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A dip in the chlorophyll fluorescence induction at 0.2–2 s in *Trebouxia*-possessing lichens reflects a fast reoxidation of photosystem I. A comparison with higher plants

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Abstract

An unusual dip (compared to higher plant behaviour under comparable light conditions) in chlorophyll fluorescence induction (FI) at about 0.2–2 s was observed for thalli of several lichen species having *Trebouxia* species (the most common symbiotic green algae) as their native photobionts and for *Trebouxia* species cultured separately in nutrient solution. This dip appears after the usual O(J)IP transient at a wide range of excitation light intensities (100–1800 μmol photons m^{-2} s^{-1}). Simultaneous measurements of FI and 820-nm transmission kinetics (I_{820}) with lichen thalli showed that the decreasing part of the fluorescence dip (0.2–0.4 s) is accompanied by a decrease of I_{820}, i.e., by a reoxidation of electron carriers at photosystem I (PSI), while the subsequent increasing part (0.4–2 s) of the dip is not paralleled by the change in I_{820}. These results were compared with that measured with pea leaves—representatives of higher plants. In pea, PSI started to reoxidize after 2-s excitation. The simultaneous measurements performed with thalli treated with methylviologen (MV), an efficient electron acceptor from PSI, revealed that the narrow P peak in FI of *Trebouxia*-possessing lichens (i.e., the I–P-dip phase) gradually disappeared with prolonged MV treatment. Thus, the P peak behaves in a similar way as in higher plants where it reflects a traffic jam of electrons induced by a transient block at the acceptor side of PSI. The increasing part of the dip in FI remained unaffected by the addition of MV. We have found that the fluorescence dip is insensitive to antimycin A, rotenone (inhibitors of cyclic electron flow around PSI), and propyl gallate (an inhibitor of plastid terminal oxidase). The 2-h treatment with 5 μM nigericin, an ionophore effectively dissipating the pH-gradient across the thylakoid membrane, did not lead to significant changes either in FI nor I_{820} kinetics. On the basis of the presented results, we suggest that the decreasing part of the fluorescence dip in FI of *Trebouxia*-lichens reflects the activation of ferredoxin–$NADP^+$–oxidoreductase or Mehler–peroxidase reaction leading to the fast reoxidation of electron carriers in thylakoid membranes. The increasing part of the dip probably reflects a transient reduction of plastoquinone (PQ) pool that is not associated with cyclic electron flow around PSI. Possible causes of this MV-insensitive PQ reduction are discussed.

Keywords: Chlorophyll fluorescence; OJIP-transient; Lichen; 820-nm transmission; Photobiont; Photosystem; Thylakoid membrane; *Trebouxia*; *Lasallia pustulata*; *Umbilicaria hirsuta*; *Hypogymnia physodes*

1. Introduction

Chlorophyll fluorescence induction (FI) measured on photosynthesizing samples (higher plants, algae) during the first second(s) of intensive light excitation (hundreds to thousands of μmol photons m^{-2} s^{-1}) following a dark-to-light transition reveals the characteristic O–$J(I_1)$–$I(I_2)$–P transient [1,2]. Parameters calculated from the transient are often used for detection of plant stresses influencing the state and function of thylakoid membranes (for review see [3–6]).

Abbreviations: CEF, cyclic electron flow around photosystem I; DCMU, 3-(3,4-dichlorophenyl)-1,1′-dimethylurea; F_O-level, chlorophyll fluorescence intensity measured when all photosystem II reaction centers are open; Fd, ferredoxin; FI, chlorophyll *a* fluorescence induction; FNR, ferredoxin-$NADP^+$-oxidoreductase; LED, light emitting diode; MV, methylviologen; OJIP transient, fluorescence induction defined by the names of its intermediate steps; P700, the primary donor of photosystem I; PAR, photosynthetically active radiation; PC, plastocyanin; $PQ(H_2)$, plastoquinone (reduced); PSI(II), photosystem I(II); PTOX, plastid terminal oxidase; $Q_{A(B)}$, the primary (secondary) quinone acceptor of photosystem II

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Whereas the O–J phase of FI reflects a reduction of the primary quinone electron acceptor of photosystem II (PSII) (Q_A) with a weak reduction of acceptors beyond Q_A, the subsequent J–I phase is related to reduction of the plastoquinone (PQ) pool by reduced Q_A (e.g. [1,7]). The I–P phase of FI has been explained by different mechanisms (see e.g. [8]). Extensive mathematical simulations of the OJIP transient based on the extended reversible radical pair model reveal that electron transport beyond the PQ pool must be taken into account to be able to explain the I–P phase [9]. Recently published experimental work focusing on the I–P phase in FI in pea leaves [8] has supported the hypothesis that the I–P phase is related to the electron flow through photosystem I (PSI) [10–12]. A traffic jam of electrons at the acceptor side of PSI due to inactive ferredoxin–$NADP^+$–oxidoreductase (FNR), leading to reduction of electron carriers in thylakoid membranes, is probably the cause of the I–P phase of FI [8].

In this study, we demonstrate that FIs measured on lichen thalli, having algae of the *Trebouxia* genus as their photobionts, and on *Trebouxia* suspensions exhibit, in addition to the OJIP transient (within 0.2 s), another fluorescence dip in the 0.2–2 s time interval. A very similar but less pronounced fluorescence dip has already been reported for FI measured on some foraminifer symbionts *in hospite* [13] and zooxanthellae of corals [14]. A part of this dip (a measurement up to 1 s) appeared also in FI of the lichen *Hypogymnia physodes* [15]. The physiological background of this phenomenon has not been studied yet. Our study focuses on the possible mechanisms responsible for this dip paying special attention to the electron flow through photosystem I and the redox state of the PQ pool.

