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REGULAR PAPER

Heat stress and the photosynthetic electron transport chain of the lichen *Parmelina tiliacea* (Hoffm.) Ach. in the dry and the wet state: differences and similarities with the heat stress response of higher plants

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Abstract Thalli of the foliose lichen species *Parmelina tiliacea* were studied to determine responses of the photosynthetic apparatus to high temperatures in the dry and wet state. The speed with which dry thalli were activated by water following a 24 h exposure at different temperatures decreased as the temperature was increased. But even following a 24 h exposure to 50°C the fluorescence induction kinetics OJIP reflecting the reduction kinetics of the photosynthetic electron transport chain had completely recovered within 128 min. Exposure of dry thalli to 50°C for 24 h did not induce a K-peak in the fluorescence rise suggesting that the oxygen evolving complex had remained intact. This contrasted strongly with wet thalli were submergence for 40 s in water of 45°C inactivated most of the photosystem II reaction centres. In wet thalli, following the destruction of the Mn-cluster, the donation rate to photosystem II by alternative donors (e.g. ascorbate) was lower than in higher plants. This is associated with the near absence of a secondary rise peak ($\sim$1 s) normally observed in higher plants. Analysing the 820 nm and prompt fluorescence transients suggested that the M-peak (occurs around 2–5 s) in heat-treated wet lichen thalli is related to cyclic electron transport around photosystem I. Normally, heat stress in lichen thalli leads to desiccation and as consequence lichens may lack the heat-stress-tolerance-increasing mechanisms observed in higher plants. Wet lichen thalli may, therefore, represent an attractive reference system for the evaluation of processes related with heat stress in higher plants.

Keywords Fast fluorescence rise OJIP · High temperature · Lichens · *Parmelina tiliacea* · M-peak

Abbreviations

Chl	Chlorophyll
DF	Delayed fluorescence
F_0	Fluorescence intensity at 20 μs
F_K	Fluorescence intensity at $\sim$0.3 ms
F_J	Fluorescence intensity at $\sim$3 ms
F_I	Fluorescence intensity at $\sim$30 ms
F_P	The maximum measured fluorescence intensity
F_M	Fluorescence intensity when all PSII reaction centres are closed
$I_{820\ nm}$	A measure for the reflected light at 820 nm
O–J–I–P–S–M	Transient fluorescence induction transient defined by the names of its intermediate steps
P680 and P700	The primary electron donors of photosystems II and I, respectively
PF	Prompt fluorescence
PI	Performance index

Introduction

Lichens are a symbiotic association between a fungus (heterotrophic mycobiont) and a photosynthetic partner

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(autotrophic photobiont). The discovery of 600 million year old lichen-like fossils indicates that fungi developed symbiotic partnerships with photoautotrophs before the evolution of vascular plants (Yuan et al. 2005). Lichens are poikilohydric organisms; they have the ability to tolerate complete dehydration, which induces a latent state characterised by a total inactivation of photosynthetic gas exchange and loss of variable chlorophyll (Chl) *a* fluorescence (Kappen and Breuer 1991; Jensen et al. 1999; Heber and Shuvalov 2005). However, dehydrated lichens can restore their photosynthetic activity following rewetting with water within a few minutes (Coxson 1988; Lange et al. 1989; Soni and Strasser 2008). Lichens are obliged to tolerate desiccation because they do not possess stomata (Woodward 1998) and, therefore, cannot regulate their evaporation in this way. Evaporative cooling is an important mechanism by which lichens maintain their thallus temperature (Hoffman and Gates 1970; Gauslaa and Solhaug 1998). However, lichens cannot stop the evaporation and this leads regularly to desiccation of the lichens. Lichens can make use of a range of adaptation strategies that allow them to cope with and survive extreme environmental conditions (Rogers 1988; Kappen 2000; Romeike et al. 2002). Dehydration-induced partitioning of amphiphilic compounds in and around the membranes and the immobilisation of the cytoplasm in a stable multicomponent glassy matrix allows lichens to maintain the functional integrity of their membranes in the desiccated state (Wolfe and Bryant 1999). Black and Pritchard (2002) reported that there are at least two key elements that determine the survival of living systems such as lichens for extended periods in a desiccated state: (1) a shutdown of their metabolism during desiccation and (2) a cellular organisation and enzymes that allow a successful start-up of the organism the moment rehydration occurs.

The Chl *a* fluorescence method was used as a non-invasive tool for the analysis of photosynthesis in lichen organisms (Barták et al. 2000, Ilík et al. 2006; Heber et al. 2011). Like all photosynthetic organisms, lichens exhibit a polyphasic OJIP Chl *a* fluorescence rise during the first second of illumination following a dark-to-light transition (e.g. Ilík et al. 2006 and see Fig. 3a for some examples of such OJIP-transients). The fluorescence rise from the minimum fluorescence intensity O to the first intermediate step 'J' that is reached after ~3 ms is generally called the photochemical phase (Delosme 1967). This phase is strongly light dependent (Neubauer and Schreiber 1987; Lazár 2006; Schansker et al. 2006) and contains information on antenna size and connectivity between photosystem II (PSII) reaction centres (Strasser et al. 2004). The J to P rise (~3 to ~200 ms) is called the thermal phase (Delosme 1967) and reflects a reduction of the rest of the electron transport chain (Schansker et al. 2005). The J-to-I-rise can be associated with the reduction of the PQ-pool (Tóth et al. 2007a) and the I-to-P-rise with electron flow through PSI (Schreiber et al. 1989; Schansker et al. 2003; 2005). The slow fluorescence kinetics that follow the P step differ between angiosperms and other organisms. The rather broad P-step observed in angiosperms is split in two peaks in diatoms (Caron et al. 1987), foramifers (Tsimilli-Michael et al. 1998) and lichens (Ilík et al. 2006). As shown by Ilík et al. (2006), the almost immediate fluorescence decline that followed the P-step was accompanied by a fast oxidation of PC and P700 suggesting that it was a consequence of a much faster activation of the ferredoxin-$NADP^+$-reductase (FNR) than in angiosperm plants. Tsimilli-Michael et al. (1998) called the two peaks of the split P-level H and G, respectively. However, we will follow here the more traditional P–S–M nomenclature of Caron et al. (1987).

Chl *a* fluorescence transients (OJIP) were employed here as an intrinsic probe (Strasser et al. 1995; Papageorgiou and Govindjee 2004; Zhu et al. 2005; Lazár 2006) to assess the properties of PSII and the photosynthetic electron transport chain as a whole to explore the behaviour of the photosynthetic system of *Parmelina tiliacea* lichens in the dehydrated and rehydrated state.

The primary objective of this study is to investigate the responses of lichens in the dry and wet state to high temperatures.

