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A study on the energy-dependent quenching of chlorophyll fluorescence by means of photoacoustic measurements

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Abstract

The mechanism of energy-dependent quenching (q_E) of chlorophyll fluorescence was studied employing photoacoustic measurements of oxygen evolution and heat release. It is shown that concomitant to the formation of q_E the yield of open reaction centers (ϕ_p) decreases indicating that q_E quenching originates from a process being competitive to fluorescence as well as to photochemistry. The analysis of heat release (rate of thermal deactivation) shows: 1. The competitive process is not given by a still unknown energy storing process. 2. If the competitive process would be a futile cycle the life-times of the involved intermediates had to be faster than 50 μs.

The results of the photoacoustic measurements are in line with the idea that q_E quenching originates from an increased probability of thermal deactivation of excited chlorophylls.

Abbreviations: F – actual fluorescence; F'_m – fluorescence yield with all PS II reaction centers closed in a light adapted state; F'_0 – fluorescence yield with all PS II reaction centers open in a light adapted state; PS – Photosystem; ϕ_p – intrinsic photochemical yield; q_E – energy-dependent quenching; q_I – photoinhibition quenching; q_N – non-photochemical quenching; q_P – photochemical quenching; q_T – state transition quenching

Introduction

It is well established that part of the quenching of chlorophyll fluorescence is associated with the energy state of the chloroplast. This 'energy-dependent' quenching is found in isolated chloroplasts as well as in intact alga cells and in leaves of higher plants. The energy-dependent quenching (q_E) has been shown to be linearly related to the extent of the inner thylakoid proton concentration (Briantais et al. 1979), though the coupling is not obligatory (Oxborough and Horton 1988, Vanselow et al. 1988). The q_E quenching is clearly different from the so-called photochemical quenching (q_P) of fluorescence which depends solely on the redox state of the primary quinone acceptor of PS II, Q_A. Thus, q_E quenching belongs to the class of the non-photochemical quenching (q_N) mechanisms. It is characterized by a considerable quenching of the maximum fluorescence F'_m (up to 70%) which is defined quantitatively by the quenching coefficient q_E. The underlying mechanism is still unknown. It has been suggested that the formation of q_E results from conformational changes within the light harvesting pigment beds of Photosystem II, possibly caused by a protonation of the lumen surface (Krause et al. 1982). These conformational changes might cause an increased rate of thermal deactivation of excited chlorophyll states.

Some complications in studying the energy-

dependent quenching can arise from the interference with other non-photochemical quenching mechanisms as there are the state transition quenching (q_T) and the photoinhibition quenching (q_I) (Horton and Hague 1988). In this study a separation of q_E quenching was achieved by making use of the different kinetic properties. After an increase in light intensity formation of q_E quenching occurs with a half-time of about 10 s (Dau and Hansen 1989). Formation (or decay) of the other non-photochemical quenching mechanisms occur on a much slower time scale (Horton and Hague 1988, Dau and Canaani 1989). Therefore a separation of q_E related changes from changes related to q_T or q_I is possible by studying q_N quenching within the first seconds after an increase in light intensity.

The progress gained by this study results from the application of the photoacoustic technique in order to analyze the effect of q_E related changes on the yield of photochemistry and thermal deactivation. Photoacoustic signals are formed upon absorption of modulated light by a sample. The photoacoustic signal in leaves was shown to comprise two components, modulated heat and modulated gas exchange, which are both transduced to a pressure wave in the bulk phase (Bults et al. 1981, Poulet et al. 1983, Canaani et al. 1988, Buschmann 1990). The photoacoustic technique is especially suited for studying the mechanism of q_E quenching since it enables both the measurement of the yield of PS II photochemistry and of the yield of thermal deactivation of absorbed light energy. At low frequencies of the modulated light the yield of PS II photochemistry can be studied by measuring the modulated oxygen evolution. At modulation frequencies above 200 Hz the photoacoustic signal gives the yield of thermal deactivation by measuring the modulated heat release.