2. Material and methods

2.1. Material

Thalli of foliose lichen species *Umbilicaria hirsuta*, *Lasallia pustulata* were collected from granitic rocks (Oslava river valley, 30 km west of Brno, Czech Republic). *Hypogymnia physodes* thalli were collected from bark of orchard trees (Moravany, 5 km south of Brno). After collection, the thalli were naturally dehydrated at room temperature under dim light and stored in the dark at 5 °C for 7 days. Prior to the measurements, the thalli were rehydrated at natural dim light at about 15 °C for 4 days. The lichen photobiont strain *Trebouxia erici* Ahmadjian, a representative of the green alga genus that is present in *Umbilicaria* and *Lasallia* lichens, was cultured in BBM3N medium at 20 °C under a 14/10 light/dark regime (20 μmol photons m^{-2} s^{-1}; PAR). Chlorophyll $a+b$ concentration in the suspension was determined spectrophotometrically using DMSO as a solvent [16]. Pea (*Pisum sativum*, cv. Ambassador) was grown in a greenhouse for 3 weeks. Mature pea leaves were chosen for the measurements. Samples were pre-darkened for 45–60 min before the measurements.

2.2. Chemical treatment

All chemicals (antimycin A, rotenone, n-propyl gallate, nigericin and tentoxin) except methylviologen (MV) were dissolved in ethanol and then added into the suspension of *Trebouxia erici* or into distilled water for the treatment with *Umbilicaria* thalli. The final ethanol concentration was ≤1%. Pieces of thalli (approximately 1 cm^2) were smeared with the solution and then kept in the contact with a filter paper (moistured with the solution) for various times. The last treatment (with tentoxin) was performed with thalli from a different collection. That is why the control FIs are slightly different from that obtained for other treatments. This distinction, however, is not significant with respect to the results we discuss.

2.3. Measuring equipment

Chlorophyll fluorescence induction and 820-nm transmittance (I_{820}) kinetics were measured simultaneously using a dual channel PEA Senior instrument (Hansatech Instruments, Norfolk, UK) as described previously [8]. FI measurements on algal suspensions were performed using a PEA fluorimeter (Hansatech Instruments, Norfolk, UK) equipped with the PEA/VA vial adapter for measurements with liquid samples (e.g. [17]). The measurements were performed with 0.5 ml alga suspension with chlorophyll $a+b$ concentration of 20 μg ml^{-1}. The sources of excitation light were ultra bright 650-nm LEDs in both PEA instruments.

3. Results

3.1. FI of lichen thalli and photobiont suspension

The upper curve in Fig. 1 shows the FI measured with a dark-adapted pea leaf under high intensity exciting light (1800 μmol photons m^{-2} s^{-1}). This FI follows the typical OJIP kinetics that can be observed in intact leaves of many higher plants and algal species [2]. The two middle FIs in Fig. 1 were measured with thalli of two lichen species *Umbilicaria hirsuta* and *Lasallia pustulata* under the same conditions. In both FIs, the J and I steps appeared at similar positions as in the FI of a pea leaf (~2 and 20 ms, respectively). The FI of a pea leaf had a rather flat P step lasting from 0.2–2 s, that is typical for FIs measured with higher plants and many algal species at higher exciting light (see [2]). In the FI of lichen thalli, on the other hand, we observed a fluorescence dip in this time range (more pronounced for

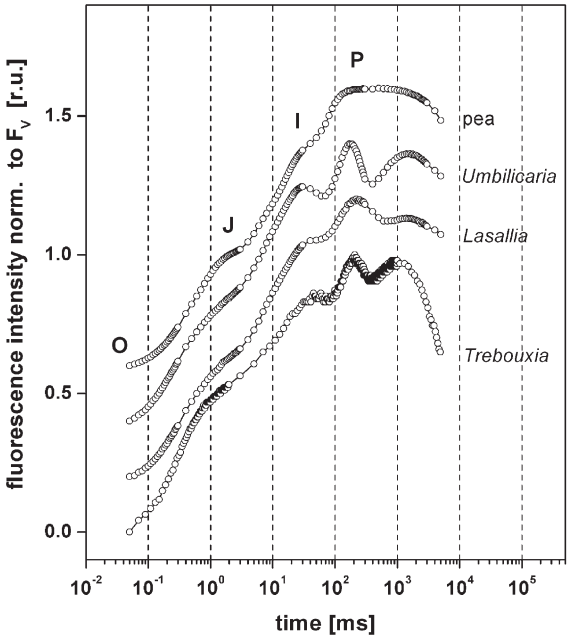

Fig. 1. FIs of a mature pea leaf, lichen thalli of *Umbilicaria hirsuta* and *Lasallia pustulata*, and their photobiont *Trebouxia erici*. The excitation intensity was 1800 μmol photons m^{-2} s^{-1}. FIs were normalized to variable fluorescence (F_V) and vertically shifted. The FI of the *Trebouxia* suspension (0.5 ml) was measured with a different instrument (see Material and methods) at a chlorophyll $a+b$ concentration of 20 μg ml^{-1}.

