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ORIGINAL ARTICLE

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Recovery of photosystem I and II activities during re-hydration of lichen *Hypogymnia physodes* thalli

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Abstract Photochemical efficiencies of photosystem I (PSI) and photosystem II (PSII) were studied in dry thalli of the lichen *Hypogymnia physodes* and during their re-hydration. In dry thalli, PSII reaction centers are photochemically inactive, as evidenced by the absence of variable chlorophyll (Chl) fluorescence, whereas the primary electron donor of PSI, P700, exhibits irreversible oxidation under continuous light. Upon application of multiple- and, particularly, single-turnover pulses in dry lichen, P700 oxidation partially reversed, which indicated recombination between $P700^+$ and the reduced acceptor F_X of PSI. Re-wetting of air-dried *H. physodes* initiated the gradual restoration of reversible light-induced redox reactions in both PSII and PSI, but the recovery was faster in PSI. Two slow components of $P700^+$ reduction occurred after irradiation of partially and completely hydrated thalli with strong white light. In contrast, no slow component was found in the kinetics of re-oxidation of Q_A^-, the reduced primary acceptor of PSII, after exposure of such thalli to white light. This finding indicated the inability of PSII in *H. physodes* to provide the reduction of the plastoquinone pool to significant levels. It is concluded that slow alternative electron transport routes may contribute to the energetics of photosynthesis to a larger extent in *H. physodes* than in higher plants.

Keywords Chlorophyll fluorescence · Absorbance changes · *Hypogymnia* · P700 oxidation · Photosystem · Plastoquinone

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Abbreviations A_0 and A_1: Primary acceptor chlorophyll and secondary electron acceptor phylloquinone · Chl *a*: Chlorophyll *a* · F_m: Maximal level of chlorophyll fluorescence when all PSII centers are closed · F_o: Minimal level of fluorescence when all PSII centers are open after dark adaptation · FR: Far-red · F_v: Variable fluorescence ($=F_m-F_o$) · F_X, F_A, and F_B: Iron–sulfur centers · MT pulse: Multiple-turnover pulse · PS: Photosystem · P700: Reaction center chlorophyll of PSI · Q_A: Primary quinone acceptor of PSII · Q_B: Secondary quinone acceptor of PSII · ST pulse: Single-turnover pulse

Introduction

Among the factors that restrict the development of photosynthetic organisms, the most important one is water deficit. When autotrophs are exposed to high salinities, high or low temperature extremes, or severe drought, they often experience acute water-deficit stress. As a consequence, the most severe form of water deficit is the desiccation stress that not only affects growth in multiple ways but also induces several biochemical and physiological responses (for review, Hanson and Hitz 1982). In higher plants, water relations are under fine control through the opening and closure of stomata. An immediate event occurring in plants that undergo desiccation is the closure of stomata, which subsequently leads to a depressed photosynthetic capacity as carbon assimilation is diminished owing to low availability of CO_2 (see Bartels and Salamini 2001 and references therein). During vegetative growth, the majority of flowering plants cannot cope with desiccation when the loss of water exceeds 85–98% (v/v) of their relative humidity, even though it is an essential part of their normal development during seed formation (Bartels and Salamini 2001).

Desiccation tolerance is limited to a small group, called resurrection plants, that includes some angiosperms (Gaff 1971) and cryptogams such as lichens,

bryophytes, and pteridophytes (Bernacchia et al. 1996; Ingram and Bartels 1996). Within this narrow range of resurrection species, the adaptative mechanisms that operate under water-deficit stress during the course of their development are highly variable and complex. Though the involvement of protective mechanisms during dehydration of photosynthetic tissues is well documented (Bernacchia et al. 1996; Bartels and Salamini 2001), the physiological changes involved during re-hydration are unknown.

When desiccation-tolerant lichens in their ecological niches grow on rocky surfaces or as epiphytes under conditions of extreme water deficit, they are often exposed to repetitive cycles of drying and re-hydration (Lange and Matthes 1981). Therefore, they serve as excellent probes to unravel the protective mechanisms and the functionality of the photosynthetic apparatus during severe desiccation. Compared to vascular plants, lichens do not possess stomata, and cannot protect their tissues from over-drying (Woodward 1998). Thus, their water content is strongly dependent on environmental conditions. To date, the most intensive studies with dehydrated and re-hydrated lichens indicate that photosynthetic activity is displayed only if they are exposed to high water vapours or supplied with liquid water, particularly when the cyanobacterial species is the photobiont (Lange et al. 1989, 2001; Green et al. 2002).

Chlorophyll (Chl) *a* fluorescence and absorbance changes at 830 nm that monitor the redox changes of the primary donor ,P700, of photosystem I (PSI) were largely employed as intrinsic probes to assess the properties of photosystem II (PSII) and PSI in dehydrated and re-hydrated states of lichens (Lange et al. 1989; Heber et al. 2000). The most important information emerging from these studies is that not only the overall photosynthetic activity, but also the light-driven redox reactions of electron transport are very sensitive to the water content in lichen thalli. PSII activity is greatly altered in dry lichen thalli in comparison with fully re-hydrated ones (Lange et al. 1989; Sass et al. 2002). Upon severe desiccation of lichen thalli, the basal level of F_o, which indicates the openness of all PSII reaction centers in the dark, is strongly decreased (Lange et al. 1989; Bilger et al. 1989). Subsequently, the light-induced changes in Chl fluorescence yield, F_v [variable fluorescence = F_m (maximal level of Chl fluorescence when all PSII centers are closed)$-F_o$)], which is a good indicator of charge separation in PSII, disappear completely in both dehydrated lichens and leaves (Lange et al. 1989; Bilger et al. 1989; Heber et al. 2000). During the course of re-hydration of dried lichen thalli, F_o increases and light-induced F_v recovers reversibly (Lange et al. 1989; Bilger et al. 1989; Heber et al. 2000), implicating the reconstitution of the photosynthetic apparatus. However, this reactivation of PSII is not found in re-hydrated leaves (Heber et al. 2000). Unlike higher plants, in which a significant loss of water causes irreversible damage of autotrophic cells, suppression of PSII activity during desiccation is reversible during re-hydration of dried lichens. Hence, the differential responses to desiccation stress indicate the operation of yet-unknown stabilizing mechanism(s) that protect the functions of the photosynthetic apparatus.