Thus, by using fluorescence and photoacoustic measurements experimental data become available on three pathways of energy flux: photochemistry, thermal dissipation and fluorescence. It is the first issue of this study to provide further evidence that q_E quenching of fluorescence results from a process being competitive to photochemistry and fluorescence. The second one is to demonstrate that the formation of q_E is coupled to an increase in thermal deactivation. Three possibilities concerning the nature of the process leading to q_E quenching are discussed along the results of the photoacoustic measurements.

Materials and methods

Plant material

Spinach (*Spinacia oleracera* L.) was grown in 11 h d^{-1} light at an intensity of 20 W m^{-2} (HQI-400 lamp, Franz Sill GmbH, Berlin) at room temperature (about 20°C). Before each measurement leaves were cut and put into the photoacoustic cell (Cahen 1981). Then the intact leaves were exposed to the measuring light, which also acts as a constant actinic light, until stable steady-state values of fluorescence and photoacoustic signal were reached.

Light sources and recording of fluorescence

a) The photoacoustic measuring light was provided by a halogen bulb and was passed through a chopper and a filter (as indicated in the text) into the photoacoustic measuring chamber.
b) The non-modulated light was provided by a light source equivalent to (a) yet without a chopper.
c) In order to measure the F_0'-level of fluorescence, far-red non-modulated light was used (halogenbulb + Schott filters RG 9 and Dal 722 nm) – at a light intensity of 3 W m^{-2}. F_0' denotes the fluorescence yield with open PS II reaction centers in a light adapted state determined as described below. F_0' has to be distinguished from the F_0-level of dark adapted plants.
d) The measuring light for fluorescence measurements was provided by a light emitting diode (Stanley H-3000 + filter Balzers DT-cyan special, a short-pass filter with a half-value wavelength of 705 nm) electronically switched on and off with a frequency of 8 kHz (light intensity of 0.2 W m^{-2}).

The intensity of (a), (b) and (c) was controlled electronically. All light sources and the fluorescence detector were connected to different bran-

ches of a light guide with the common end positioned in front of the light entrance of the measuring chamber (Cahen 1981). The given light intensities were measured at the leaf surface.

For fluorescence measurements, the fluorescence emission of the leaf was passed through a filter (Schott RG 9) and was detected by a home-made photodiode detector. The lock-in amplifier used the 8-kHz signal which modulated the light emitting diode as reference signal.

The determination of the maximum level F'_m and minimum level F'_0 of fluorescence was done as described previously (Dau and Hansen 1988). Briefly, lights (a), (b) and (d) were continuously supplied. For determination of F'_m, a saturation pulse of 1.2 s duration was supplied by a sudden increase of the intensity of light (b) to 800 $W\,m^{-2}$. For determination of F'_0, light (a) and (b) were switched off and far red light (c) was switched on for 3 s.

For the determination of the time-courses of F'_m and F'_0 as given in Fig. 1 the following 'sampling' technique was applied: Every 4 minutes the additional actinic light (b) was applied for 40 s. At a certain time after the increase in light intensity either F'_0 or F'_m was determined. The time of determination of F'_m or F'_0 was different in different runs.

$\bar{F}'_m$, $\bar{F}'_0$, $\bar{\phi}_p$ in Fig. 1 are the final values which are obtained by extrapolation of the related curves in Fig. 1.

The photochemical quenching coefficient q_P and the intrinsic photochemical yield ϕ_p were calculated according to the following equations. (F and r give the fluorescence and oxygen evolution yield, respectively.)

$$q_P = (F'_m - F)/(F'_m - F'_0) \quad \text{(a)}$$

$$\phi_p = r/q_P \quad \text{(b)}$$

Recording of the photoacoustic signal

A measuring chamber as described by Cahen (1981) was used. The modulated measuring light caused pressure changes of the same frequency inside the measuring chamber which were detected by a microphone (BL 1785, Knowles Electronics). The amplitude and the phase of the microphone signal were determined by a lock-in amplifier (Brookdeal, Ortholoc, 9502) operated with a reference signal from the chopper.