Umbilicaria), "splitting" the typical P step into two narrow peaks with maxima at about 0.2 and 1–2 s. A similar dip was also found in the FI measured with a suspension of the green alga *Trebouxia erici* (the natural photobiont of the *Lasalia* and *Umbilicaria* lichens), which was cultivated separately in nutrient solution (Fig. 1, bottom curve). This implies that the observed fluorescence dip in the FI does not depend on the photobiont–mycobiont interaction within the lichen thallus and thus might be attributed exclusively to the algal photobiont. We also observed the dip in FI of other lichens having different *Trebouxia* species as their photobionts (e.g. *Hypogymnia physodes* possessing *Trebouxia jamesii* as a photobiont) or of a suspension of other symbiotic green algae (e.g. *Trebouxia irregularis*) (data not shown).

3.2. FI and I_{820} transients at various intensities of exciting light

To characterize the fluorescence dip in the FI of *Trebouxia*-possessing lichens, we measured the FI at various intensities of exciting light (100–1800 μmol photons m^{-2} s^{-1}). Simultaneously, the transmission kinetics at 820 nm (I_{820}), monitoring mainly the redox state of P700 and plastocyanin (PC) (e.g. [18]), were detected. For practical reasons – the highest I_{820} signal/noise ratio – we chose *Umbilicaria hirsuta* for the simultaneous measurements. For comparison, the same measurements were performed with leaves of pea—as a representative of higher plants. The changes of I_{820} in *Trebouxia* suspensions were below the detection limit of the used instrument.

Our FIs measured with pea leaves at various intensities of exciting light (Fig. 2B) are very similar to those reported earlier [2] and represent a common excitation intensity profile of FI of higher plants and many algal species ([2], our unpublished data). A gradual change of the OIP to OJIP transient, a shift of the P step to shorter times and its increase (relative to the F_O level) with increasing exciting light belong to the typical characteristics. A comparison of Fig. 2B and D also shows that in the lichen the J, I and P levels saturate slightly faster than in pea-leaves indicating that PSII activity in the lichen is not more limiting than in the pea leaves. Fig. 2A shows the I_{820} transients corresponding to the FIs of pea (Fig. 2B). Whereas the initial I_{820} decrease (up to the I step in the corresponding FIs) is attributed to transient oxidation of PC and P700 due to PSI activity, the subsequent increase in I_{820} (I–P phase at FI) reflects their reduction by electrons generated by PSII and the transient block at the acceptor side of PSI (see [8,18]). These two phases of the I_{820}-signal create a dip with a minimum at about 20–30 ms that is more pronounced for higher intensities of exciting light (Fig. 2A). In addition, it can be observed that the increase of I_{820} is faster for higher exciting light intensities, which corresponds to the acceleration of the I–P phase in FI (Fig. 2B).

FIs measured with *Umbilicaria* thalli (Fig. 2D) show that the presence of the unusual dip (0.2–2 s) in FI is not dependent on the intensity of the exciting light. This implies that the dip represents rather a biochemical than a photochemical reaction. The position of the P step in the lichen FI shifts from 250 to 150

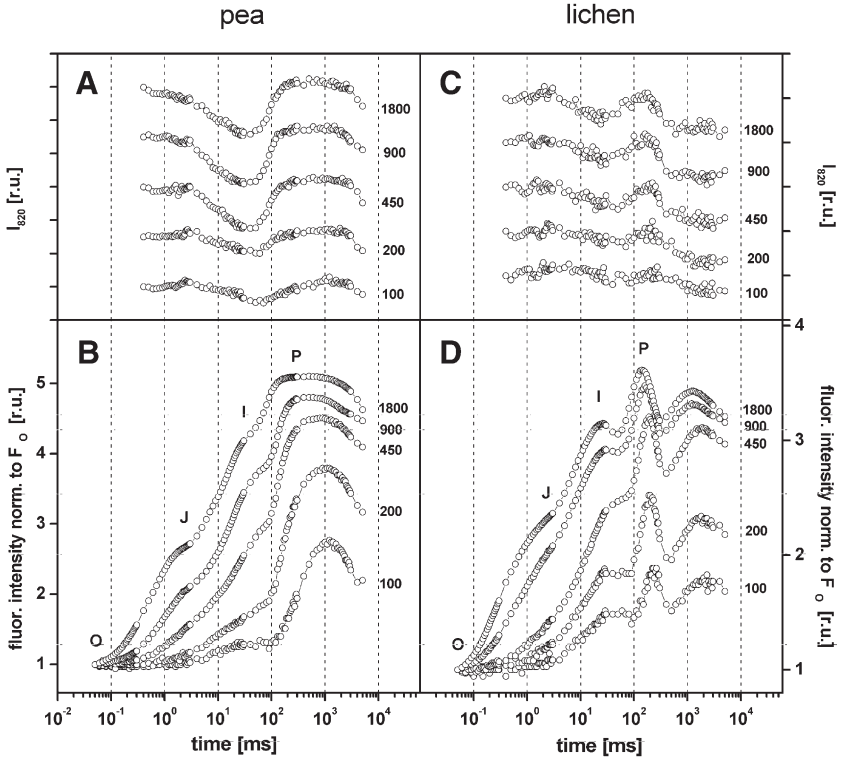

Fig. 2. FIs (B, D) and transmission changes at 820 nm (I_{820}) (A, C) for pea leaves (A, B) and *Umbilicaria* thalli (C, D) during 5-s excitation at different intensities of exciting light (1800, 900, 450, 200 and 100 μmol photons m^{-2} s^{-1}). I_{820} kinetics was measured at the same intensity of 820-nm light; the kinetics are vertically shifted. FIs were normalized to the fluorescence intensity at 50 μs ≅ the minimal fluorescence level F_O [2].

ms changing the light intensity from 100 to 1800 μmol m^{-2} s^{-1}, a shift that was more pronounced in pea (1 to 0.2 s) in the same light intensity range (compare Fig. 2B and D). The position of the step following the P step in FI of *Umbilicaria* is also shifted to shorter times with increasing intensity of the exciting light (from about 2–3 to 1 s).