The amount of light energy absorbed by Chl molecules is the same in hydrated and dehydrated lichens, and thus the protection of the photosynthetic apparatus against desiccation is crucial for their survival under conditions of extreme light regimes. Protection against damages caused by the combined stress of desiccation and photoinhibition in lichen thalli is important not only for PSII, but for PSI as well. Photoprotective mechanisms such as those operating in plant chloroplasts are functional in hydrated lichen tissues (Heber et al. 2000). They include light-induced acidification of intra-thylakoid space, with concurrent increase in thermal dissipation of absorbed quanta in PSII antenna (Horton et al. 1996; Heber et al. 2000) and other deactivation pathways. Despite the fact that the question of energy transfer to effective fluorescence quenchers is still unresolved, it has been argued that the decreased Chl fluorescence in dehydrated poikilohydric lichens is caused mainly by an enhanced thermal dissipation of energy (Heber et al. 2000, 2001). One school of thought claims that the energy transfer from light-harvesting antenna pigments to the reaction centres of PSII is curtailed (Bilger et al. 1989) while another hypothesis corroborates the preferential excitation of PSI complexes in desiccated lichens (Sigfridsson and Oquist 1980; Jensen and Feige 1987). Also, it is unclear how energy migration within the antenna, restoration of active PSII and PSI centers, and non-photochemical quenching of absorbed energy processes are co-ordinated during re-hydration.

The primary objective of this study is to investigate the efficiency of photochemical processes in dry lichens and during the course of re-hydration. For this purpose, the light-induced redox reactions in PSI and PSII in *Hypogymnia physodes* L. were examined using the techniques of Chl *a* fluorescence and absorbance changes at 830 nm. We demonstrate here the progressive increase in the light-driven redox reactions of both PSII and PSI during re-hydration. In this process, the photochemical activity of PSI recovered more rapidly than that of PSII. In those slowly developing active PSII centers, the recovery of a functional antenna preceded charge separation. During re-hydration, and even in fully hydrated thalli, the electrons generated through linear electron transport involving PSII were not sufficient to reduce all PSI centers and about one-third of PSI units received electrons via alternative routes to maintain the energetics of photosynthesis.

Materials and methods

Plant material

Thalli of *Hypogymnia physodes* L. were taken from the bark of trees in Trois-Rivières. The phycobiont partner of these lichens is

the trebouxioid green alga with a pyrenoid-containing chloroplast (Smith and Griffiths 1998). Thalli were re-hydrated and stored in water in the dark for 6 h. Then, they were kept for several days in the dark under atmospheric air to obtain air-dried material. Procedures of re-hydration are described in the text for each particular experiment.

P700 absorbance changes

Photochemical activity of PSI was characterized using light-induced absorbance changes at 830 nm using an ED-P700DW dual-wavelength unit connected via a PAM-101 fluorometer (Walz, Effeltrich, Germany). The ED-P700DW dual-wavelength emitter–detector unit detects the differential absorbance changes (810 nm minus 860 nm) attributed to the absorption of the $P700^+$ cation radical (Klughammer and Schreiber 1991). The acquisition of the signal of ΔA_{830} detected by ED-P700DW was mediated through a PAM Data Acquisiton System (PDA-100) with the aid of Wincontrol software (Walz). White light from a Fiber-Lite light source (Microview, Canada) was passed through an RG-9 filter (Schott, Mainz, Germany) to excite lichen samples with far-red (FR) light. An electronic shutter (Ilex Optical Co, USA) was used to control the onset and termination of FR light or white actinic light. To investigate the charge recombination between $P700^+$ and PSI electron acceptor(s) in dry thalli, XMT-103 and XST-103 power/control units together with an XF-103 flash lamp (Walz) were utilized to generate multiple-turnover (MT; 50 ms) and single-turnover (ST) saturating flashes of white light, and the absorbance changes at 830 nm were acquired at the rate of 300 μs/point.

During measurements, the bottom surface of the lichen thallus was placed on a dry filter paper, while the upper surface was covered with a glass plate. The optic-fiber guide of the PAM fluorometer was tightly fixed to the glass plate to prevent any changes in geometry of the thallus. In addition, enough care was taken to avoid any baseline drifts. When the changes in ΔA_{830} were recorded, the instrument was calibrated for true reference signal levels for every sample of dried or hydrated thallus.

Recently, it was shown that the optical properties of PSII membrane fragments remain unaltered if dehydrated as thin films (Kaminskaya et al. 2003). Hence the change in the optical path length of light is unlikely to affect the absorbance changes at 830 nm in measurements with dried thalli. This is evident from minor changes in absorbance changes at 830 nm during re-hydration of dry thalli. To avoid high scattering of the excitation beam, we recorded the absorbance changes at 830 nm in reflection mode. Besides, the use of a dual-wavelength detector permits the elimination of scattering effects to a larger extent than the conventional single-beam method of detection (Klughammer and Schreiber 1998). The difference between the magnitudes of the oxidized (the signal developed upon the onset of strong FR or white actinic light) and reduced state of P700 was considered as a relative measure of PSI activity. The kinetics of $P700^+$ reduction was analyzed using semi-logarithmic plots of acquired data.

Chlorophyll *a* fluorescence induction

Chl *a* fluorescence induction curves were recorded at room temperature using a Plant Efficiency Analyser (Hansatech, King's Lynn, Norfolk, UK). The array of red light peaking at 650 nm from six light-emitting diodes at an intensity of 4,000 μmol photons m^{-2} s^{-1} (predetermined from the analysis of light saturation for F_m) was used to excite lichens in the dry state or during re-hydration at specific time intervals. Importantly, this intensity of excitation light used to saturate maximal fluorescence in these lichen thalli is more than 2-fold relative to the fluence rates of red actinic light (1,600–1,800 μmol photons m^{-2} s^{-1}) required to saturate maximal fluorescence (F_v/F_m ratio of approx. 0.830) in leaves of healthy plants. The excitation light was focused on the surface of the lichens in order to obtain a homogenous illumination. The Chl *a* fluorescence rise was recorded during an induction period of 1 s with a data acquisition rate of 10 μs for the first 2 ms and 1 ms for the rest of the period. The fluorescence signal at 50 μs, the earliest measurement free of any artifacts related to the electronics of the instrument, was considered as F_o (Haldimann and Strasser 1999).

To compare the time course of the development of PSII and PSI activities during re-hydration, the parameters, ΔA_{830}, F_o, and F_v were recorded in the dry thalli of the same lichen. For this purpose, the experiments were performed as follows. 50 μl of water was carefully added to the bottom surface of a thallus sample placed on a filter paper to initiate slow re-hydration. ΔA_{830} was measured at different time intervals after re-hydration had been initiated. The emitter–detector unit was rapidly changed and Chl fluorescence was measured within about 30 s after recording absorbance changes. After the measurements of ΔA_{830} and Chl fluorescence in a partially re-hydrated thallus sample had been completed, 500 μl of water was added to the upper surface of the sample. Then, all three parameters were measured again after a 15-min dark incubation that produced the fully re-hydrated state. The values of ΔA_{830}, F_o, and F_v obtained for a thallus sample in the partially re-hydrated state were normalized to those for fully hydrated thalli. When dark relaxation of the light-induced F_v was analysed, PAM 101 was employed.