The light-induced pressure changes consist of two components, a photothermal one and a photobaric one, with the second one due to oxygen evolution (Bults et al. 1981, Bults et al. 1982, Poulet et al. 1983, Canaani et al. 1988, Buschmann 1990). The two components are not in phase (Poulet et al. 1983). In order to measure the yield of oxygen evolution the following procedure was applied: Before each experiment the phase of the thermal component was detected by application of saturating background light (about 1500 $W\,m^{-2}$, white light). Under these conditions the phase of the lock-in amplifier was adjusted in such way that the output signal was zero. This adjustment of the phase enabled the measurements of the component perpendicular to the thermal component, thus eliminating the thermal component in subsequent measurements. It is assumed that the phases of the oxygen component and of the thermal component remain constant during the course of one experiment. Thus the remaining signal can serve as a measure of the oxygen evolution. Oxygen evolution due to non-modulated background light does not contribute to the photoacoustic oxygen signal since only the modulated part of oxygen evolution is detected. Thus, the photoacoustic oxygen signal can serve as a measure of the yield of oxygen evolution (Poulet et al. 1983). Another consequence of measuring modulated oxygen evolution is the elimination of the influence of photorespiration since a modulation of the photorespiratory oxygen uptake with 65 Hz is highly unlikely. It is still unknown whether or not the Mehler reaction contributes to the signal (Malkin 1987, see also Kolbowski et al., this issue).

At modulation frequencies above 200 Hz the photobaric component becomes negligible compared to the photothermal one (Bults et al. 1982, Poulet et al. 1983). Thus the high-frequency photoacoustic signal gives the yield of heat release. In the case of measurements of the high-frequency photoacoustic signal the phase of the lock-in amplifier was adjusted for maximum amplitude.

The standard deviation σ given in Table 1 was

estimated according to the criteria that 85% of the amplitudes are within 2σ (see Fig. 4B).

Results

Figure 1 shows responses to an increase in light intensity from 10 to 30 W m^{-2}. Before starting the experiment the leaf was exposed to two measuring lights for 30 min. One of it was employed for fluorescence measurements (0.2 W m^{-2}, LED) and the other one for measuring the photoacoustic oxygen signal (10 W m^{-2}, BG 28 filter (Schott) 65 Hz). Thus, the leaf was adapted to the overall light intensity of 10.2 W m^{-2}. At $t = 0$, an additional non-modulated light of 20 W m^{-2} was applied. As shown by Fig. 1A, complex kinetics of the fluorescence yield f, oxygen yield r and of the photochemical quenching factor q_P were induced. A more detailed analysis of these kinetics is given elsewhere (Dau and Hansen 1989). The maximum fluorescence F'_m and the level of instantaneous fluorescence F'_0, however, show a simple first order decay as demonstrated by Fig. 1B. This decay corresponds to the so-called τ_4-component in Dau and Hansen (1989). It is caused by the formation of the high-energy state.

One point of special interest is the behavior of the intrinsic photochemical yield ϕ_p as defined by Weis and Berry (1987). The oxygen yield r depends on two factors: the conformation of PS II (absorption cross-section, rate constants of thermal deactivation, etc.) and the degree of PS II trap closure. The intrinsic photochemical yield ϕ_p is obtained by dividing the oxygen yield by q_P. ϕ_p gives the (fictive) yield of open reaction centers. Thus changes in ϕ_p do not reflect variations in the degree of trap closure but do reflect conformational changes. According to Fig. 1, ϕ_p decreases with time after the increase in light intensity. The decrease can be described by a single exponential and proceeds with the same time-constant as in the case of the F'_m- and the F'_0-decrease as shown in Fig. 1B.

In Fig. 2, the relationship between the quenching of the F'_m fluorescence and the 'quenching' of the intrinsic photochemical yield is shown. The relation between F'_m and ϕ_p is given by a straight line.