Materials and methods

Sample collection

Apparently healthy thalli of *P. tiliacea* (Hoffm.) Ach. were collected in April 2009 from the south sides of oak trunks, around the laboratory in Jussy, Geneva (Switzerland). *Parmelina* is a genus of lichenised fungi with a widespread distribution. The photobiont is *Trebouxia jamesii*. All lichens were brought to the laboratory the same day. The collected lichen thalli were air-dried for 48 h at room temperature under low light conditions (~12 µmol photons m^{-2} s^{-1}).

Sample preparation

For each experiment, fragments (four or five samples) of a single thallus were used. Before the start of each experiment one cm long pieces of the youngest lobes of selected thalli for each measurement were cut. With respect to the fluorescence measurements we note that (1) the fluorescence transients measured on the dry thalli did not show any rise kinetics suggesting that the photosynthetic apparatus was in an inactive state (Fig. 1a) and (2) following re-

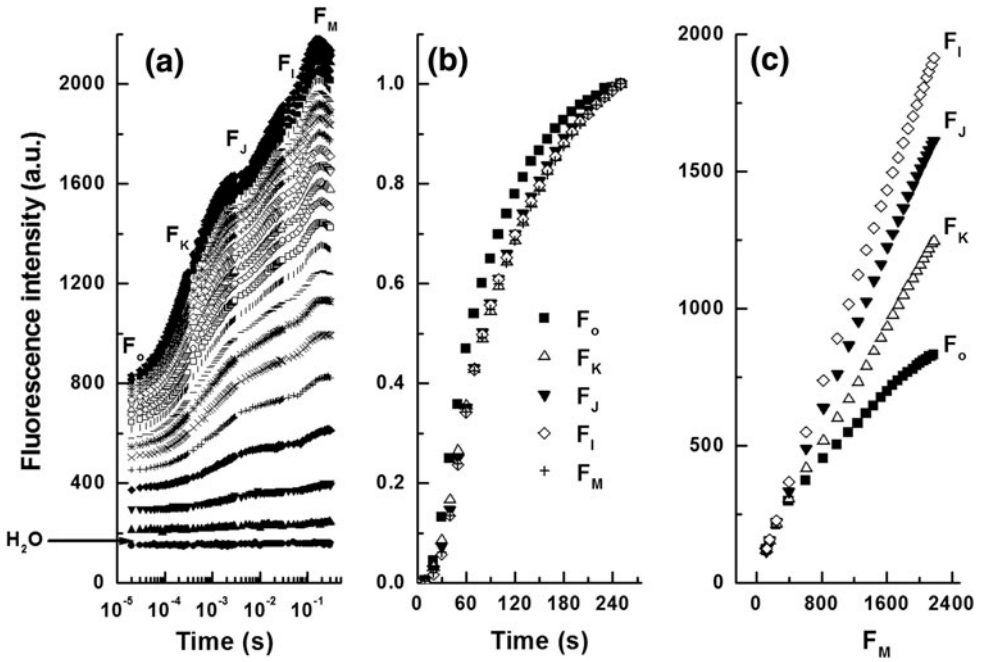

Fig. 1 Activation kinetics of the photosynthetic electron transport chain of air-dried thalli of *P. tiliacea*, as reflected by the recovery kinetics of the OJIP fluorescence rise, following the addition of a drop of water. **a** Measurements of polyphasic OJIP following addition of water to the upper surface. The fluorescence transients were induced by 300 ms pulses of strong light (~3,000 μmol photons m^{-2} s^{-1}) separated by 9.7 s of darkness. **b** Fluorescence intensities of the parameters $F_{0\ (20\ \mu s)}$, $F_{K\ (300\ \mu s)}$, $F_{J\ (2\ ms)}$, $F_{I\ (30\ ms)}$ and F_M as a function of the time after the addition of water. The values of the fluorescence parameters were double normalised between the minimum value obtained at time = 0 min and maximum value obtained after completion of the activation process. **c** *Scatter plots* of the parameters shown in Fig. 1b as a function of F_M. The second fluorescence measurement was made 1–2 s after the addition of a drop of water. The fluorescence transients are the averages of five repetitions of the experiment

activation there were no differences in the kinetics of OJIP-transients measured either after 5 min of dark adaptation or 1 h of dark adaptation (A Oukarroum, unpublished observation), suggesting that the dry thalli, for all practical purposes, were in the dark-adapted state.

Pea plants

The seeds of pea (*Pisum sativum* L. cv. Ambassador) were allowed to germinate for 48 h on a sheet of Whatman filter paper placed in Petri dishes. The germinated seeds were transplanted into plastic pots (4 l) containing commercial peat soil with three plants per pot and in five replicates. In the growth chamber experiment, the plants were grown in a controlled environment with day/night air temperature of 25/18°C, under long-day conditions (16 h light, 8 h dark) and by giving additional light if needed (OSRAM HQIT 400 W lamps were used). Mature leaves of 2–3-week-old pea plants were used. For the measurements of Fig. 3, detached pea leaves were submerged in water of the indicated temperature for 40 s, kept for 2 min at room temperature and then measured using 5000, 10000 and 15000 μmol photons m^{-2} s^{-1} light.

Measuring equipment

All Chl *a* fluorescence measurements were conducted on thalli of *P. tiliacea* with a fluorometer (Plant Efficiency Analyser, Hansatech Instruments Ltd, King's Lynn, UK) with high time resolution (10 μs) with a maximum pulse intensity of ~3,000 μmol photons m^{-2} s^{-1} provided by three light-emitting diodes with a peak emission at 650 nm.

For the measurements of the prompt fluorescence (PF) and the delayed fluorescence (DF) and 820 nm signal, respectively, an M-PEA instrument (Hansatech Instruments Ltd, UK) was used. For further technical details of the M-PEA instrument see Strasser et al. (2010). The red actinic light of the M-PEA had a peak emission at 627 nm. For the DF-measurements the average of the DF-intensities measured between 10 and 30 μs following lights off was used in Fig. 4. The effective light intensity during simultaneous Chl *a* fluorescence and DF-measurements was considerably lower than the effective light intensity of the simultaneous Chl *a* fluorescence and 820 nm reflection measurements, because the light was periodically turned off to allow the recording of the DF-signal.