The I_{820} transmission kinetics (compare Fig. 2A and C) and FIs (Fig. 2A and C) of *Umbilicaria* thalli and pea up to about 0.2 s are similar. Unfortunately, the S/N ratio of the I_{820} signal was considerably lower for the lichen than for pea leaves, which is most apparent for the transients measured at the lower exciting light intensities. However, it can be clearly seen that the decreasing phase of the fluorescence dip at 0.2–0.4 s coincides with a decrease of I_{820}, i.e., an oxidation of P700 and PC (compare Fig. 2C and D). Such an oxidation of PSI induces an oxidation of other electron carriers in the thylakoid membrane and leads to a decrease of the fluorescence level. Interestingly, the subsequent fluorescence increase in FI (0.4–2 s) is not connected with a change in the I_{820} kinetics. Our findings indicate that for the lichen, the traffic jam of electrons caused by a transient block at the acceptor side of PSI, reflected in the P peak in FI (see Introduction), is relatively short lived in comparison with that of pea leaves.

3.3. FI and I_{820} kinetics after 2-s dark adaptation

To study the fluorescence dip in FI of lichens in more details, we used two 5-s pulses separated by a 2-s dark interval and simultaneously measured the FI and I_{820} kinetics (Fig. 3). Again, measurements with pea leaves were performed for comparison. The 2-s dark period was chosen to be sufficient for a full recovery of the I_{820} kinetics (re-oxidation of the ferredoxin (Fd) pool) in pea leaves [18]. Fig. 3A and B show that whereas the I_{820} kinetics for a pea leaf really recovered after the 2-s darkness, the corresponding FIs differed substantially in their shape. The second FI recorded after the dark period resembles the FI of samples that were made anaerobic [19]. This observation indicates that the 2-s dark interval was not long enough for the reoxidation of the PQ pool in thylakoid membranes of pea leaves.

Quite different results were obtained in the double pulse experiment with *Umbilicaria* thallus (Fig. 3C and D). In the measurement after the 2-s dark period, we did not observe any increase in the I_{820} at 20–200 ms, i.e., no transient re-reduction of P700 and PC occurred. This implies that the electron transport is not limited at the acceptor side of PSI. Concomitantly, the P peak and the subsequent peak in the FI were absent. These observations agree with the results of Fig. 2 implying that a rapid activation of electron outflow from PSI occurred after 0.2 s of irradiation. It also indicates that the P peak in the FI of *Trebouxia*-possessing lichens reflects a transient limitation of the electron transport at the acceptor side of PSI similarly to that in higher plants (see above). The absence of the transient re-reduction of PC^+ and $P700^+$ during the second measurement reveals that 2 s of darkness were insufficient to undo the activation of the electron flow beyond

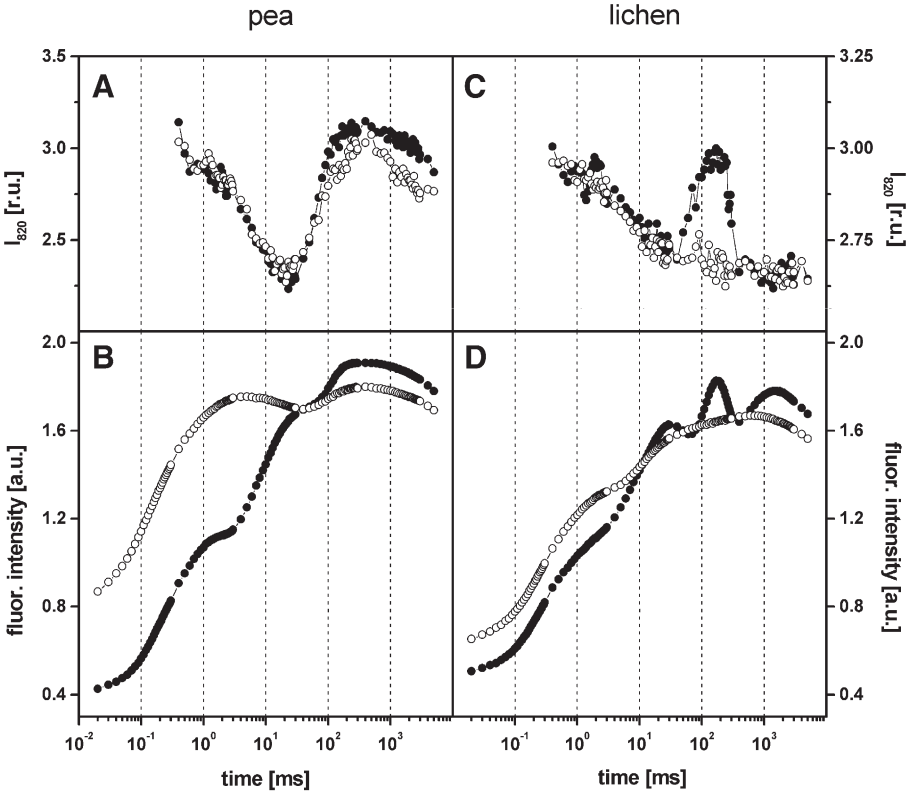

Fig. 3. FIs and I_{820} kinetics for an *Umbilicaria* thallus and a pea leaf during 5-s excitation (filled circles) and that measured subsequently after 2-s dark period (open circles). Excitation intensity was 1800 μmol photons m^{-2} s^{-1}.