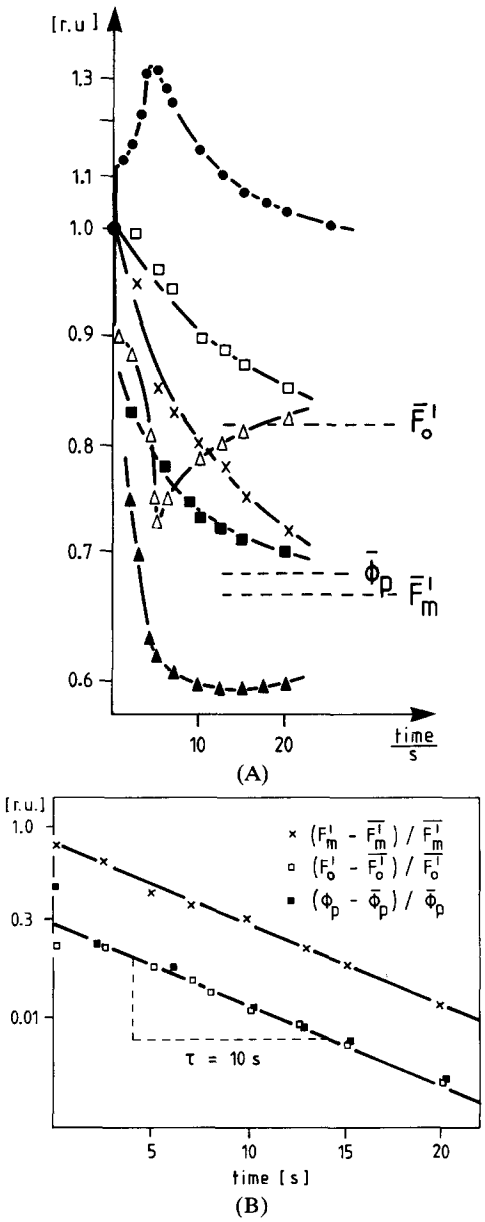

Fig. 1. Responses of the fluorescence yield F (closed circles), the minimum fluorescence F'_0 (open squares), the maximum fluorescence F'_m (crosses), the quenching coefficient q_P (open triangles), the intrinsic photochemical yield ϕ_p (closed squares) and the oxygen yield r (closed triangles) to an increase in light intensity. q_P and ϕ_p were computed according to Eqs. (a) and (b), respectively. At $t = 0$ the light intensity was increased stepwise from 10.2 to 30.2 W m^{-2}. (A) Plot of the responses with a linear scale of the ordinata. All amplitudes are normalized to their respective value before increasing the light intensity. (B) Semilogarithmic plot of F'_m, F'_0 and ϕ_p. The normalized differences as defined in the upper left corner of (B) are plotted vs. time. $\bar{F}'_m$, $\bar{F}'_0$ and $\bar{\phi}_p$ are given by the broken lines in (A).

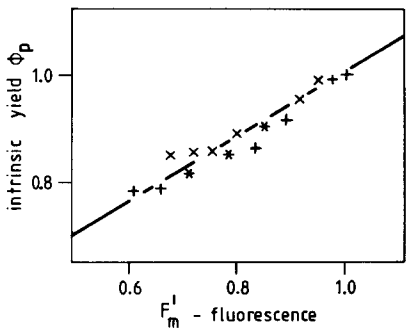

Fig. 2. Relationship between F'_m and the intrinsic photochemical yield ϕ_p. The three different symbols corresponds to experiments on different leaves. The experimental conditions are the same as in Fig. 1. The data points were obtained by using the corresponding F'_m and ϕ_p values measured at different times ($t = 0 \ldots 40$ s) after the increase in light intensity.

The decrease of the fluorescence yield and of the intrinsic photochemical yield might originate from an increase in the probability of thermal deactivation of absorbed light energy. For studying this possibility several experiments with the high-frequency photoacoustic signal (thermal signal) were performed.

In Fig. 3 the response of the high-frequency (thermal) photoacoustic signal A to an increase in light intensity is shown. The thermal signal increases concomitant to the decrease in fluorescence F which originates from the formation of q_E. However, the increase in the rate of thermal