Results

Development of reversible redox reactions in PSI and PSII during re-hydration of dry thalli

A consequence of a low availability of water to poikilohydric lichens is the massive decline in their intracellular water content that triggers the changes in physical and chemical properties at the cellular level, leading to a shift towards the dehydrated state in which most of the physiological functions are inactive. In this process, changes in photosynthetic membrane organization are not excluded. Therefore, we determined the photochemical efficiencies of PSI and PSII in lichen thalli that experienced severe desiccation to evaluate the recovery processes during re-hydration. For this purpose, we analysed the absorbance changes at 830 nm (ΔA_{830}) and Chl fluorescence by applying 1-s strong white light pulses in series separated by 2-min dark intervals. In dehydrated thalli, the first 1-s pulse induced an irreversible decrease in A_{830}, whereas following pulses did not produce any additional absorbance changes (Fig. 1, upper trace). Thus, irreversible charge separation occurred in PSI with the stabilization of P700 in its oxidized form, indicating the complete suppression of PSI activity in lichen tissues exposed to desiccation stress. Upon addition of 50 μl of water to the bottom surface of dry thalli to initiate the re-hydration process, application of a series of 1-s light pulses resulted in the appearance of reversible ΔA_{830} after an initial lag phase (Fig. 1, upper trace). P700 was oxidized with 1-s light pulses and completely re-reduced in darkness by electrons arriving from PSII-mediated linear electron transport and PSI cyclic electron transport pathways. Thus, the efficiency of both the reducing and oxidizing sides of PSI units was greatly regained in re-hydrated thalli. Also, only a small drift in the baseline was observed during re-hydration of

dry thalli, indicating rather small changes in their optical properties.

When a series of 1-s strong white light pulses separated by 2-min dark intervals was applied to dehydrated lichen thalli, no appreciable change in Chl fluorescence yield was observed (Fig. 1, lower trace) as the basal level of Chl fluorescence was quenched and variable fluorescence, F_v, was completely abolished. On the other hand, when re-hydration of dry thalli was initiated as mentioned for reactivation of PSI, energy migration in the antenna and light-induced charge separation in the PSII reaction centers were restored. This is indicated by a gradual increase in the basal level of Chl fluorescence and by the appearance of fluorescence spikes upon applications of 1-s light pulses. The gradual increase in F_v demonstrates the reduction of the primary quinone acceptor of PSII, Q_A, under light followed by re-oxidation of Q_A^- in the dark. Maximum magnitudes of reversible light-induced ΔA_{830} and F_v, as depicted in Fig. 1, were reached about 30 min after dehydrated thalli were supplied with water. However, it must be emphasized that the development of reversible photochemical reactions in both PSI and PSII was significantly enhanced if the re-hydration process was accelerated by addition of a large amount of water (up to 500 µl) to the upper surface of dry thalli.

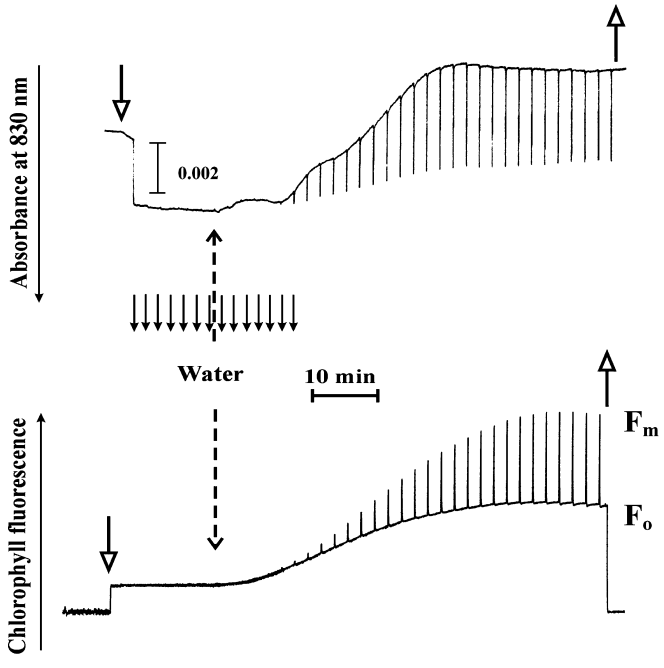

Fig. 1 Simultaneous measurements of changes in absorbance at 830 nm (*upper trace*) and Chl fluorescence (*lower trace*) in dry thallus of *Hypogymnia physodes* after addition of 50 µl of water to its bottom surface. During measurement, 1-s pulses of strong white light (7,400 µmol quanta m^{-2} s^{-1}) separated by 2-min dark intervals were applied. *Downward dark arrows* indicate the position of these light pulses until reversible spikes in absorbance and fluorescence appeared. *Downward and upward open arrows* indicate weak modulated light on and off, respectively

Action of single and 50-ms MT pulses on the P700 redox state in dry and hydrated thalli

As already shown in Fig. 1, irradiation of a dehydrated thallus with a 1-s strong white light pulse caused irreversible P700 oxidation. Application of a 50-ms MT pulse resulted in some irreversible P700 photooxidation as well (Fig. 2, trace 1). After such a pulse, a predominant part of ΔA_{830} increased, thus indicating the stabilization of $P700^+$. However, only part of the signal was rapidly reversed in the dark. When the desiccated thalli were re-hydrated and illuminated with a series of 50-ms pulses, the shape and kinetics of the P700 oxidation–reduction curve were markedly transformed. In these samples, the magnitude of ΔA_{830} induced by such pulses was severalfold higher compared to dehydrated thalli (Fig. 2, trace 2) indicating higher accumulation of oxidized P700. Importantly, the absorbance rise at 830 nm relaxed rapidly and almost completely after the pulses. Evidently, the rapid phase of relaxation was due to electron transfer from PSII to PSI with concomitant reduction of $P700^+$, as this fast relaxation was delayed when the linear electron transfer from Q_A to Q_B was blocked with diuron (Fig. 2, trace 3).

In contrast to the estimates of P700 oxidation observed with application of 50-ms MT pulses in dehydrated thalli, continuous FR light produced a much higher absorbance rise at 830 nm (Fig. 2, trace 1). As FR light preferentially excites PSI, the oxidation of P700 was greatly enlarged. At first sight, this finding indicates that the population of P700 is weakly oxidized with 50-ms MT pulses. Especially, the dark relaxation of ΔA_{830} after the cessation of FR illumination was completely eliminated in dehydrated thalli as $P700^+$ re-reduction was prevented due to perturbations in the electron