Fluorescence parameters

Maximum quantum yield of primary photochemistry φ_{Po} (F_V/F_M) and performance index [PI = $[(1 - F_0/F_M)/(M_0/V_J)] \cdot [(F_M - F_0)/F_M].[(1 - V_J)/V_J)]$, where $M_0 = 4 \cdot (F_{270\ \mu s} - F_0)/(F_M - F_0)$ and $V_J = (F_J - F_0)/(F_M - F_0)$] were used to study the recovery of heat-stressed dry thalli following rehydration. The parameter φ_{Po} corresponds to the efficiency by which an absorbed photon will be trapped by PSII reaction centres (Krause and Weis 1991). The parameter PI is designed as a Nernst equation and consists of an antenna, reaction center trapping, and PSII acceptor side related component (Strasser et al. 2004). The F_V/F_M-value is probably the most widely used fluorescence parameter in stress research, but it has the disadvantage that it probes only one property of PSII that is sensitive to only a limited number stress conditions. The PI probes three different properties and is, therefore, sensitive to a wider range of stress conditions. Drought stress is a good example of this difference in sensitivity (Oukarroum et al. 2006, 2007).

Rehydration experiments

Rehydration experiments were conducted on dry thalli, following the addition of water. To follow the rehydration process a series of 300 ms pulses (3,000 µmol photons $m^{-2}\ s^{-1}$) spaced 9.7 s apart was given to the samples and the emitted fluorescence was measured.

High temperature treatments

Experiments on dry thalli

Dry thalli of *P. tiliacea* were kept at various temperatures (25, 30, 35, 40, 45 and 50°C) for 24 h in darkness. After the temperature treatments the thalli were transferred to room temperature conditions. The Chl *a* fluorescence measurements were made immediately (5 s) after the transfer to room temperature. Rehydration was initiated adding a drop of water to the thalli and the Chl *a* fluorescence was measured again after 4, 16, 64 and 128 min. After 128 min the thalli were still in the wet state.

Experiments on hydrated thalli

To heat stress hydrated thalli, dark-adapted hydrated thalli of *P. tiliacea* were submerged in water of various temperatures for 40 s in the dark to obtain a homogeneous heating of the samples. Following the heat treatment, the thalli were taken out of the water and transferred to room temperature. After 2 min of acclimation to the room temperature conditions, Chl *a* fluorescence—induced by a 10 s light pulse of 3000, 5000, 10000 and 15000 µmol photons $m^{-2}\ s^{-1}$—was measured. For the assessment of the donor side of PSII only thalli heat stressed at 45 and 50°C were studied. In this case, the fluorescence measurements consisted of two 5 ms pulses spaced a time Δt apart with Δt ranging between 2.3 and 500 ms. To study the regeneration of the K-peak the F_K–F_0 amplitude was plotted as a function of Δt; this yields an exponential curve of which the halftime was determined (Tóth et al. 2007b). For PSII reaction centres lacking a functional manganese cluster, the time dependence of the regeneration of the K-peak following the first pulse is a measure for the re-reduction kinetics of Tyr_Z^+ (Tóth et al. 2007b, 2009).

Results

Dry *P. tiliacea* thalli did not show an increase in their fluorescence intensity on a dark-to-light transition (Fig. 1a). Following the addition of water, the fluorescence intensity started to increase after a short lag-time (20–30 s), exhibiting the OJIP fluorescence transient within 3–4 min with all intermediate steps F_J, F_I, F_M as shown before (Soni and Strasser 2008). The recovery kinetics of the $F_{0\ (20\ \mu s)}$, $F_{K\ (300\ \mu s)}$, $F_{J\ (2\ ms)}$, $F_{I\ (30\ ms)}$ and F_M fluorescence intensities on rehydration showed similar kinetics (Fig. 1b). Only the minimum fluorescence F_0 showed slightly faster relative recovery kinetics than the other parameters. Figure 1 b also illustrates that during the first 30 s following the addition of a drop of water there is little change in the fluorescence intensities. The fact that F_J, F_I and F_P have relative recovery kinetics that are nearly identical suggests that the photosynthetic electron transport chain beyond PSII was maintained in a potentially active state. The close parallelism between the recovery kinetics of the different fluorescence induction steps is further illustrated in Fig. 1c, where the fluorescence intensities F_0, F_K, F_J, F_I are shown as a function of F_M.

To assess the heat resistance of dry *P. tiliacea* thalli, they were exposed for 24 h to different temperatures (25–50°C). Following the addition of water and during the initial steps of the recovery process a gradual regeneration of the OJIP transient was observed. After 128 min of recovery, all samples exhibited again the full OJIP kinetics (data not shown). In Fig. 2, these recovery kinetics are illustrated using the F_V/F_M and the PI. For both the F_V/F_M and the PI full recovery was observed 128 min after the samples were wetted for all treatment temperatures. After 4 min of recovery all temperature treatments showed their own particular effect as reflected by the measured F_V/F_M and PI values. At longer recovery times the thalli treated at 25, 30 and 35°C and the thalli treated at 40, 45 and 50°C,

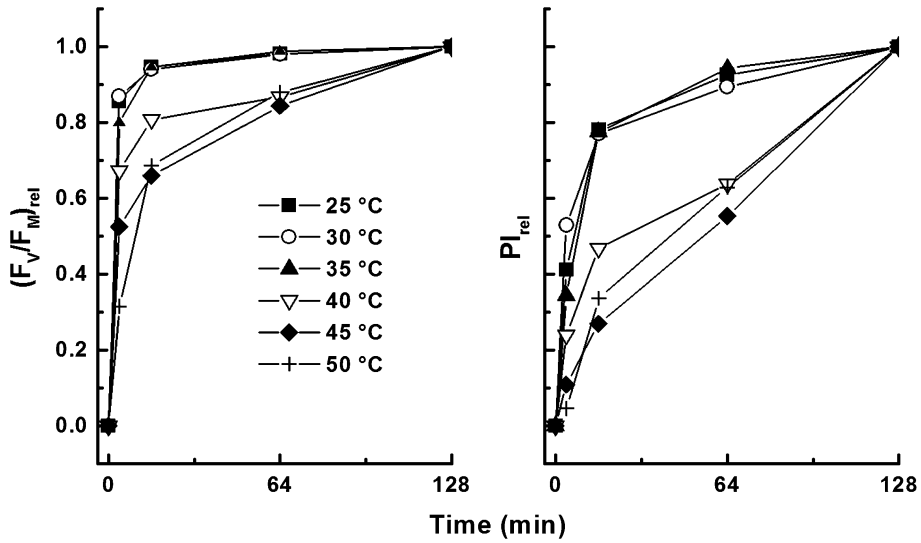

Fig. 2 Recovery of the maximum quantum yield of primary photochemistry (F_V/F_M) and PI of air-dried thalli of *P. tiliacea* following 24 h of exposure to different temperatures, addition of a drop of water and 0, 4, 16, 64 and 128 min recovery. The water was added immediately after the samples were transferred to room temperature and the first measurement (time 0) was made ~5 s after the addition of the drop of water. The measurements are the averages of 3–4 repetitions of the experiment

