in motion additional mechanisms of nonphotochemical quenching. Methylviologen is an electron transport mediator in the Mehler reaction and can generate a sufficient ΔpH to induce xanthophyll cycle dependent nonphotochemical quenching (Neubauer and Yamamoto 1992, Thiele and Krause 1994).

Table 3. Characteristic times of photothermal signal amplitude decay, for *Taraxacum officinale* leaves treated with methylviologen.

Place of sample collection	Time [s]	
	AR	AR + SR
Sominy	391 ± 70	162 ± 21
Sopot	370 ± 81	148 ± 23
Gdynia	321 ± 51	137 ± 24

AR (25 $\mu Em^{-2}s^{-1}$) – actinic radiation,

SR (2500 $\mu Em^{-2}s^{-1}$) – saturating radiation

The established large variability in photoacoustic signal kinetics accompanied by the high level of signal to noise ratio allowed us to determine not only the Rd but also the characteristic time of quenching. In general, the nonphotochemical relaxation can be decomposed into, at least, three exponential components (Horton and Hague 1988, Lavergne and Briantais 1996) that are denoted qT, qE, qI. These contributions have also been correlated with the irradiance intensity range at which they saturate: qT in low light, qE at an intensity which saturates photosynthesis and qI at higher intensities. The characteristic times (one exponential decay) obtained from the photoacoustic signal decay curves using the best-fit procedure (a Lavenberg – Marquardt algorithm) are collected in Tab. 3. They are approximately equal to 6.5 min. at low measuring light beam intensities and are in agreement with the typical times of fluorescence quenching of the qT type (about 5 min., as reported by Lavergne and Briantais (1996)). A majority of authors interpret nonphotothermal quenching at this light intensity as related to a state 2 transition (Horton and Hague 1988). As it s well known its role is

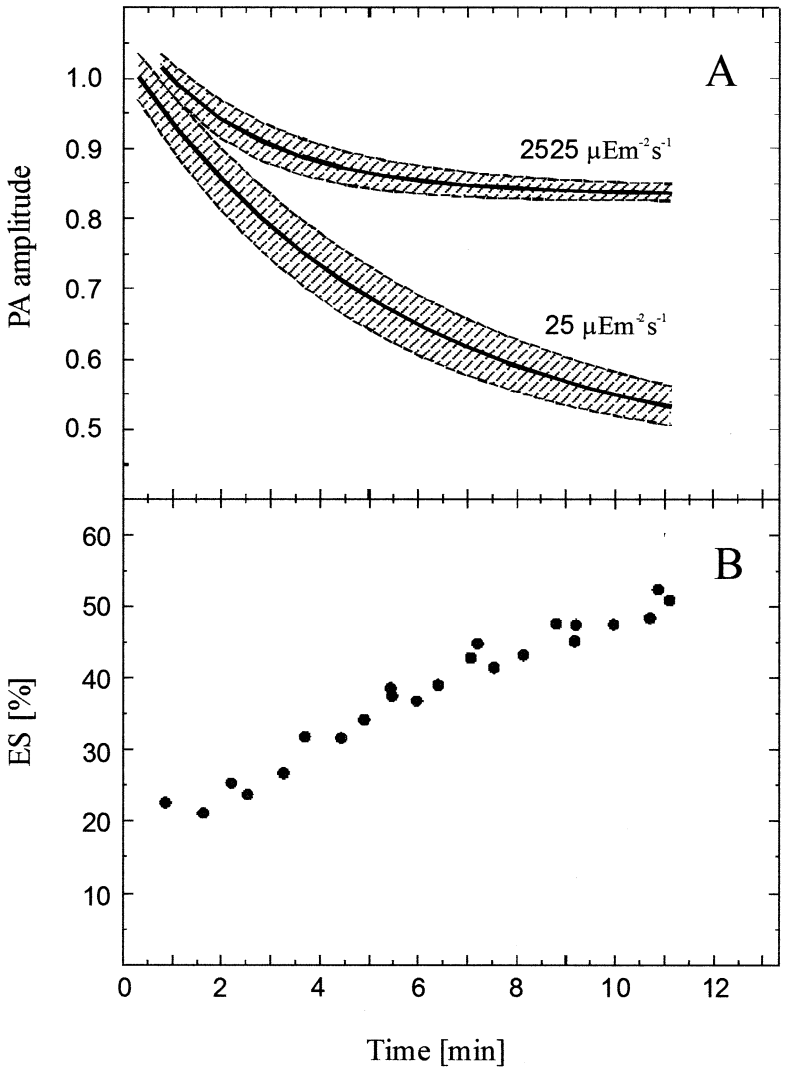

Figure 1. The normalized photoacoustic signal amplitude time decay plots, for dandelion (*Taraxacum officinale*) leaves collected at Gdynia and treated with methylviologen, measured at different illuminating light intensities (**A**). Shaded areas correspond to the measurement significance levels of the plots. The continuous increase of energy storage ES versus elapsed time of photoacoustic measurement (**B**). The measuring light beam was modulated at a 20 Hz frequency and the time constant of the lock-in amplifier was 3s.

principal in unicellular green algae and cyanobacteria, whereas it is a relatively minor factor in higher plants (Lavergne and Briantais 1996).

The characteristic times obtained in our studies (Table 3) at the light intensity which saturates photosynthesis (2525 $\mu Em^{-2}s^{-1}$) appear several times higher than these corresponding the fluorescence quenching process of qE type. The component of qE *in vivo* is rapid relaxation of fluorescence with a half-time of around 10 seconds. In higher plants at physiological irradiances, qE is the major component of quenching. The molecular mechanism of qE is still controversial (Van Wijk and Hasselt 1990). The photoacoustic signal amplitude both at low and at saturating photosynthesis light intensities decays slower than the corresponding changes of fluorescence intensity do. It is well understood since the photothermal signal contains a slowly decaying (for short times of dark adaptation) photochemical quenching component which is not present in the fluorescence measurements (compare the 'light doubling' fluorescence technique; Bradbury and Baker, 1984). One of the limits which does not allow the faster signal change to be observed could have been a time constant of the system (lock-in amplifier) response, about 3 seconds.

Exemplary decay plots of the photoacoustic signal with the significance ranges specified at low and high light intensities, for dandelion leaves samples collected in Gdynia, are depicted in Fig. 1A. Such changes in the photoacoustic signal amplitude have an apparent effect on the measured ES value (Fig. 1B). As a consequence of different kinetics applicable to the signal decay at low and high intensities of light illuminating the sample, the ES value evaluates in elapsing time of each measuring run. It grows about 3 percent per minute on average. However, methylviologen processing allows ES measurement at low light modulation frequencies (Bukhov et al. 1996, Szurkowski 2002) under higher signal to noise ratio conditions, but simultaneously leads to a rather fast increase of the ES values observed within the first dozen minutes. In our measurements the ratio initial ES/steady-state is less then 0.42 compared to 0.9 (for 5 min of dark adaptation) obtained by Malkin et al. (1992) for non-treated leaves. In ES measurements using a methylviologen sample treatment, measurement duration should be taken into account as a resulting factor.

Acknowledgements. This work was supported by the University of Gdańsk under Grant No. BW/5200-5-012-3.

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