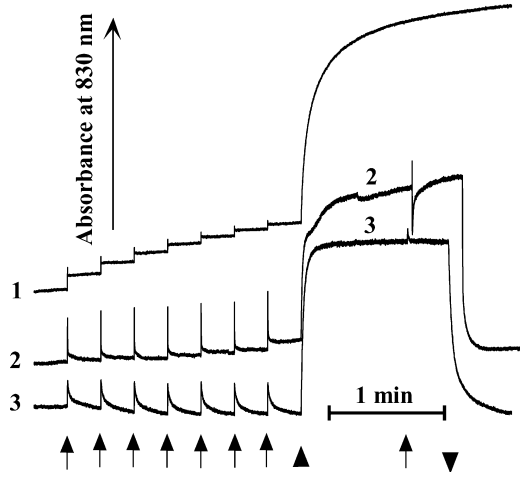

Fig. 2 Original traces of absorbance changes at 830 nm induced by 50-ms MT pulses or by continuous FR light in dry *H. physodes* thallus (*trace 1*) or in thallus fully hydrated with water (*trace 2*) or with 100 µM diuron solution (*trace 3*). *Arrows* indicate the applications of MT pulses; *upward and downward triangles* indicate FR light on and off, respectively. Data acquisition rate: 10 ms/point

donation pathways, including those coming from PSII. Following FR irradiation, P700 photooxidation proceeded more slowly in dehydrated thalli relative to the hydrated state in which the reversibility of ΔA_{830} was faster (Fig. 2, compare traces 1 and 2). When a 50-ms MT pulse was applied to hydrated thalli under an FR light background, a rapid decline in the absorbance at 830 nm proceeded as the electrons coming from PSII (Fig. 2, traces 2 and 3) reduced $P700^+$. However, the absorbance signal recovered to its original level observed before the application of the 50-ms MT pulse owing to the re-oxidation of P700 under background FR light. These findings implicate the complete recovery of PSI units during the re-hydration of dehydrated thalli.

In dehydrated lichen thalli, the application of a 50-ms MT pulse weakly photooxidized P700 when compared to the photooxidation of P700 by continuous FR irradiation (Fig. 2 trace 1). Upon termination of the 50-ms pulse, dark relaxation of ΔA_{830} was only partial. Therefore, we sought to analyse the redox changes of P700 elicited by an ST pulse in dried thalli. Figure 3a shows the kinetic curves of ΔA_{830} induced by an ST pulse and a 50-ms MT pulse. Though, the amplitudes of the absorbance signals were almost similar, the extent of ΔA_{830} reversibility was severalfold higher with ST pulse excitation. In both cases, the dark relaxation of ΔA_{830} was monoexponential with half-times of 3.7 ms and 6.8 ms for excitations with an ST pulse and a 50-ms MT pulse, respectively (Fig. 3b).

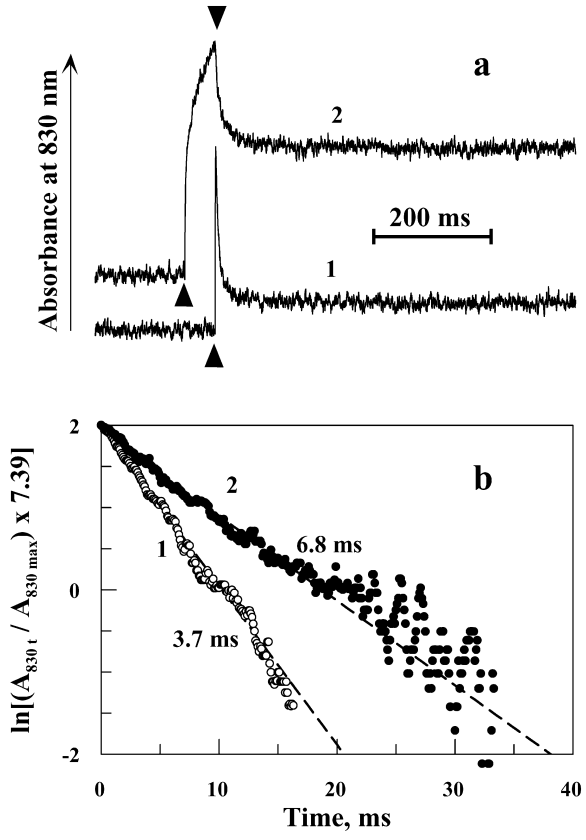

Fig. 3 a Original traces of absorbance changes at 830 nm induced by either an ST pulse (*trace 1*) or a 50-ms MT pulse (*trace 2*) in dry *H. physodes* thalli. *Arrows* indicate the application of ST or MT pulses. **b** Semilogarithmic plots of the dark recoveries. The magnitudes of ΔA_{830} were normalized to 100%. Data acquisition rate: 300 μs/point

Changes in the kinetics of $P700^+$ reduction during re-hydration of desiccated thalli

As shown in Figs. 1, 2, and 3, this electron transfer chain is not operational in the desiccated state but it is reactivated during the re-hydration phase. Therefore, we attempted to characterize the kinetics of $P700^+$ reduction during the course of re-hydration through the analysis of ΔA_{830} dark decay after a 2-s irradiation with white light of 175 W m^{-2}. To evaluate this pattern, ΔA_{830} was measured with thalli kept in darkness for various time intervals (in the range 40–300 s) after addition of 50 μl of water, henceforth referred to as partially hydrated thalli. Then, 500 μl of water was added to the partially dehydrated thalli, which were stored in the dark for 15 min to achieve complete recovery of PSI. Subsequently, these fully hydrated samples were used to record dark relaxation of ΔA_{830}. The magnitudes of absorbance changes obtained from partially hydrated thalli were normalized to the magnitude obtained from fully hydrated samples. Figure 4 illustrates the kinetics of ΔA_{830} dark decay observed after a 2-s irradiation with white light of 175 W m^{-2}. In general, the kinetics of ΔA_{830} dark relaxation was fitted by the sum of three exponentially decaying components with highly different half-times (Fig. 4). The half-times for the slow, middle, and fast components varied in the range 1,600–2,100 ms, 195–220 ms, and 22–30 ms, respectively (Fig. 4b,c) and did not depend on the degree of ΔA_{830} restoration during re-hydration.

The semi-logarithmic plots of ΔA_{830} relaxation registered after a 10-s irradiation by FR light in thalli with various extents of restoration of a reversible absorbance change signal are shown in Fig. 5. To avoid the irreversible part of ΔA_{830}, dry thalli were irradiated by a 2-s pulse of strong white light prior to the addition of 50 μl of water. Only two components were found in these plots. Remarkably, their half-times corresponded to those found for the middle and slow components of ΔA_{830} relaxation after irradiation of thalli by white light (compare Figs. 4 and 5).