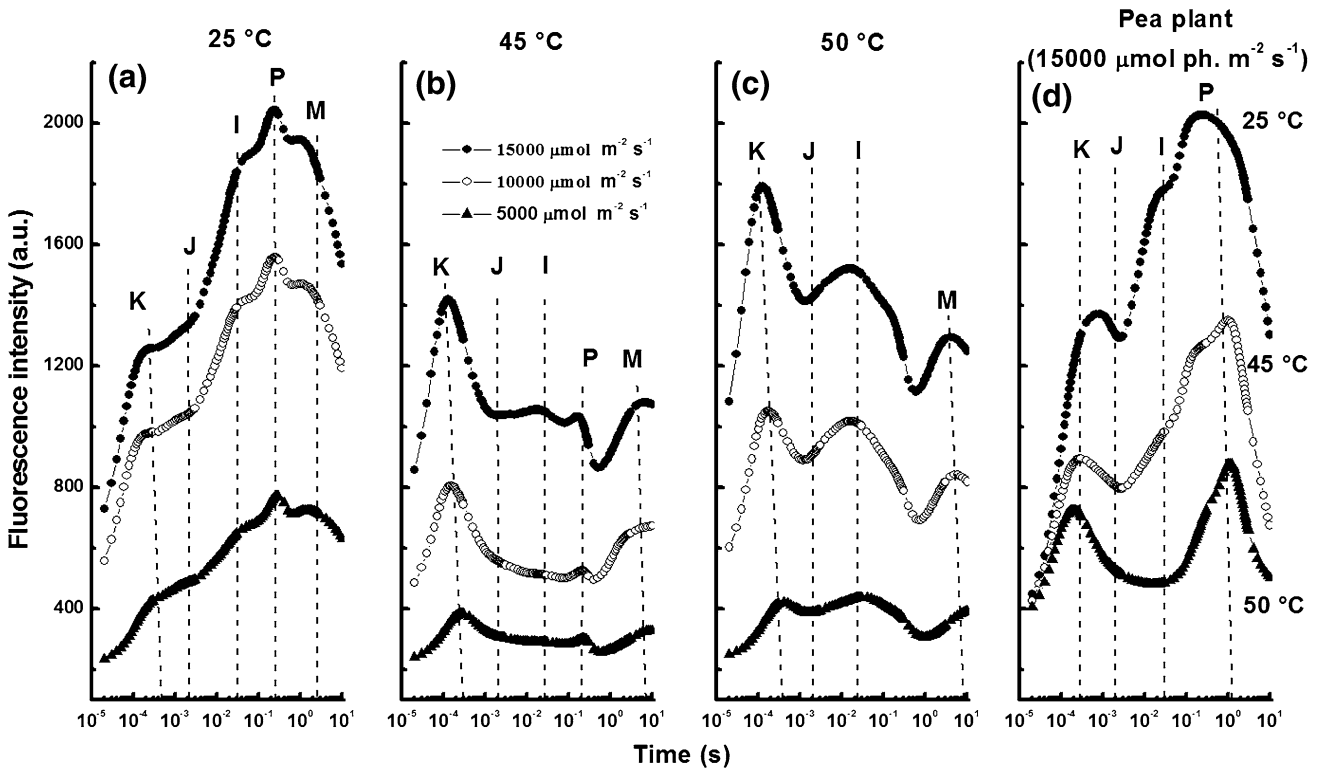

Fig. 3 Comparison of OJIP transients of untreated (25°C) (**a**) and heat-treated [40 s at 45 (**b**) or 50°C (**c**)] wet *P. tiliacea* thalli and of control (untreated) and heat-treated (40 s of 45 and 50°C) pea leaves (**d**). The fluorescence transients were measured at 25°C and induced by a 10 s light pulse of 5000, 10000 and 15000 µmol photons m^{-2} s^{-1}. The OJIP transients measured on the pea leaves were all induced by a 10 s pulse of 15,000 µmol photons m^{-2} s^{-1}. The transients are the averages of five independent measurements

respectively, started to recover together with the same time dependence.

Leaves of angiosperms measured at room temperature (25°C) yield a typical OJIP rise with a rather flat P step (0.2–2 s) (Strasser et al. 1995). For *P. tiliacea* thalli, the P-level splits into two peaks (cf. Ilík et al. 2006), the first peak P (0.2 s) is followed immediately by a decrease of the fluorescence intensity to a dip S (~0.7 s) and a subsequent rise to a peak M reached after ~2 s of illumination (Fig. 3). In Fig. 3, the effects of light intensity and temperature on the OJIP-kinetics are illustrated and a comparison is made with similar measurements on pea leaves.

OJIP transients were measured at light intensities of 5000, 10000 and 15000 μmol photons m^{-2} s^{-1} following a treatment at 25, 45 and 50°C for *P. tiliacea*. For the pea leaves the same treatments were applied (to untreated pea leaves), but the fluorescence transients were only measured at 15,000 μmol photons m^{-2} s^{-1} (Fig. 3d). Comparing the 25°C treatments a striking difference is observed in the K–J-region of the fluorescence rise. In pea leaves at high light intensities a dip around 2–3 ms of illumination is observed that is probably due to a transient increase in the $P680^+$ concentration in reaction centres in the S_4 state (Schansker et al. 2011). This dip is missing in lichens (Fig. 3a); on increasing the light intensity from 5,000 to 15,000 μmol photons m^{-2} s^{-1} there is no qualitative change in the O–K–J–I-rise. Another difference with the pea leaves is that in lichens both the K and J steps can be observed in a single measurement in thalli kept at 25°C. For the heat stressed thalli it was observed that on increasing the light intensity from 5,000 to 15,000 μmol photons m^{-2} s^{-1}, the relative intensity of the K peak increased and its peak position gradually shifted from 0.3 to 0.16 ms as observed by Tóth et al. (2007b) in barley plant treated at 48°C. The peak around the I-step that is so prominent following a heat treatment at 50°C (Fig. 3c) is observed in response to illumination of the thalli treated at 45°C with 15,000 μmol photons m^{-2} s^{-1} as well (Fig. 3b).

In severely heat stressed barley plants [and pea leaves as well (Fig. 3d)], a second peak analogous to P (accompanied by a re-reduction of $P700^+$ and PC^+) is observed around 1 s, which was an indication of a further accumulation of reduced Q_A. This peak is small following a heat treatment at 45°C and missing in the fluorescence transients of lichen thalli treated at 50°C. Instead, there is secondary rise phase both for thalli treated at 45 and 50°C peaking around the M-peak (Fig. 3b, c) that is missing in the pea leaves (Fig. 3d). The position of this M-peak shifts to shorter times as the light intensity is increased.