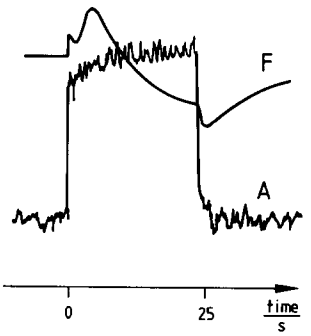

Fig. 3. Responses of the high frequency (thermal) photoacoustic signal A and of fluorescence yield F to an increase in light intensity. At $t = 0$ s the intensity of the photoacoustic measuring light (Balzers DT-cyan special filter, modulated with 276 Hz) was increased from 10 to 30 W m^{-2}. At $t = 25$ s the intensity of the measuring light was decreased to 10 W m^{-2}. Both signals, A and F, were recorded with a time-constant of 100 ms of the related lock-in amplifier.

deactivation as shown in Fig. 3 might originate from an increased heat emission of PS I in consequence of PS I trap closure. Therefore it appears to be difficult to get further insights in the mechanism of q_E formation by studying such transients.

In order to simplify the situation a different experimental approach was used. We studied the formation of q_E following an increase in light intensity up to a level leading to complete saturation of photochemistry (800 W m^{-2}). It is reasonable to assume that under these high-light conditions all PS II and PS I traps are closed. The photochemical yield approaches zero. The fluorescence yield F approaches the F'_m level.

Figure 4A and 4B show the responses of fluorescence yield F and of the thermal photoacoustic signal A to an increase in light intensity up to more than 800 W m^{-2}. In both experiments an additional non-modulated light was added at $t = 0$ (800 W m^{-2}, shortpass filter Balzers DT-cyan special). In the case of Fig. 4B the intensity of the modulated light (which was employed for measuring the photoacoustic signal) was increased from 10 to 100 W m^{-2} simultaneously to the application of the additional non-modulated light. By virtue of this method the kinetics of the photoacoustic signal as induced by the increase in light intensity can be studied with a ten times better signal to noise ratio as in Fig. 4A.

As shown in Fig. 4A the additional saturating light causes a fast increase in the thermal photoacoustic signal. This well-known phenomenon (Bults et al. 1981, Poulet et al. 1983) reflects the increase in the rate of thermal deactivation as a consequence of the vanishing photochemical yield. The fluorescence signal F increases up to the level F'_{m1}. This increase is also a consequence of the vanishing photochemical yield (trap closure). Then F decreases and reaches the F'_{m2} level at $t = 40$ s. This decrease reflects the formation of the high-energy state (Laasch 1987). In Fig. 4A no changes of the rate of thermal deactivation are detectable which occur concomitantly to the q_E-related decrease of fluorescence. However, by virtue of the improved signal to noise ratio such changes are apparent in Fig. 4B. Concomitant to the decrease of fluorescence by about 60% the rate of thermal deactivation increases by about 5%.

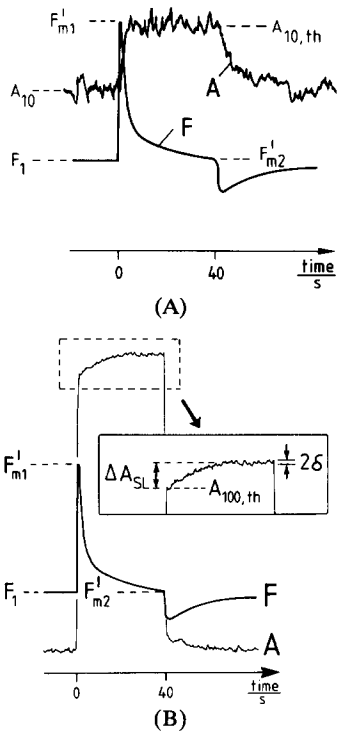

Fig. 4. Responses to the application of saturating light of the high frequency (thermal) photoacoustic signal A and of the fluorescence yield F. At $t = 0$ s a non-modulated background light (800 W m^{-2}, Balzers DT-cyan special) was added, at $t = 40$ s it was removed again. In (A) the intensity of the measuring light (10 W m^{-2}, Balzers DT-cyan special, 276 Hz) remains unchanged in the course of the experiment. In (B) the intensity of the modulated light was increased from 10 to 100 W m^{-2} simultaneously with the addition of the non-modulated background light. Both lock-in time-constants for recording F and A were set on 100 ms both.