PSI. Increasing the dark period between the two measurements we found that at least 3 min of dark adaptation after the first light pulse were necessary for full re-establishment of the transient block at the acceptor side of PSI (data not shown).

From the comparison of Fig. 3B and D, it is obvious that the O–J–I transient of the second FI of *Umbilicaria* after 2 s of darkness resembles the O–J–I transient of the first FI to a considerable extent. Reducing the length of the first pulse to 100 or 200 ms did not change the FI on the second pulse, which may point to a high plastoquinone oxidase activity (data not shown). However, after the activation of electron flow at the acceptor side of PSI a fast reoxidation of the plastoquinone pool can also be explained by forward electron transport to PC^+ and $P700^+$ (see Fig. 3A and C). This was also observed in pea leaves treated with MV [8]. Comparing Fig. 3B and D, it can also be observed that the suppression of the maximum fluorescence level in the second pulse is very similar in *Umbilicaria* thalli and pea leaves. This fluorescence quenching is probably due to the energy dependent component of non-photochemical quenching (qE) induced by an acidification of the lumen during the first pulse. If the dip is ignored, the FI in the 30-ms to 5-s range looks very similar in both species also with respect to the kinetics.

3.4. FI and I_{820} kinetics in MV-treated lichen thalli

To test whether the fluorescence dip at 0.2–2 s is sensitive to the presence of an extra electron sink at the acceptor side of PSI, we performed simultaneous measurements of the FI and I_{820} kinetics in *Umbilicaria* thalli treated with 1 mM methylviologen (MV) for 0–5 h. MV is an electron acceptor, which effectively competes with Fd for electrons at the acceptor side of PSI (e.g. [20]). It is evident that prolonged treatment with MV led to a gradual disappearance of the P step in FI. The change was accompanied by a disappearance of the transient increase in I_{820}, i.e., by the re-reduction of $P700^+$ and PC^+ (Fig. 4A and B). This result clearly shows again that the P step in FI peaked at about 0.2 s reflects a transient reduction of the Fd-pool at the acceptor side of PSI. It is further evident that the disappearance of the P step does not prevent the appearance of the fluorescence increase at about 0.4–2 s, i.e., the increasing phase of the fluorescence dip. This finding indicates that this phase of the dip, not accompanied by redox changes at PSI (see also Fig. 2), does not reflect a reduction of PQ pool via the Fd-dependent cyclic electron flow around PSI (CEF).

3.5. FI of samples treated with various inhibitors

To further characterize the unusual dip appearing in FI of *Trebouxia*-possessing lichens and the alga *Trebouxia* itself, we performed FI-measurements with suspensions of *Trebouxia erici* treated with inhibitors of CEF and plastid terminal oxidase (PTOX) in thylakoid membranes. Rotenone (200 μM) was used as the inhibitor of NAD(P)H-dehydrogenase and antimycin A (100 μM) for the inhibition of Fd-PQ reductase (see [21]). FIs of *Trebouxia* treated with these inhibitors were very similar to FIs

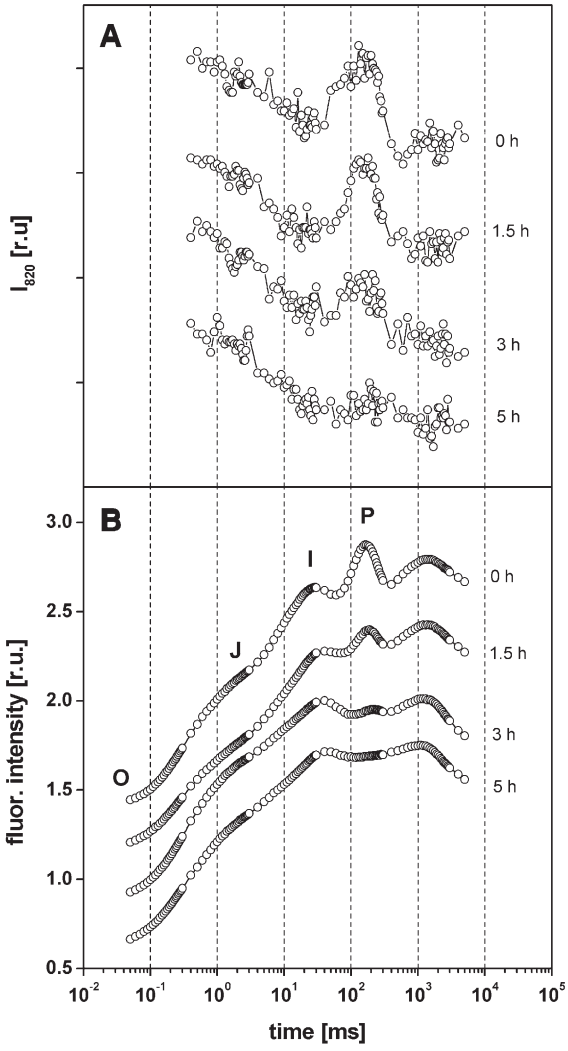

Fig. 4. I_{820} kinetics (A) and FIs (B) for *Umbilicaria* thalli treated with 1 mM MV for 0, 1.5, 3 and 5 h. Excitation intensity was 1800 μmol photons m^{-2} s^{-1}. The FIs represent vertically shifted raw curves. F_V/F_P ratios calculated from the FIs were 0.72, 0.66, 0.63 and 0.62, respectively. The P level was taken as a global fluorescence maximum in the FI.

of the untreated *Trebouxia* even if the incubation with these inhibitors at the high concentrations lasted for 2.5 h (Fig. 5). In the presence of antimycin A, a non-specific quenching of FI after the J step was observed. Very similar results were obtained for the treated *Umbilicaria* thalli (results not shown). These results indicate that neither Fd-dependent CEF nor NAD(P)H-dependent reduction of the PQ pool were responsible for the fluorescence dip in the FI of *Trebouxia*. This conclusion is also supported by the observed insensitivity of the increasing phase of the dip in the FI of *Umbilicaria* to MV (Fig. 4). The treatment with 2 mM n-propyl gallate, used as an inhibitor of PTOX in the thylakoid membrane [22], did not influence the dip in FI of *Trebouxia* either (Fig. 5). This indicates that possible chlororespiratory electron flow does not affect the studied fluorescence dip in FI.