Changes in PSII activity during re-hydration of dry thalli

Figure 6a illustrates that F_v dark relaxation in lichen tissues was fitted by a single exponentially decaying component irrespective of the degree of re-hydration. Its half-time varied from 25 to 33 ms for different thalli and did not depend on the extent of F_v restoration (Fig. 6b, closed circles). Importantly, the values of half-times of

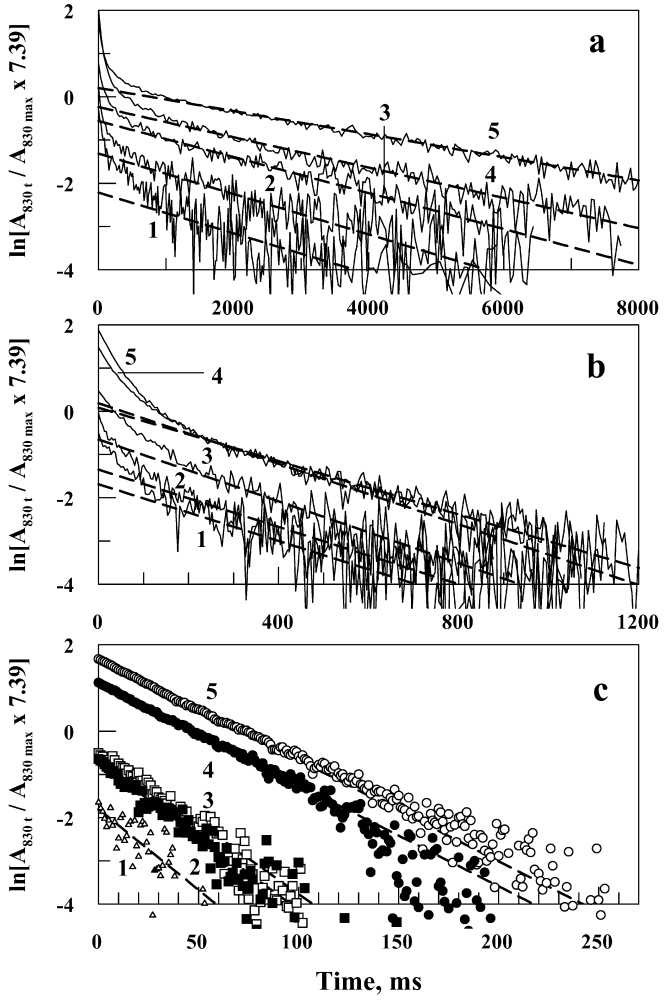

Fig. 4a–c Semi-logarithmic plots of the dark decay of ΔA_{830} observed after a 2-s irradiation by white light of 175 W m^{-2} in partially (*traces 1–4*) or fully (*trace 5*) hydrated lichen thalli. **a** Original traces; **b,c** deconvoluted slow and middle components of kinetics, respectively. The percentage of recovery of the ΔA_{830} signal compared to fully hydrated samples was 10% (*1*), 17% (*2*), 29% (*3*) and 76% (*4*). Data acquisition rate: 1 ms/point

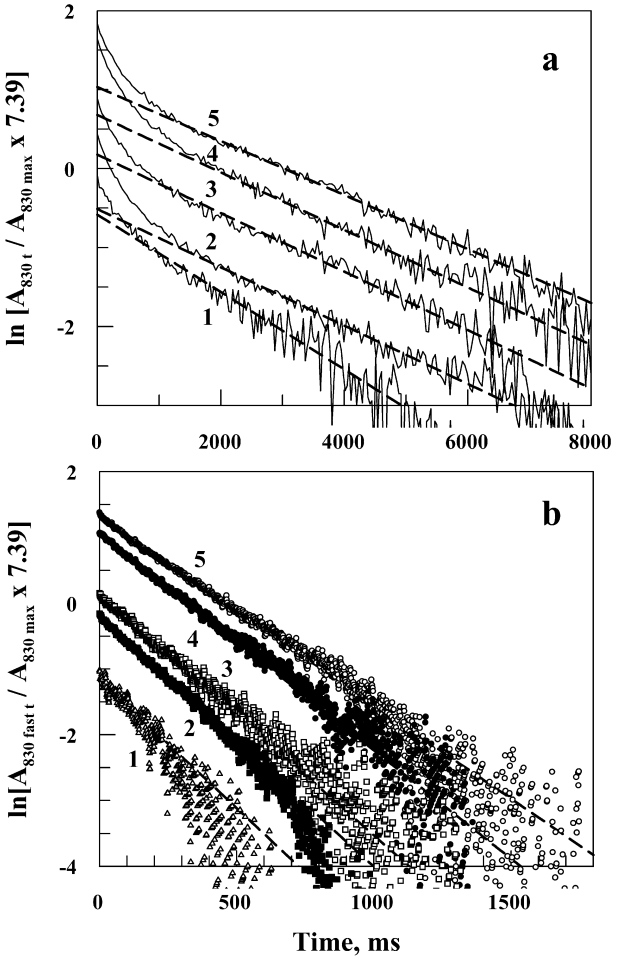

Fig. 5a,b Semi-logarithmic plots of dark decay of ΔA_{830} measured after a 10-s irradiation by FR light in partially (*traces 1–4*) or fully (*trace 5*) hydrated lichen thalli. **a** Original traces; **b** deconvoluted slow components of kinetics. The percentage of recovery of ΔA_{830} signal compared to fully hydrated samples was 12% (*1*), 21% (*2*), 33% (*3*), and 70% (*4*). Data acquisition rate: 1 ms/point

F_v decay were similar to those found for the fast component of $P700^+$ re-reduction after irradiation with white light (Fig. 6b, open circles).

Figure 7 shows the kinetic curves of the polyphasic rise of Chl fluorescence recorded with different pieces of the same dry thalli at various time intervals after addition of 500 µl of water. During the initial steps of re-hydration, which involved the reactivation of PSII centers, the light-induced fluorescence transients were non-monotonous despite the amplitude of F_o being increased (Fig. 7, traces 1–4). Thus, a clear segregation of the photochemical and thermal phases represented by the fluorescence increase from O-level (F_o) to J-level (I1), and by the J–I–P (I1–I2–F_m) levels, respectively, was not observed (Strasser et al. 1995). This trend continued until the magnitude of F_v reached about 25% of the maximum observed in a fully hydrated sample. With further increase in the extent of F_v, these non-monotonous light-induced transients (Fig. 7, traces 5 and 6) were transformed into O–J–I–P transients (Strasser et al. 1995). Unlike higher-plant chloroplasts, the Chl fluorescence rise at I- and P-levels was indicated by the appearance of well-resolved peaks followed by a much sharper dip (Fig. 7, traces 5 and 6).