In Fig. 4, a comparison is made between the PF and DF signals (Fig. 4a, c) and the fluorescence and 820 nm reflection signal (Fig. 4b, d) of thalli kept at 25°C (Fig. 4a, b) and of thalli treated at 45°C (Fig. 4c, d). The effective light intensity was in the case of the DF measurements less and, therefore, it was not possible to compare all three signals at the same time. Prompt fluorescence is the fluorescence that is emitted at room temperature in response to illumination. Decay of the PF-signal is practically complete within 5 ns (Jursinic 1986). Delayed fluorescence is induced by recombination reactions within PSII. The DF-emission between 10 and 30 μs of darkness monitors the recombination between Q_A^- and $P680^+$ (Christen et al. 1998; Grabolle and Dau 2007). With respect to the 820 nm measurements a decrease of the 820 nm signal reflects an oxidation of PC and P700 and an increase of the signal a reduction of PC^+ and $P700^+$ (Schansker et al. 2003). Figure 4b shows that for thalli kept at 25°C the I–P–S transition follows perfectly the reduction and oxidation of PC and P700. Following a treatment at 45°C (Fig. 4d), there is still a peak in the fluorescence intensity that coincides with the 820 nm signal, however, the parallelism between the re-reduction of $P700^+$ and PC^+ and the fluorescence rise in the same time domain is partially lost. For the heat-treated thalli, there was an increase in the 820 nm signal paralleling the S–M-rise that was not observed in thalli kept at 25°C. A further increase of the temperature to 50°C eliminated the increase in the 820 nm signal during the S–M-rise (Fig. 5). For the thalli kept at 25°C the DF-signal decreased during the I–P-rise, did not change during the P–S-decline and increased during the S–M-rise (Fig. 4a). For the thalli that were treated at 45°C an increase of the DF-signal during the I–P-rise and a decrease starting halfway during the S–M-rise was observed (Fig. 4c).

Following a heat treatment at 50°C a peak appears around the I-step (Fig. 3c). Such a change can, for example, be due to a change in the redox state of the photosynthetic electron transport chain. In barley the destruction of the oxygen evolving complex causes a slowdown of the re-reduction of $P700^+$ and PC^+ from 20 to 80 ms as measured by 820 nm transmission measurements (Tóth et al. 2007b). As shown in Fig. 5 heat stress had the opposite effect on lichen thalli: the re-reduction phase (30–200 ms of illumination) shifted to shorter times as the heat stress treatment became more severe: following a heat treatment at 45°C the re-reduction phase starts already after ~20 ms of illumination (instead of ~30 ms for thalli kept at 25°C) and following the 50°C treatment the 820 nm transmission signal starts to increase again after 5 ms of illumination, suggesting that already at that point in time electrons start to reach PSI that compensate the transfer of electrons to the acceptor side of PSI. The most likely source of these electrons is the PQ-pool and these measurements suggest that the PQ-pool is more reduced in darkness following a heat treatment at 50°C. In Fig. 6, the donor side of PSII was probed by two short (5 ms) pulses of light spaced 2.3, 75.3 and 500 ms apart (Tóth et al. 2007b; Oukarroum et al. 2009). The destruction of the oxygen evolving complex leads to a fluorescence transient that peaks after ~0.3 ms (the K-peak, reflecting the creation of the state Tyr_Z^+/Q_A^-), followed by a decline on of the fluorescence intensity (formation of the state Tyr_Z^+/Q_B^-). In Fig. 6a (lichen thalli treated at 45°C), the amplitude of the fluorescence change induced by the second pulse of light is much smaller because in PSII reaction centres lacking a functional Mn-cluster there are no further electrons that can be donated to Q_A^-. Increasing the time interval between the pulses the K-peak is regenerated (Fig. 6a–c). Using the method of Tóth et al. (2007b, 2009) a halftime for the re-