A possible explanation for the increasing part of the dip could be that after activation of the electron flow at the acceptor side of PSI and the subsequent fast reoxidation of the

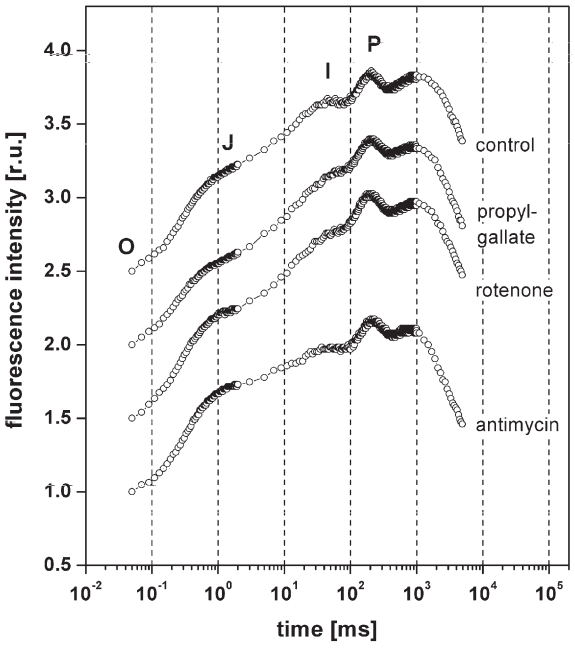

Fig. 5. FIs of *Trebouxia erici* suspension (control) treated with 2 mM n-propyl gallate, 200 μM rotenone and 100 μM antimycin A for 2.5 h in the dark. The FIs represent the raw curves, vertically shifted. Excitation intensity was 1800 μmol photons m^{-2} s^{-1}. Chlorophyll *a*+*b* concentration was 20 μg ml^{-1}.

electron transport chain a rapid acidification of the lumen occurs. This could happen especially near the cyt b_6/f complexes slowing down the re-oxidation of PQH_2 [23], which in turn would lead to a more reduced PQ pool and higher fluorescence level. At the same time the slow down of the re-oxidation of PQH_2 will also lead to a more oxidized state of PC and P700 or keep them oxidized as is observed in Fig. 3. To verify this idea, we approached this question in several ways: (i) FIs were measured after incubation with 5 μM nigericin to dissipate the pH-gradient; (ii) FIs were measured after incubation with tentoxin to inhibit ATP-synthase and to increase the pH-gradient; (iii) The re-reduction kinetics of PC^+ and $P700^+$ were determined in untreated *Umbilicaria* on turning off the light after various times of irradiation. Fig. 6A and B show the results of double pulse measurements separated by 2-s darkening for *Umbilicaria* thalli treated with 5 μM nigericin for 2 h. No significant changes in FIs and I_{820} kinetics of treated thalli in comparison with control ones were observed. The only change seemed to be a slightly more pronounced dip between I and P steps in the first pulse, which was not reflected in the change of I_{820}. Further extension of the treatment with 5 μM nigericin to several hours led to a progressive depression of the dip between I and P steps and to a disappearance of the P peak in FI. Even these fluorescence changes were not reflected in the change of I_{820} (results not shown). However, this nigericin-induced fluorescence quenching of variable fluorescence, which has been already reported for higher nigericin concentrations [24] probably represents a side effect of nigericin [25]. The 2-h treatment of *Umbilicaria* thalli with 5 μM tentoxin led to a faster induction of qE (Fig. 6D). This was especially apparent during the second pulse indicating that tentoxin inhibited the function of ATP-synthase. A more acid lumen is expected to lead to a lower re-oxidation rate of PQH_2. Tentoxin did, however, not lead to a more pronounced dip or steeper/higher increasing part of the dip. There were also no significant effects observed on the I_{820} kinetics (Fig. 6C). Finally, the re-reduction kinetics of $P700^+$ and PC^+ were studied on turning off the light after 1.2 s (increasing part of the dip) and after 100 s in untreated thalli. For the longer irradiation time a lower light intensity was used (540 μmol photons m^{-2} s^{-1}) to avoid photoinhibition. In both cases full re-reduction took 40–60 ms (data not shown) and most of the differences in the kinetics were probably due to the fact that P700 and PC were in a slightly less oxidized state after the 100 s of irradiance. All the three approaches indicate that there was little effect of the lumen pH on neither the electron transport rate from the PQ pool to P700 nor on the increasing part of the dip.