Relationship between the development of reversible light-induced redox changes in PSI and PSII

It is clear from Figs. 1 and 7 that the changes proceeding during re-hydration of dry thalli occurred both in the antenna and in the reaction centers of PSII. The former were characterized by changes in F_o level of Chl fluorescence, and the latter by the magnitude of light-induced F_v. We examined the development of the two above parameters of Chl fluorescence during re-hydration of dry thalli in order to evaluate whether the changes in PSII antenna and in reaction centers were

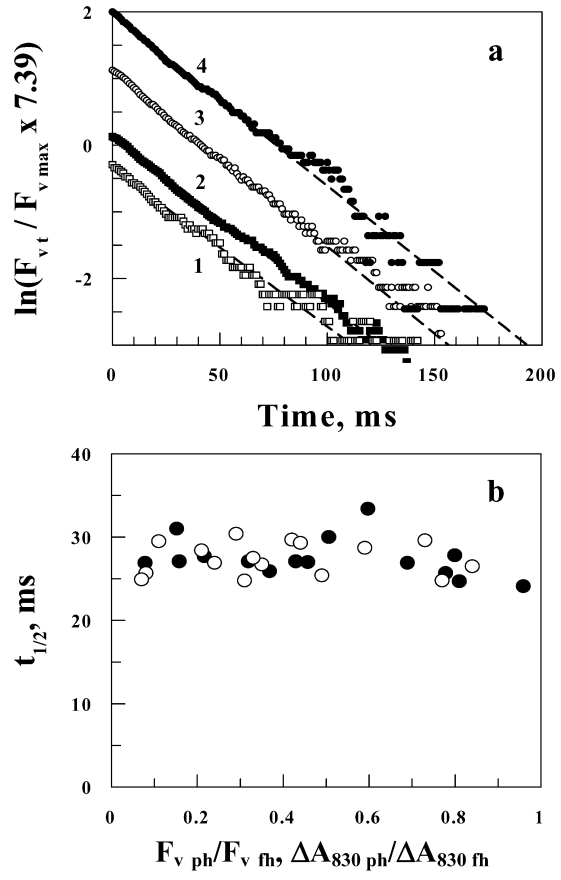

Fig. 6 a Semi-logarithmic plots of F_v decay after a 2-s irradiation of partially (*traces 1–3*) or fully (*trace 4*) hydrated thalli. The percentage of F_v recovery for samples was 10% (*1*), 15% (*2*), and 41% (*3*). Data acquisition rate: 1 ms/point. **b** Dependence of half-times of F_v dark decay (*open circles*) or fast component of ΔA_{830} dark relaxation (*closed circles*) on the extent of restoration of the corresponding activity during re-hydration compared to fully hydrated samples. *ph* Partially hydrated *fh* fully hydrated

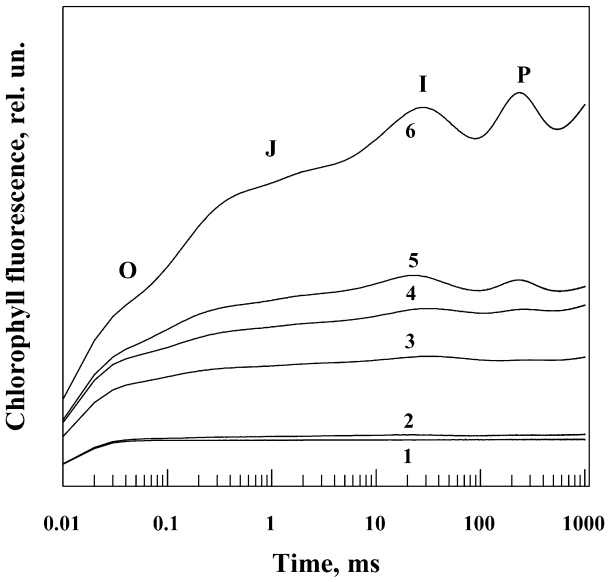

Fig. 7 Original traces of the fast rise of Chl fluorescence in air-dried *H. physodes* thalli (*1*) or in partially rehydrated thalli, in which the maximal extent of F_v was recovered to 2% (*2*), 14% (*3*), 24% (*4*), 33% (*5*), and 100% (*6*). Fluence rate of red actinic light was 4,000 μmol quanta m^{-2} s^{-1} using Plant Efficiency Analyser. For other details see Materials and methods

proceeding synchronously during the restoration of PSII activity. Figure 8 shows the relationship between the changes in F_o and F_v during re-hydration of dry lichen thalli. F_o and F_v were primarily recorded with partially hydrated thalli at various time intervals after they had been supplied with 50 μl of water. The values obtained for partially hydrated thalli were then normalized to corresponding values registered in the same thallus kept for 15 min in the dark after addition of 500 μl of water (fully hydrated). Measurements of F_o and F_v were done with both PAM (closed circles) and PEA (open circles) Chl fluorometers. The data obtained with both instruments were similar (Fig. 8). The initial step of re-hydration was characterized by the restoration of about 40% of F_o observed in fully hydrated thallus whereas the restoration of F_v was less than 20% of the maximum F_v. Thus, the restoration of F_o and F_v were not closely co-ordinated during this phase of re-hydration. Obviously, this is explained by a clear separation between the recovery of PSII antenna functions and charge separation in PSII. During further re-hydration, a linear increase in the restoration of F_o and F_v was observed. The above indicates that the absorbed light energy is utilized for primary photochemical reactions in PSII during the recovery process. This is confirmed by the increase in F_v/F_o by severalfold, a direct measure of the functional PSII centers (Babani and Lichtenthaler 1996; Kaňa et al. 2002), in close correlation with water oxidation in fully hydrated lichen thallus (data not shown).

Figure 9a shows that the reversible photochemical changes of PSI were recovered earlier than those of PSII during the first steps of re-hydration: almost 60% of maximum ΔA_{830} was already attained, when only 30% of maximum F_v was achieved. A more rapid development of PSI activity in the course of re-hydration was not found if the F_o level was compared with ΔA_{830}: the increase in F_o was accompanied by a nearly proportional appearance of reversible ΔA_{830} (Fig. 9b). The above is consistent with the data presented in Fig. 8 where the recovery of F_v and F_o are compared.

Discussion

The major aspect of desiccation tolerance in plants is their ability to develop strategies to protect the co-ordinated functions of the photosynthetic machinery, which includes both antenna systems and various components of the different electron transport chains, against severe loss of water. This is essential in resurrection species like poikilohydric lichens when they undergo repeated cycles of dehydration and re-hydration in nature. Indeed, the present investigation characterizes

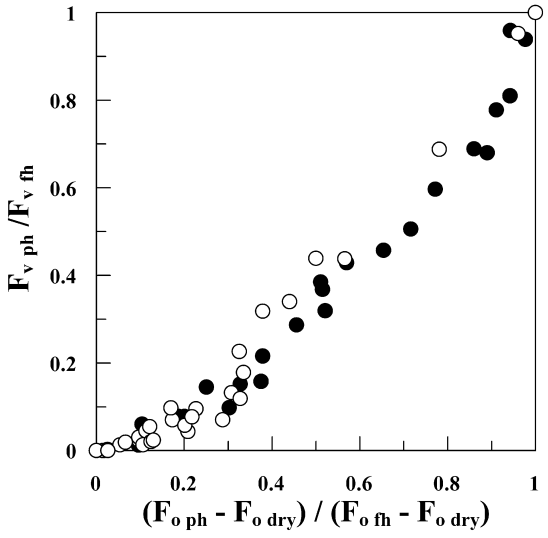

Fig. 8 Relationship between the changes in F_o level of Chl fluorescence and the extent of F_v ($=F_m-F_o$) during re-hydration of dry lichen thalli. F_v was induced with 2-s pulses of strong white light. Each measurement was done with a new sample using either PAM (*closed circles*) or PEA (*open circles*) Chl fluorometers. Each F_o and F_v value obtained for partially re-hydrated thallus was normalized to a corresponding value obtained for the same thallus in the fully hydrated state reached after its 15-min incubation in the dark. *F_o ph*, *F_o fh* and *F_o dry*, basal level of fluorescence of partially hydrated, fully hydrated, and dry thallus, respectively; *F_v ph* and *F_v fh*, variable fluorescence of partially hydrated and dry thalli, respectively