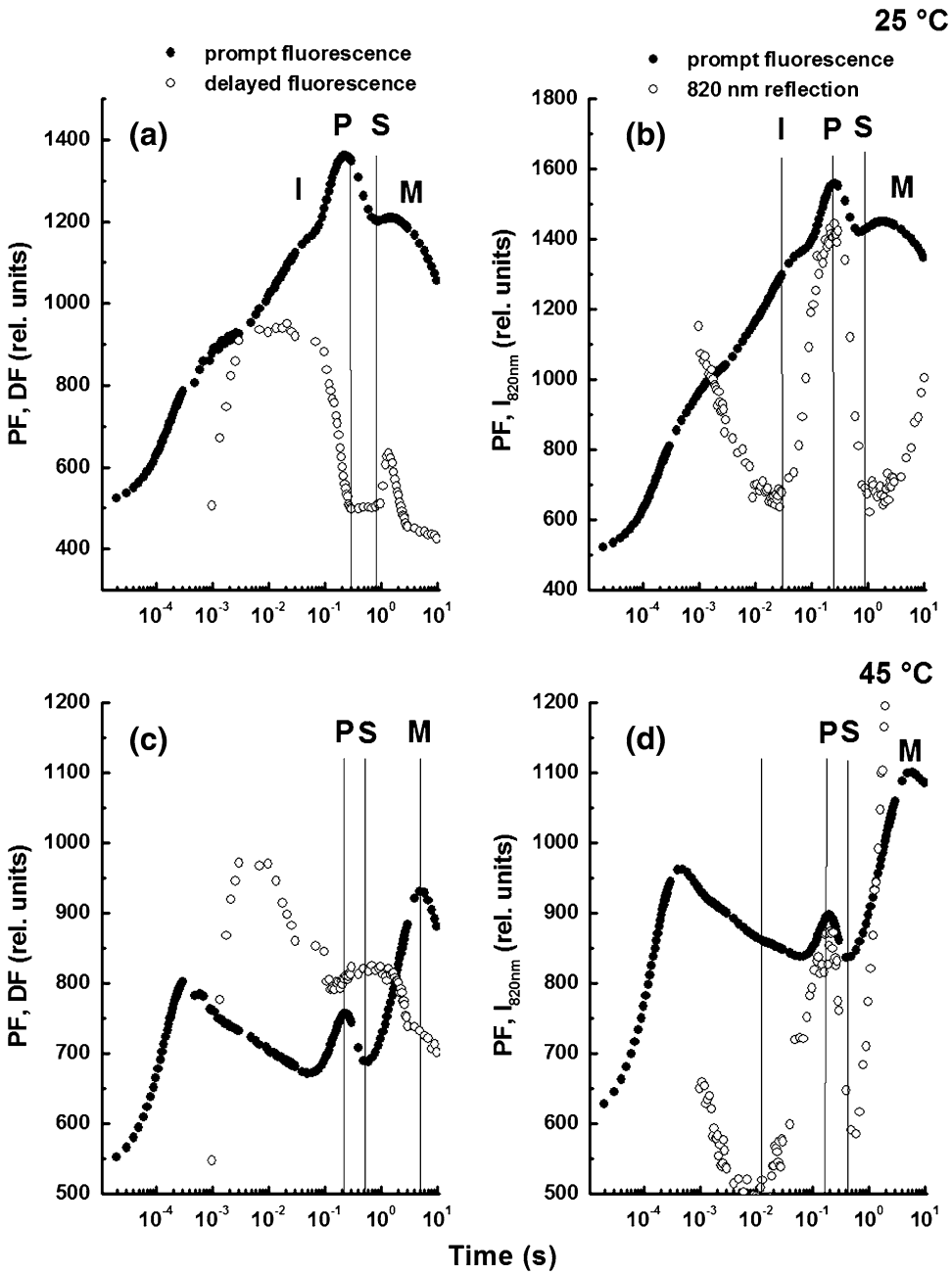

Fig. 4 Simultaneous measurements of prompt (PF) and delayed fluorescence (DF) and PF and 820-nm reflection induced by 10 s pulses of strong red actinic light (5,000 μmol photons m^{-2} s^{-1}) on untreated (**a**, **b**) and heat-treated (45°C) (**c**, **d**) thalli of *P. tiliacea*. The transients are the averages of five independent measurements

reduction of Tyr_Z^+ by external donors of ~50 ms can be calculated. For the lichen thalli treated at 50°C the observed pattern is very different (Fig. 6d–f). The fluorescence peaks only after 0.6/0.7 ms and the fluorescence intensity remains high after reaching its peak value, suggesting that there is little electron transfer towards Q_B. This is confirmed by fluorescence transients induced by the second pulse where the fluorescence intensity remained high as well. Comparing Fig. 6b and e it can be observed that the initial fluorescence level dark adapts more quickly in the leaves treated at 50°C.

Discussion

Lichens are poikilohydrous organisms whose internal water content rapidly equilibrates with the water potential of the surrounding environment (Lange et al. 1994). Recently, several studies were carried out on the absence of variable Chl *a* fluorescence in dry lichen thalli. Veerman et al. (2007) described a desiccation-induced quencher with a peak at 740 nm in dry thalli of *Parmelia sulcata*. Slavov et al. (2011) confirmed the existence of the 740 nm fluorescence band but in addition concluded that the more efficient component of

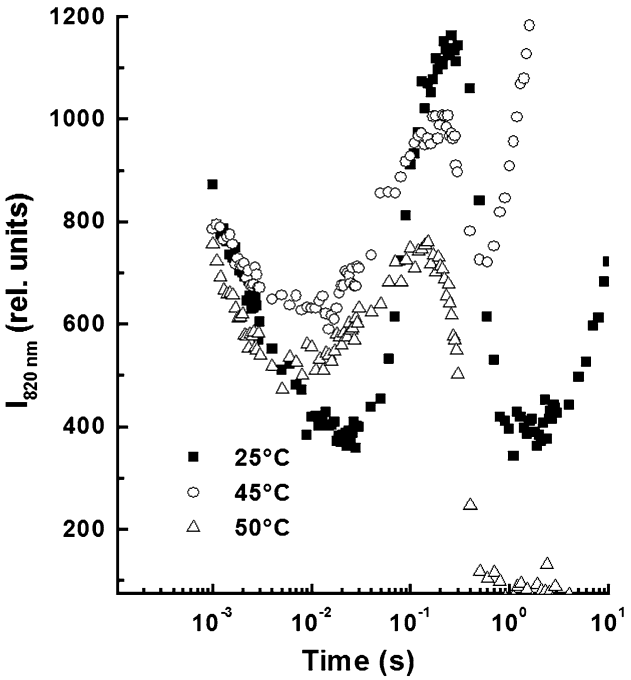

Fig. 5 Assessment of the oxidation/reduction kinetics of PSI of heat-treated (45 and 50°C) and untreated thalli (25°C) of *P. tiliacea* measuring the 820 nm reflection signal reflecting the oxidation/reduction of plastocyanin and P700. The transients represent the average of five independent measurements

the fluorescence quenching is due to spill over between the PSII and PSI antenna with $P700^+$ acting as the effective quencher. On addition of water, the measured variable fluorescence starts to increase following a lag-time of about 20–30 s. It suggests that fully functional photosynthetic PSII units can be reformed on short notice. Of all the parameters that were followed (Fig. 1b) only the F_0-value recovered somewhat faster than the other parameters that were related to the variable fluorescence. This is in agreement with the observation of Bukhov et al. (2004) that F_0 recovers more quickly than F_V on rehydration. The fact that the recovery of F_I and F_M follow the recovery of F_K and F_J closely, suggests that on activation of PSII the photosynthetic electron transport chain is functional again. Several PSII reaction centres feed the same PQ-pool (Siggel et al. 1972). The J-to-I-rise has been shown to parallel the reduction of the PQ-pool and non-synchronous reactivation of the PSII reaction centres feeding the individual PQ-pools would be expected to cause a slower reduction of the PQ-pools and, therefore, a slower J-to-I-rise (Schreiber and Neubauer 1987). This is for example observed in partially DCMU-inhibited samples (Heber et al. 1988). The close parallelism between the recovery of the different fluorescence steps suggests that this is not the case and that the recovery time reflects the time needed for the water to reach each and every cell of the lichen tissue. Within a minute after the addition of water, a normal OJIP rise as observed in any dark-adapted oxygen evolving system (Strasser et al. 1995) was re-established. The resistance of PSII to desiccation and the fast recovery of functional photosynthetic units is of great ecophysiological significance since dehydration of lichens is occurring frequently in the field and the ability of lichens to cope with repetitive cycles of desiccation and rehydration is crucial to their survival (Eickmeier et al. 1993).

Under heat stress conditions the oxygen evolving complex of desiccated thalli remains intact

Parmelina tiliacea in the dried state regained its photosynthetic electron transport activity nearly immediately following addition of water to thalli treated for 24 h at temperatures up to 35°C as indicated by the recovery kinetics of the shape of the Chl *a* fluorescence rise, F_V/F_M and the PI (Fig. 2). Increasing the temperature further (40–50°C), *P. tiliacea* needed more time following the addition of water to reach complete activation. This suggests that these more severe conditions induced further changes in the lichens that required more time to reverse. In this respect, our results differ from those of Gauslaa and Solhaug (1999) who observed for the lichen *Lobaria pulmonaria* that dry thalli kept for 24 h at 49°C and that were subsequently allowed to recover in darkness for 48 h, had an F_V/F_M value of less than 0.2 suggesting that the PSII reaction centres in these thalli were mainly in an inactive state.