Table 1. Effect of saturating light on chlorophyll fluorescence and the high-frequency photoacoustic signal. The upper line gives the frequency of the photoacoustic measuring light. The meaning of A_{10}, $A_{10,th}$, $A_{100,th}$, A_{th}, F_1, F'_{m1}, F'_{m2} is given in Fig. 4. σ which gives the standard deviation of the $A_{100,th}$ signal was determined as described in Materials and Methods. The experimental conditions are the same as in the case of the experiments shown by Fig. 4

Frequency/Hz	276	457	1100	2900
$L = 1 - A_{10}/A_{10,th}$	0.29	0.26	0.25	0.25
$\Delta A_{SL}/A_{100,th}$	5.1%	5.9%	5.2%	5.5%
$\sigma/A_{100,th}$	0.7%	1.2%	1.8%	2.5%
F_1/F'_{m1}	0.39	0.40	0.40	0.41
F'_{m2}/F'_{m1}	0.40	0.42	0.41	0.42

The first column of Table 1 shows the results of the measurements of Fig. 4 in quantitative terms. The results given in columns 2 to 4 were obtained by using the same experimental protocol, but with different frequencies of the measuring light as indicated in the upper line. All measurements were done on the same spinach leaf. The saturating light was applied for 40 s with an interval of 10 min in between.

The third line of Table 1 gives the normalized standard deviation of the photoacoustic signal. This scatter factor seems to indicate a considerable uncertainty in the values of $\Delta A_{SL}/A_{100,th}$ at higher frequencies of the modulated light. However, A_{SL} and $A_{100,th}$ were determined by an eye-fit of the timecourse shown in the inset of Fig. 4B. Thus the whole curve was taken into account for the determination of A_{SL} and $A_{100,th}$. We estimate that even at the highest frequency of the modulated light the actual uncertainty in the values given in the second line of Table 1 is less than 25%.

For all frequencies the photochemical loss L was detected as shown in Fig. 3A. L does not decrease significantly with increasing modulation frequencies. There seems to be no significant increase in artefacts like contribution to the photoacoustic signal arising from absorption by the wall of the measuring cell. Otherwise L would tend to decrease with modulation frequency. For a further discussion of Table 1 see below.

Measurements on spinach leaves of different batches and on leaves of *Aegopodium podagraria* led to identical results.

Discussion

Relationship between the photochemical yield and q_E

Results of Weis and Berry (1987) and Peterson et al. (1988) strongly suggest that the q_E quenching of maximum fluorescence F'_m is coupled to a 'quenching' of the intrinsic photochemical yield ϕ_p. This suggestion is strengthened by the data shown in Figs. 1 and 2.

In the studies mentioned above, correlations between the steady-state values of F'_m and of ϕ_p were found. Both F'_m and ϕ_p are influenced by

several non-photochemical quenching effects. Thus, not the relation between q_E and ϕ_p but the relation between $q_N(=q_E + q_T + q_I + \cdots)$ and ϕ_p were studied. In contrast, we try to separate the q_E quenching effect from other non-photochemical quenching mechanisms by analyzing individual components of the kinetics of the F'_m, F'_0 and ϕ_p changes as induced by an increase in light intensity.

In the range of seconds, a mono-exponential decay of the three signals with a common time-constant of about 10 s was observed (Fig. 1). This time-constant corresponds to the time-constant τ_4 of previous investigations. There is considerable evidence that it originates from an increase in thylakoid pH-gradient (Hansen et al. 1987, Dau and Hansen 1989, Vanselow et al. 1988, Vanselow et al. 1989). Thus, the increase in thylakoid pH-gradient with the time-constant τ_4 leads to the formation of q_E causing the decrease of F'_m, F'_0 and ϕ_p shown in Fig. 1. The formation of q_I and q_T, however, have been found to occur on a much slower time scale (range of minutes). In Fig. 2 only changes of F'_m and ϕ_p are considered which occur within 40 s after the increase in light intensity. Therefore the data shown in Fig. 2 should be scarcely influenced by non-photochemical quenching mechanisms different from energy-dependent quenching.