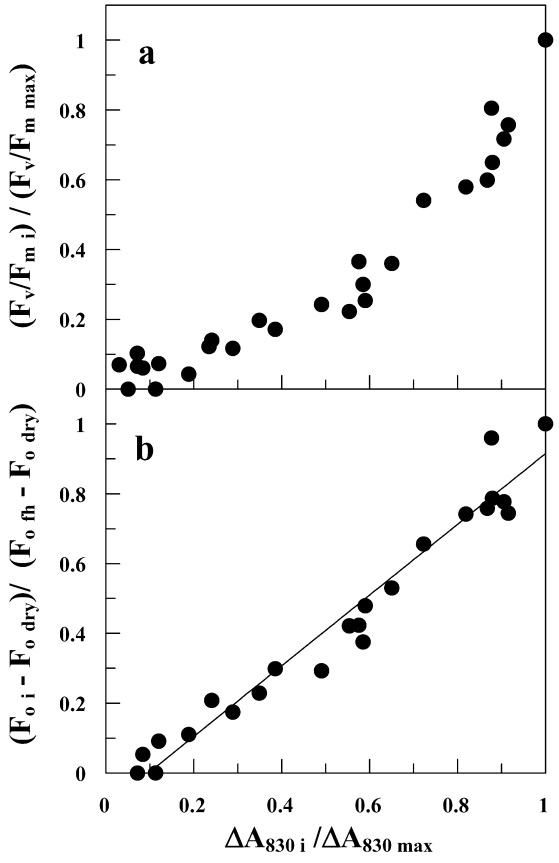

Fig. 9a,b Relationship between the development of reversible ΔA_{830} changes and F_v (**a**) or F_o (**b**) during re-hydration of dry thalli of *H. physodes*. *i* Sample index

the main negative effects of desiccation stress on photochemical reactions and their recovery during re-hydration in the lichen, *H. physodes*. These desiccation/re-hydration-induced changes in the photochemical efficiencies of both PSI and PSII will be discussed in detail further below. The data presented here demonstrate that the irreversibility of P700 photooxidation under continuous irradiation in dry thalli of *H. physodes* is caused by a suppressed input of electrons to $P700^+$ from the intersystem chain. In dry thalli, no component with a half-time of 20–30 ms corresponding to Q_A^- re-oxidation was found in the kinetics of $P700^+$ reduction after the application of an ST or a 50-ms MT pulse. Also, the rates of $P700^+$ reduction after an ST or a 50-ms MT pulse were several times higher than those observed after continuous white light. This substantiates the idea that the partial reversibility of P700 photooxidation was caused by recombination between $P700^+$ and electron acceptor(s) on the PSI reducing side rather than by electron transport from PSII, which is completely inactivated in dry state.

The back-reaction from the reduced $[F_A/F_B]$ cluster (iron–sulfur centers of PSI) to $P700^+$ in intact PSI complexes is characterized by half-times of about 40 ms (Sonoike et al. 1995; Teicher et al. 2000). If $[F_A/F_B]$ centers are destroyed or chemically reduced, a back electron transfer proceeds from the acceptor F_X^- to $P700^+$ with a characteristic time of about 1 ms (Golbeck and Bryant 1991). In desiccated lichens, the half-time of $P700^+$ reduction after an ST pulse was found to be 3.7 ms (Fig. 3b), which is close to the characteristic time of recombination in the $[P700^+\ F_X^-]$ pair. We can thus conclude that the electron transfer on the acceptor side of PSI is restricted to the reduction of F_X in some population of PSI units. Irreversible stabilization of $P700^+$ in dry thalli can be explained as follows. For every event of light-induced charge separation in the PSI reaction center, there is a probability that the electron will be delivered from F_X^- to oxygen. Clearly, the amount of PSI reaction centers losing an electron increases with the number of light-induced turnovers in the reaction centers. This corresponds to a much larger irreversible P700 oxidation state provided by ST pulses compared to MT pulses (see Fig. 3a).

Two important observations presented in Fig. 2 for dry thalli of *H. physodes* were, first, that the ability of MT pulses to oxidize P700 declined with increasing number of pulses. Secondly, the rate of P700 oxidation under FR light (as well as under moderate white light, data not shown) was significantly slower compared to hydrated samples. The first finding indicates that redox turnovers provided by 50-ms MT pulses were not sufficient to completely oxidize P700 despite the fact that the irreversible component of its kinetics dominated. The ability of FR light to exhibit a much larger extent of

P700 oxidation (Fig. 2) suggests that the acceptor side of PSI was active in the population of PSI centers weakly oxidized by the 50-ms pulses. Thus, the most plausible explanation for these two phenomena is that the acceptor F_X could not be reduced in a major fraction of PSI reaction centers in dry thalli. Recombination between $P700^+$ and reduced acceptor A1 (secondary acceptor of PSI) is known to proceed with characteristic times of 3–5 μs (Shuvalov et al. 1986; Golbeck 1987). This is much below the time resolution of our experimental system. Nevertheless, we propose that rapid back reaction in the pair [$P700^+$ $A1^-$] does not allow oxygen to be reduced with high efficiency under continuous FR light. The above must determine the slow rate of P700 oxidation in dry thalli.

It has been proposed that controlled backflow of electrons in PSI either through recombination or accelerated rates of PSI cyclic electron transport might protect the damages to PSI under specific stress conditions (see Kim et al. 2001 and references therein). However, the present study clearly demonstrates that the latter mechanism is fully suppressed in dehydrated lichens (Fig. 2). Therefore, it appears that the major mechanism to protect PSI in dehydrated lichens is the recombination that culminates in the accumulation of oxidized P700. In addition, it should be mentioned that the recombination between $P700^+$ and $A1^-$ leads directly to the ground state of P700 in PSI reaction centers in the absence of F_X, F_B and F_A (Warren et al. 1993). This protection strategy would permit efficient $P700^+$-mediated dissipation of absorbed energy into heat. Two lines of evidence strengthen this view. Firstly, oxidized P700 is highly stable (Hihara and Sonoike 2001). Secondly, $P700^+$ does not initiate photooxidative damage as its redox potential is much lower compared to P680, the primary donor of the PSII reaction center (Hihara and Sonoike 2001).