In photosynthetic organisms exposed to high temperatures (40–50°C), the kinetics of the OJIP transients change, with as a prominent new feature the appearance of fluorescence peak around 0.3 ms, the K-step (Guissé et al. 1995; Srivastava et al. 1997). On hydrating dry *P. tiliacea* thalli that had been kept for 24 h at 50°C the K-step was not observed (data not shown). The appearance of the K-peak under heat-stress conditions is a reflection of the destruction by heat of the OEC, which eliminates the electron source of PSII and reduces the number of stable charge separations to one and allows only a transient accumulation of Q_A^- (Strasser 1997; Tóth et al. 2007b). Our data, therefore, suggest that a 50°C treatment for 24 h of *P. tiliacea* thalli does not lead to the destruction of the OEC. In barley leaves, it has been shown that PSII reaction centres with a destroyed reaction centres are degraded and new reaction centres are subsequently formed, a process that takes 24–48 h (Tóth et al. 2005). Retaining an intact OEC is no doubt one of the reasons the lichens can recover so quickly from the heat stress (Fig. 2).

Light intensity dependence of the fluorescence rise of *Parmelina* thalli suggests differences in the electron transport properties inside PSII compared to pea leaves

Comparing Fig. 3a and d, the main difference is observed in the K-J-region. In pea leaves, especially around

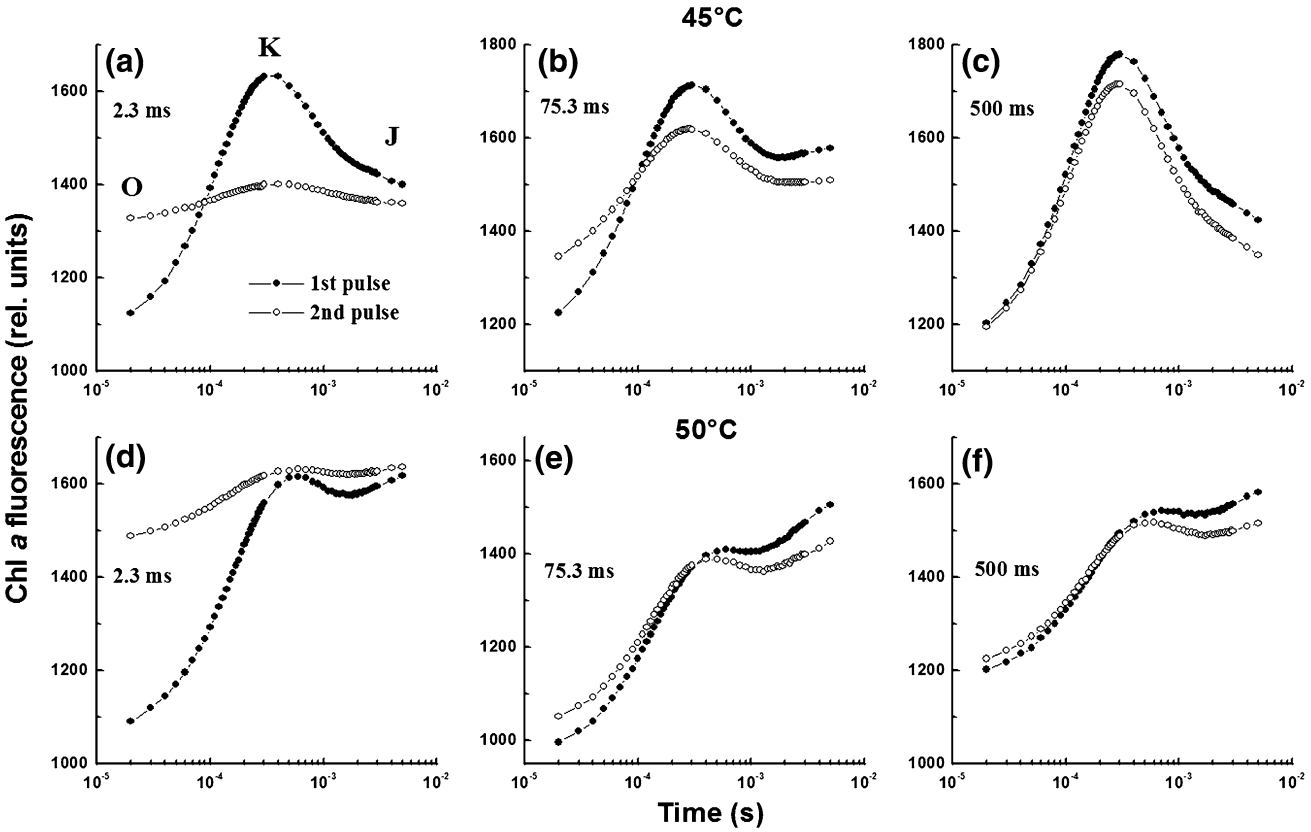

Fig. 6 Assessment of the PSII donor side activity of heat-treated *P. tiliacea* thalli using a pair of two 5-ms pulses of light. Heat treated thalli (40 s at 45°C and 50°C) were illuminated by two 5 ms light pulses spaced 2.3 (**a**, **d**), 75.3 (**b**, **e**) and 500 ms (**c**, **f**) apart. The rate with which the original kinetics were regenerated is a measure for the rate of re-reduction of Tyr_Z^+. The fluorescence transients are the averages of 4–5 independent measurements

3,000 µmol photons m^{-2} s^{-1} the rise to the J-step is smooth and the K-step is hidden (e.g. Schansker et al. 2006). In *P. tiliacea* thalli both the K and the J-step are observed in a single measurement (Fig. 3a). A second feature is observed at high light intensities, where in pea leaves a dip is observed after 2–3 ms of illumination (Fig. 3d) that has been associated with a transient accumulation of $P680^+$ as the majority of reaction centres is in the S_4 state (Schansker et al. 2011). This dip is missing completely in the case of the lichen measurements. On increasing the light intensity from 5,000 to 15,000 µmol photons m^{-2} s^{-1} there is absolutely no qualitative change in the kinetics of the OJI-fluorescence rise. These differences suggest that some rate constants or equilibria on either the donor or acceptor side of lichen PSII differ from those in e.g. pea leaves.