Despite of the different experimental approaches the results obtained by means of a kinetic analysis are in line with the results of Weis and Berry (1987). A linear relationship between F'_m and ϕ_p is found (Fig. 2). However, for smaller values of F'_m we cannot exclude a nonlinear relationship as found by Peterson et al. (1988) because of the limited range of the ϕ_p and F'_m changes shown in Fig. 2.

As discussed above, the present study and studies of other groups indicate that the formation of q_E decreases fluorescence as well as the intrinsic photochemical yield. For instance, Fig. 1B shows that F'_0, the fluorescence of open PS II centers, decreases to the same extent as ϕ_p, the photochemical yield of open PS II centers, does. The most likely explanation for this behavior is that a process being competitive to fluorescence and photochemistry causes the quenching of both. In the following three basically different

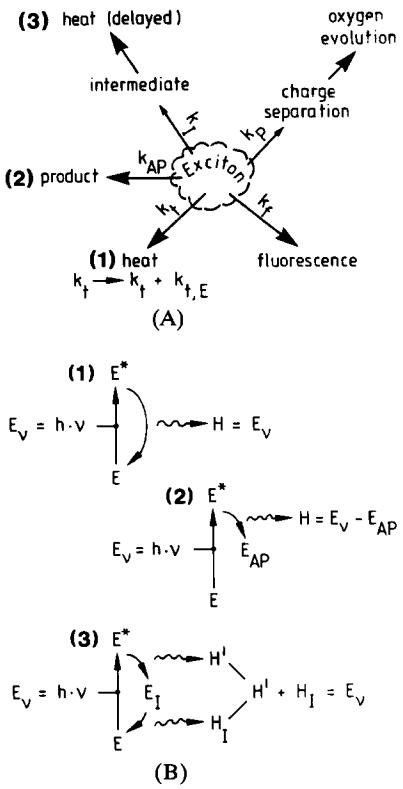

Fig. 5. Schemes of energy levels for possible processes leading to q_E quenching of fluorescence. H denotes the energy released in form of heat. (1) The energy of the absorbed photon (E_ν) leads to an excited chlorophyll state (E*). The lifetime of the excited chlorophyll state decreases because of an increased probability of thermal deactivation. If the exciton decays thermally the energy E_ν is immediately and completely released in form of heat. (2) Part of the excitation energy E_ν is stored by a (still unknown) photochemical process not leading to oxygen evolution. Thus the excitation energy is only partially released in form of heat. (3) Part of the excitation energy is used for building up an excited state of an intermediate. This excited state decays and the energy $E_I - E$ is released thermally. Thus the exciton energy is completely released in form of heat. However, the dissipation of $E_I - E$ is delayed by the lifetime of the intermediate state.

possibilities concerning the nature of this competitive process are discussed along the results given in Table 1. A survey of the three alternatives is given in Fig. 5.

1. *Increase in the probability of thermal deactivation.* (Fig. 5-1)

The competitive process is given by an increased thermal deactivation of antenna or reaction cen-

ter chlorophylls. The thermal deactivation occurs immediately after absorption, i.e., within less than 5 ns.

The formation of q_E was studied under saturating light conditions. Therefore, the photochemical yield approaches zero. We have to consider only two pathways of dissipation of absorbed energy, the thermal deactivation and fluorescence emission. Thus the following continuity equation holds. (The yields of absorption, thermal deactivation and fluorescence are ϕ_a, ϕ_t and ϕ_f, respectively.)

$$\phi_a = \phi_t + \phi_f \quad (1)$$

If we assume that ϕ_a remains constant we obtain Eq. (2).

$$\Delta\phi_t = -\Delta\phi_f \quad (2)$$

Changes of ϕ_t and ϕ_f were detected by the photoacoustic signal and the fluorescence signal, respectively. According Table 1 we observe an increase in the yield of thermal deactivation by about 5% concomitant to the q_E quenching of the fluorescence yield by about 60%. In the following it is shown that these values are of reasonable magnitudes.

The insertion of the relative changes into Eq. (2) leads to:

$$0.05\phi_{t,0} = 0.6\phi_{f,0} \quad (3)$$

By insertion of (3) in (1) we get:

$$\phi_{f,0} = 0.076\phi_{a,0} \quad (4)$$

Thus, under saturating conditions in the absence of the high-energy state (F'_{m1}-level) 7.6% of the absorbed light energy is emitted by the leaf in form of fluorescence light. This is a reasonable value compared to the values of about 10% as found by other authors (Lavorel and Etienne 1979).