4. Discussion

4.1. Reoxidation of PSI reflected in FI

The presented results describe some characteristics of the unusual dip in FI of dark adapted *Trebouxia*-possessing lichens and *Trebouxia* itself appearing at 0.2–2 s after the onset of exciting light. From a simple comparison of FIs measured with *Trebouxia*-possessing lichens (*Trebouxia*) and pea leaves (Fig. 1), it seems that both FIs are nearly the same, only some additional transient fluorescence quenching at 0.2–2 s can be seen for *Trebouxia*. The simultaneous measurement of FI and I_{820} kinetics at various intensities of exciting light (Fig. 2) and the double pulse measurements separated by a dark period (Fig. 3) showed that the responses of both species were very different. For *Trebouxia*, a mere 0.2-s excitation activated an effective outflow of electrons from PSI reflected by a drop in I_{820} transient and the decreasing part of the dip (Figs. 2–4). This fast PSI oxidation enables PQ pool to be reoxidized in a subsequent 2-s dark period as detected using the second measurement of FI (Fig. 3B and D). The kinetics of the FI in the second measurement (Fig. 3D) are very similar to the FIs of the second measurement observed in pea leaves infiltrated with MV [8]. In [8] it was shown that at the end of a 1-s measurement in the presence of MV, PC and P700 were mainly in the oxidized state and quickly re-reduced by electrons from the plastoquinone pool. A similar acceleration of the re-oxidation of Q_A^- was observed by Bukhov et al. [26] who created a partially oxidized electron transport chain by either MV or low light intensities.

In pea leaves, the fluorescence and I_{820} levels decrease only very gradually after reaching the maximum fluorescence level (Fig. 3A and B), possibly indicating that the activation of the electron outflow from PSI is a gradual process. As a consequence, the electron transport chain is still nearly reduced after the 5-s measurement and the PQ pool cannot become oxidized by forward electron transport. Instead it has to be

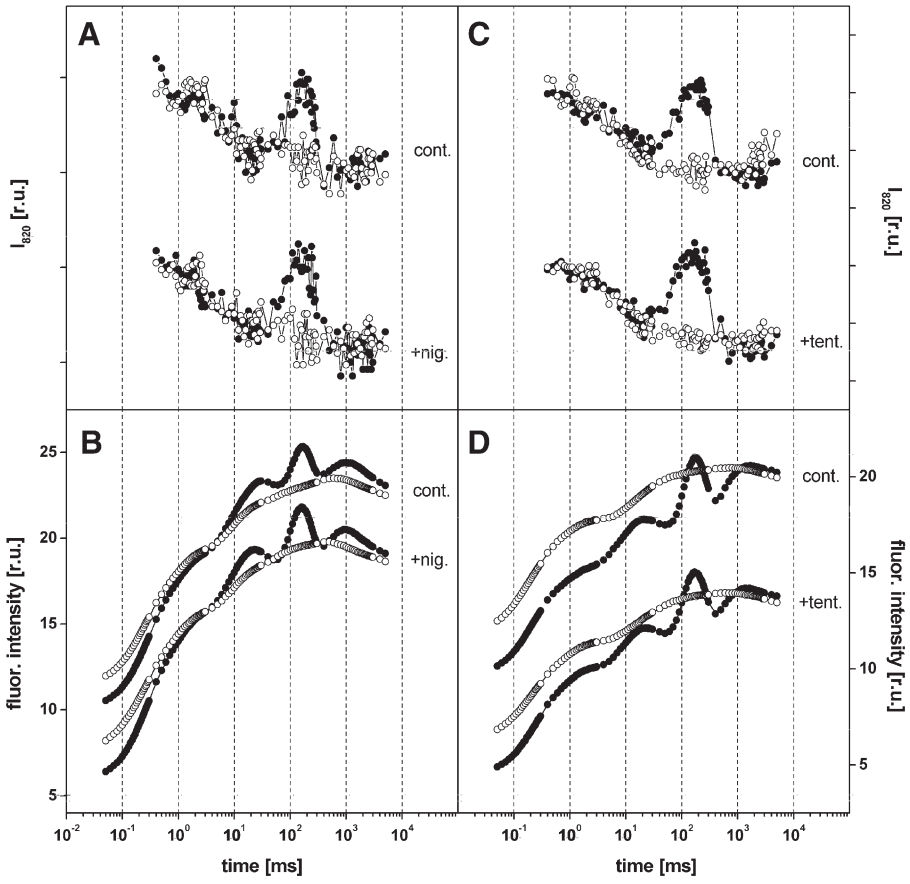

Fig. 6. I_{820} kinetics (A, C) and FIs (B, D) for *Umbilicaria* thalli either untreated or treated with 5 μM nigericin (A, B) or 5 μM tentoxin (C, D) for 2 h. The protocol consisted of two 5-s pulses spaced 2 s apart (first pulse: filled circles and second pulse: open circles). The transients of the treated thalli were vertically shifted. Excitation intensity was 1800 μmol photons m^{-2} s^{-1}. Each treatment was performed with a thallus from a different collection (see also Material and methods). That is why their control responses are slightly different.

oxidized by PTOX-activity, which takes much longer than 2 s [27,28].