The DF-signal as a probe for the redox state of the photosynthetic electron transport chain and cyclic electron transport around PSI

DF is a reflection of charge recombination processes in PSII and its intensity in the tens of µs range depends strongly on the concentrations of $P680^+$ and Q_A^- (see Goltsev et al. 2009 for a recent review). Figure 4a shows that in unstressed lichen thalli the PF and the DF-signal change in an anti-parallel way between I and P. It suggests that the DF-signal in this time domain is determined by reduction of the photosynthetic electron transport, which reduces the turnover rate of Q_A and decreases the $P680^+$ concentration (Schansker et al. 2011). Beyond P, a remarkable feature is the transient DF-rise that accompanies the S–M-rise. This DF-peak reflects a temporary increase in the number of charge pairs that can recombine within PSII. If this peak would have been due to an increased concentration of $P680^+$ caused by a temporary increase of the electron transport rate we would have expected that the peak would have paralleled the P–S-decline that is accompanied by an oxidation of P700 and PC allowing electrons to flow from the PQ-pool to PSI. The fact that the DF-peak occurs later suggests that another process is responsible for it and cyclic electron transport round PSI would be a good alternative candidate. However, further experiments would be needed with respect to this point since it disagrees with the observations of Ilík et al. (2006). For thalli heat-stressed at 45°C the situation is quite

different. Here, there is not such a clear relationship between the DF and the PF-signal (Fig. 4c). Under heat-stress conditions the electron donation capacity of all PSII reaction centres together is gradually reduced and as a consequence the photosynthetic electron transport chain remains relatively oxidized. The destruction of the Mn-cluster by heat also means that the lifetime of Tyr_Z^+ becomes much longer [halftime of re-reduction of ~50 ms for thalli treated at 45°C (see above)] and as a consequence, due to the equilibrium between Tyr_Z and P680, the concentration of $P680^+$ will be relatively high at any time during the illumination. This means that the intensity of the DF-signal will be determined mainly by the Q_A^- concentration of which the formation in heat-stressed thalli is PSII donor side limited. This explains why the DF-intensity remains relatively high during the whole illumination period in heat-stressed thalli (Fig. 4c) in contrast to the untreated thalli, where a sharp drop of the DF-intensity is observed towards the end of the illumination period (Fig. 4a).

Nature of the fluorescence peak around the I-step observed after a 50°C heat treatment

As shown in Fig. 3c, a 50°C heat-stress treatment induces a peak around the I-step that is absent in the transients treated at 45°C (Fig. 3b). Such a peak would be expected if the PQ-pool would be more reduced in darkness following a 50°C heat-stress treatment. In untreated higher plants a more reduced PQ-pool raises the J-step (Schansker et al. 2005, Tóth et al. 2007a), but in severely heat-stressed lichens with a limited electron donation rate to the donor side of PSII (Fig. 6) this effect is expected to occur after longer illumination times. The presence of a more reduced PQ-pool was confirmed by the experiments illustrated in Figs. 5 and 6: the re-reduction phase of the 820 nm signal occurred at shorter times following heat stress (Fig. 5), the fluorescence intensity remains high at the end of the 5-ms pulses (cf. Fig. 6a–c with d–f) and the initial fluorescence intensity dark adapts more quickly in the thalli treated at 50°C than in those treated at 45°C (cf. Fig. 6b with e). Both experiments support the idea that the PQ-pool remains much more reduced in darkness following a heat treatment at 50°C.

Low activity of alternative electron donors to heat inactivated PSII in P. tiliacea and its consequences for the interpretation of the experiments

Tóth et al. (2007b, 2009, 2011) showed that in photosynthetic organisms of which the OEC had been destroyed by heat-stress ascorbate can donate electrons to the donor side of PSII with halftimes of 15–30 ms depending on the species tested. In hydrated thalli treated at 45°C this halftime was ~50 ms, a value close to the ~55 ms halftime observed for the vtc2-1 mutant of *Arabidopsis thaliana* that has an approximately seven times lower ascorbate content than the wild type plants (Tóth et al. 2009). In heat-treated barley leaves electron donation by ascorbate led to a second fluorescence rise peaking around 1 s. Another characteristic of this peak was that its position was insensitive to the light intensity. In *P. tiliacea* this peak was very small (the P-line in Figs. 3b and 4d). In the case of the 50°C heat treatment it was at best a shoulder on the fluorescence decline around 100 ms of illumination (Fig. 3c). Instead, a fluorescence rise phase was observed in the same time domain where the S–M rise occurred in thalli kept at 25°C and this rise phase was light intensity dependent (Fig. 3b–d). These observations have two implications: (1) the small peak around 1 s suggests that ascorbate or any other alternative electron donor to PSII cannot drive a measurable electron transport in lichen thalli and (2) the apparent absence of linear electron flow in heat-stressed lichens suggests that the fluorescence rise in the S–M-domain must be due to cyclic electron transport. A stronger non-photochemical donation of electrons to the PQ-pool following heat stress in lichens is also supported by Fig. 5c where it was observed that the $P700^+$ and PC^+ re-reduction phase of the 820 nm signal started earlier following heat stress at 45 and 50°C. This in contrast to heat-treated barley plants were this re-reduction phase started considerably later than in control leaves (Tóth et al. 2007b). Ilík et al. (2006) rejected a relationship between the M-peak and CET for unstressed lichens, since rotenone had no effect on it. However, it cannot be entirely excluded that the rotenone never reached its target or that it does not inhibit CET in lichens. Our present data suggest that the fluorescence rise in the S–M time domain is probably due to CET in heat-stressed thalli. If the S–M fluorescence rise is also due to CET in samples kept at 25°C remains an open question.

Conclusion

In lichens, due to evaporative cooling during desiccation (Hoffman and Gates 1970), heat stress will normally affect desiccated thalli. Dry thalli kept for 24 h at 50°C showed on hydration no K-peak and were able to recover normal OJIP-rise kinetics within a few hours (Fig. 2). In barley, a 40 s treatment at 48–50°C induces a K-peak and necessitates a 1–2 day recovery period related to a degradation and re-assembly of the PSII complexes (Tóth et al. 2005, 2011). The desiccated state in lichens clearly protects the Mn-cluster of plants against heat-induced destruction. In the wet state, the situation is completely different. A short heat treatment of *P. tiliacea* at 45°C (40 s) was enough to

inactivate nearly all PSII reaction centres (Figs. 3b, 6a) suggesting that under these conditions the lichens were as sensitive (if not more) to heat stress as pea (Fig. 3d) or barley leaves (Tóth et al. 2005, 2007b). In addition the re-reduction of Tyr_Z by ascorbate, which has been suggested to give the photosynthetic apparatus some protection against the oxidative power of the $Tyr_Z^+/P680^+$ couple (Tóth et al. 2009, 2011), is in lichens much slower than in higher plants (halftime of ~50 ms in lichen, based on a set of measurements of which some examples are shown in Fig. 6) compared with 15–30 ms in higher plants (Tóth et al. 2009). The lack of alternative donors to PSII is also illustrated by the near absence of the secondary rise peak around 1 s in the fluorescence transient (Fig. 3b). These data suggest that mechanisms that increase the tolerance of higher plants to heat stress are absent or less well developed in lichens probably related to the fact that wet thalli would under natural condition not be exposed to heat-stress conditions making wet thalli an attractive reference species for the evaluation of potential heat stress related protection mechanisms in photosynthetic organisms.

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