In conclusion, the photoacoustic data on thermal deactivation is in line with the suggestion that the energy-dependent quenching of fluorescence originates from an increased probability of thermal deactivation in the antennas or in the reaction centers of PS II.

2. *Alternative energy storing process* (Fig. 5-2)

Part of the absorbed light energy might be stored by a (still unknown) alternative photochemical pathway. This alternative process would compete with fluorescence and with the photochemistry which leads to oxygen evolution.

Such a mechanism is consistent with the results on the relationship between the intrinsic photochemical yield and q_E as discussed above. However, if the q_E quenching of fluorescence originates from activation of a competitive energy storing process, a decrease in the yield of thermal deactivation should occur, since an increasing part of the absorbed light energy is dissipated neither by fluorescence nor by means of thermal deactivation. Such a decrease is not observed, thus we can exclude that the q_E quenching of fluorescence as induced by application of saturating light is caused by an alternative energy storing process.

3. *Delayed thermal deactivation by a futile cycle* (Fig. 5-3)

Part of the absorbed light energy is transfered to an intermediate. The energized intermediate has a limited life-time T_I and the decay leads to a delayed heat emission. (Such a process might be a cyclic way of electron transport around PS II).

The photoacoustic signal gives the yield of the modulated heat emission. Only that part of thermal deactivation is detected which occurs within a certain 'time window'. The time window depends on modulation frequency (Malkin and Cahen 1979). At low modulation frequencies we can detect also the heat emission which is delayed by an intermediate. Thus a behavior as described under (1) has to be expected. However, at high modulation frequencies – higher than $(2\pi T_I)^{-1}$ – the delayed heat emission is not detected in photoacoustic signal. Thus the situation resembles the energy storing case already discussed above (2).

In Table 1 the results are given for different frequencies of the modulated light ranging from 276 to 2900 Hz. At all frequencies of the modulated light we observe the same behavior, i.e., the formation of q_E leads to an increased thermal deactivation (see 1) within the time window de-

fined by the modulation frequency. Thus, there are no hints that the q_E formation originates from the activation of a futile cycle. However, a faster cycle can not be excluded. If a futile cycle would cause the q_E quenching of fluorescence in the experiments shown in Fig. 3 the lifetimes T_I of involved intermediate states had to be shorter than 50 μs (T_I smaller than $(2\ \pi\ 2900)^{-1}$).

Conclusions

The comparison of the intrinsic photochemical yield ϕ_p and F_m' (Figs. 1 and 2) strongly suggests, that the q_E quenching of fluorescence originates from a process being competitive to fluorescence as well as to photochemistry. The analysis of q_E formation by studying the thermal deactivation by means of photoacoustics is leading to results which are in line with the proposal that the q_E quenching originates from an increase in the probability of thermal deactivation of excited antenna or reaction center chlorophylls. The possibility that the competitive process is a still unknown energy storing process can be excluded. There are also no hints that the competitive process behaves like a futile cycle (e.g., a cyclic way of electron transport around PS II). However, by the data given in Table 1 this possibility cannot be excluded completely. If such a cycle would exist, the lifetimes of the intermediate states would have to be less than 50 μs.

It has to be mentioned that results of Oxborough and Horton (1988) can be interpreted in terms of two different mechanisms which lead to a decrease of the intrinsic photochemical yield concomitant to the formation of the thylakoid pH-gradient. One mechanism seems to be coupled to the q_E quenching of fluorescence, the other one does not. Thus, the relation between ϕ_p and F_m' (Fig. 2) may result from two different pH-dependent effects or states. In the case of the experiments under saturating light conditions (Fig. 4, Table 1), however, only the q_E mechanism could be studied. The fact that according to the experiments of Oxborough and Horton the other mechanism does not influence F_m' despite its influence on ϕ_p indicates that it is saturated by high light intensities, i.e., its yield approaches zero.

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