4.2. Fluorescence dip in FI and CEF

The decreasing part of the dip in FI of *Trebouxia*-possessing lichens and *Trebouxia* itself, reflecting a fast outflow of electrons from PSI, was followed by the fluorescence increase forming a dip at about 0.3–0.5 s and another maximum at about 1–2 s (Fig. 2D). A simple explanation for this phenomenon would be the induction of CEF that returns electrons from PSI to the PQ pool and of which the fluorescence increase would be a reflection. However, the dip was neither sensitive to antimycin A nor to rotenone — inhibitors of the antimycin-sensitive Fd-dependent and NAD(P)H-dependent CEF, respectively (for review see [28]). Moreover, the treatment with MV that is assumed to prevent any Fd-dependent CEF (also possible antimycin-insensitive) did not influence the increasing part of the dip. From these results, we can conclude that the Fd- and NAD(P)H-dependent CEF are not responsible for the increasing part of the dip in FI. Other CEFs have been proposed. In [29] it was suggested that phylloquinone A_1 in PSI can react with oxygen to form superoxide that can be scavenged by PQ-9 and the Joliots [30,31] proposed an unspecified antimycin-insensitive pathway in stromal thylakoids. However, in all these CEFs, it would be expected that the addition of MV, markedly increasing the outflow of electrons from PSI (see the disappearance of the P step in FIs in Fig. 4), should change the increasing part of the fluorescence dip. Fig. 4 demonstrates that the presence of MV causes the disappearance of the P step but does not affect the increasing part of the fluorescence dip. It has been shown that MV suppresses CEF [32] and therefore, on the basis of the antimycin A, rotenone and MV-data together, CEF can be ruled out as an explanation for the fluorescence dip in FI. The lack of the effect of n-propyl gallate (Fig. 5) on FI of *Trebouxia* indicates that a possible chlororespiration (the reaction of PTOX with PQH_2) is too slow to affect the FI measurements.

4.3. Causes of the decreasing part of the dip in FI

The decreasing part of the dip in FI of *Trebouxia*-lichens and *Trebouxia* itself appearing after the P step at 0.2–0.4 s (Figs. 1–6) reflects a fast PSI reoxidation (see section 4.1). The fast outflow of electrons from PSI can be explained by the fast light-dependent activation of ferredoxin–$NADP^+$–oxidoreductase (FNR). The light period required for the FNR activation can be estimated from the kinetics of $NADP^+$ photoreduction after the

onset of exciting light. For isolated chloroplasts, NADPH-formation starts from about 0.5 s [33] to several seconds [12,34]. FNR activation takes place through a conformational change of the enzyme initiated by light-induced alkalization of the stroma [35]. We could, therefore, propose that the pH-optimum in *Trebouxia* FNR is shifted to lower pH-values allowing faster activation. Another cause for the fast PSI reoxidation in *Trebouxia* can be the Mehler-peroxidase reaction at the stromal side of thylakoids, which was reported to be a short-term valve for electrons during induction phase of photoassimilation after dark–light transition [36]. This reaction is autocatalytically stimulated by a gradual ΔpH formation across the thylakoid membrane [37]. The stimulation is based on the light-driven reduction of monodehydroascorbate [38] that is formed from ascorbate in the peroxidase reaction [39] and on an alkaline shift of pH-optimum of the peroxidase reaction [37].

4.4. Causes of the increasing part of the dip in FI

The increasing part of the dip in FI of *Trebouxia*-lichens and *Trebouxia* itself appearing at 0.4–2 s (Figs. 1–6) was not followed by the change in the redox state of P700 and PC (Figs. 2–4). Thus, this fluorescence increase does not reflect a traffic jam of electrons on the PSI level as it does during the I–P phase of FI (see Introduction). A mechanism that causes a transient reduction of redox components preceding PC leading to a more reduced Q_A should be responsible for this fluorescence increase. A role for CEF in this fluorescence increase could be ruled out (see section 4.2) and our experiments provide also no evidence for a role of the lumen pH. The nigericin, tentoxin and re-reduction kinetics (see section 3.5) indicated that the lumen pH was not involved. A possible explanation for the observed phenomenon could be that the amount of cyt b_6/f present in the thylakoid membranes is not sufficient to keep up with a free outflow of electrons from PSI. In that respect the dip could represent a similar process as that observed in anaerobic leaves where the reduced PQ-pool induced by the anaerobic treatment leads to a transient imbalance between PSII and PSI and a dip in the FI (see [8] and references therein). In *Trebouxia*, the fast activation of the acceptor side of PSI in the presence of a reduced PQ pool may trigger such an imbalance. It is important to note that replacing the dip by a line connecting the two maxima gives a transient that is nearly identical in shape as that observed in pea leaves.

5. Conclusion

In this study, we describe an unusual dip recorded in FI of *Trebouxia*-possessing lichens and the photobiont *Trebouxia* sp. itself that is not reported to live independently in nature [40]. We found that the decreasing phase of the dip appearing at 0.2–0.4 s after the onset of exciting light reflects a fast outflow of electrons from PSI that can be attributed to the activation of FNR or Mehler-peroxidase in thylakoid membranes. Such a fast reoxidation-phase was not observed in FI of dark-adapted higher plants [2,8,18]. We suggest that the FI of *Trebouxia*-lichens and *Trebouxia* itself up to the time of the dip (O–(J)–I–P–dip transient) reflects a typical linear electron flow (from PSII to PSI and through PSI) in thylakoid membranes. The cause for the subsequent fluorescence increase (at about 0.4–2 s) could not be established. A possible explanation given in Discussion is an imbalance between PSII and PSI triggered by the fast activation of the acceptor side of PSI. In contrast to *Trebouxia*, in higher plants, the FI at 0.2–2 s reflects a limitation of the electron flow at the acceptor side of PSI (high fluorescence and I_{820} levels).

The dip in FI described here for *Trebouxia* has been observed at similar times in corals and foraminifera (see Introduction) that contain other species of symbiotic photosynthetic algae. This indicates that symbiotic algae, in comparison to the non-symbiotic ones and higher plants, regulate their electron flow through PSI in a different way during a dark-to-light transition. This finding may be of particular interest for future more detailed studies of the functioning of photosynthetic photochemical processes in green symbiotic algae under control and stress conditions. It can be mentioned that in higher plants under special light conditions (intermittent light grown bean leaves transferred to continuous light) a dip beyond the P-level (in the time range of several seconds) has also been observed in FI [41].

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