Unlike PSI, which exhibits light-induced redox changes even in dehydrated lichen thalli, PSII reaction centers seem to be photochemically inactive in such a state. In these conditions, a severalfold decline in F_o indicates an ineffective PSII antenna system with a greatly enhanced thermal dissipation yield of absorbed quanta (Figs. 1, 7). The decrease in F_o should be construed as the operation of a photoprotective mechanism associated with the quenching of absorbed energy in the dehydrated state of lichens, whereas the increase in F_o, usually observed in dehydrated higher-plant leaves (Heber et al. 2001), is correlated with damaged PSII (Cleland et al. 1986). Therefore, this response seems to be of great importance for the very survival of lichens in the dehydrated state, as it provides photoprotection of PSII under conditions when absorbed light cannot be utilized photochemically (Heber et al. 2001).

Upon re-hydration of dehydrated lichens, the removal of antenna quenching and restoration of charge separation in PSII is indicated by a progressive increase in F_o and F_v (Figs. 1, 7, 8). This is a clear demonstration of the reconstitution of PSII units during re-hydration. However, the disappearance of PSII antenna quenching and the increase in the quantum yield of primary photochemistry of PSII are not synchronous as this phenomenon exhibited temporal separation (Fig. 8). With the availability of water, the initial steps of re-hydration represent the recovery of a functional antenna system so as to ensure the absorbed light energy is fully utilized in photochemistry. Furthermore, the data presented in Fig. 8 demonstrate clearly the existence of a close correlation between the progressive increase in F_o and F_v in the latter phase of recovery during re-hydration. We cannot rule out, however, that a non-proportional relationship between the development of F_o and F_v levels during re-hydration could be related to the curvilinear dependence of F_o and F_v quenchings in the antenna or reaction center of PSII (Butler and Kitajima 1975). If the recoveries of PSII and PSI are compared, the latter develops much earlier (Fig. 9a). The relatively slow restoration of PSII may be due to a requirement for more elaborate reactivation processes such as photoactivation of the Mn cluster in the water-oxidizing complex.

A large difference was found between hydrated lichen thalli and leaves of higher plants in the kinetic curves of $P700^+$ reduction and QA^- re-oxidation following brief white light illumination, which saturated F_v thus indicating full QA^- reduction. Indeed, in higher plants, the kinetics of $P700^+$ reduction after continuous white light irradiation is monoexponential and characterized by half-times of 6–12 ms (Harbinson and Hedley 1989; Laisk and Oja 1994), while the kinetics of QA^- re-oxidation is complex and consists of several components (Eaton-Rye and Govindjee 1988; Etienne et al. 1990; Bukhov and Carpentier 1996; Bukhov et al. 2001a). However, opposite results were obtained in the partially or even fully hydrated lichen *H. physodes*, in which the re-oxidation of QA^- was fast and monophasic, whereas the contribution of the middle and slow components to $P700^+$ reduction was significant (see Figs. 4, 6). The two above components of $P700^+$ re-reduction were obviously not related to electron donation to PSI from PSII since they were observed in the kinetics of $P700^+$ re-reduction after irradiation by FR light (compare Figs. 4 and 5). Recently, two components were found in the dark reduction of P700+ in leaves of higher plants treated with methyl viologen and irradiated with FR light (Bukhov et al. 2001b, 2002). They were ascribed to the different routes of non-photochemical electron donation to the plastoquinone pool, from which reducing equivalents reached $P700^+$ in one of two different types of PSI unit. Similar conclusions appear to be valid for lichen. Indeed, the ratio of half-times between the slow and fast components of $P700^+$ re-reduction after FR light varied in the range 8.4–9.5 for different lichen thalli, while the ratio of their magnitudes approached 0.7–0.9. Thus, the existence of two components in the kinetics of $P700^+$ dark re-reduction cannot be explained by simple kinetic competition between two pathways of non-photochemical donation of electrons to the same

PSI unit. From the ratio of their half-times, a ratio of 1:9 can be deduced for the magnitudes of the slow component to that of the middle one. Thus, our data demonstrate that two independent types of PSI unit exist in *H. physodes*.

When partially or fully hydrated lichen thalli were irradiated with strong white light, which simultaneously excites both PSII and PSI, the half-times for the fast component of $P700^+$ reduction varied between 22 and 30 ms in partially or fully hydrated thalli (Figs. 4, 6b). This component was completely abolished by diuron and was not observed after irradiation of partially or completely hydrated thalli by FR light (see Fig. 2, traces 2 and 3, and Fig. 5). These findings support the view that the electrons injected by PSII determine fast $P700^+$ reduction after irradiation with white light in lichens. The values of 22–30 ms are higher than those reported for characteristic times of electron transfer from PSII to PSI in higher plants (Harbinson and Hedley 1989; Laisk and Oja 1994). Thus, the intersystem electron transport is much slower in *Hypogymnia physodes* than in higher plants. This likely constitutes an adaptive mechanism to sustain electron donation through alternative routes to a population of PSI units during the initial steps of rehydration.

Another line of evidence shows that the contribution from the sum of the slow and middle components to the total ΔA_{830} approached one-third (Fig. 4). This is a clear demonstration of the inability of PSII to provide reducing power sufficient to saturate all PSI units with electrons as discussed above. Therefore, the autotrophic component of *H. physodes* is different in comparison with higher-plant chloroplasts (Kaňa et al. 2002). In the remaining PSI units, relatively slow electron flow to $P700^+$ proceeded from reductants localized in the chloroplast stroma. The kinetics of QA^- re-oxidation represented by a single fast component in the lichen *H. physodes* strongly support this conclusion (Fig. 6a). Such kinetics consist of several components in higher plants, in which PSII activity is in excess compared to the electron transport capacity between the two photosystems (Eaton-Rye and Govindjee 1988; Etienne et al. 1990; Bukhov and Carpentier 1996; Bukhov et al. 2001a). The appearance of slower components is explained by the presence of a highly reduced pool of plastoquinone with corresponding lack of oxidized plastoquinone molecules to occupy the Q_B-binding (secondary quinone acceptor of PSII) site following QA^- re-oxidation (Eaton-Rye and Govindjee 1988, Bukhov et al. 2001a). Under such conditions, a major fraction of reduced Q_A species is re-oxidized by recombination with components of the PSII donor side (Bennoun 1970). In lichens, the reducing power generated by PSII is not sufficient to completely fill up the plastoquinone pool with electrons, and access of oxidized plastoquinone molecules to the Q_B-binding site is efficient enough to provide relatively fast Q_A^- re-oxidation in all photochemically competent PSII centers.

In summary, our results demonstrate that reversible light-driven redox transformations are restored in both PSII and PSI during re-hydration of dehydrated *Hypogymnia physodes*, and that transformation of PSI develops faster. In fact, P700 can be photooxidized even in dry thalli through irreversible reactions. In partially or fully hydrated thalli, PSII is not capable of reducing all PSI centers, and about one-third of PSI units receive electrons via alternative routes.

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