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Evidence for Wavelength-Dependent Light Screening of Cyanobionts and Phycobionts in *Lobaria* during Dehydration

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Abstract

Lichens have little control over their hydration status, but rapid physiological and optical responses allow them to thrive through repeated dehydration cycles. Chlorophyll fluorescence and thallus optics were monitored during progressive dehydration of two ecologically similar old forest epiphytic lichens of the genus *Lobaria* harbouring different photobionts. Thalli of *Lobaria scrobiculata* (cyano-lichen) and *L. pulmonaria* (phyco-lichen) were harvested from a red maple (*Acer rubrum*) forest in southeastern New Brunswick, Canada. Simultaneous excitation during dehydration-rehydration cycles with amber light (590 nm) preferentially absorbed by phycobilisomes and blue light (450 nm) preferentially absorbed by chlorophyll was used to measure wavelength-selective changes in excitation of photosystem II activity of thallus samples. The ratio of PSII steady-state fluorescence yield from these two excitation sources (ϕ_{590}/ϕ_{450}) declined in the cyano-lichen during dehydration, but rose in the phyco-lichen. These dual-wavelength excitation experiments did not however support the hypothesis of specific decoupling of the light harvesting phycobilisomes in the cyano-lichen during dehydration. Instead, the opposing patterns of ϕ_{590}/ϕ_{450} in the cyano- and phyco-lichens suggest a symbiotic interaction in which the mycobiont cortex develops wavelength-dependent screening of light transmission during dehydration to

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generate photoprotection specific to the absorbance spectra of the particular photobionts' light harvesting antennae. Changes in reflection from and transmission of light by the thallus support the hypothesis that optical changes in the thallus cause this screening.

Keywords: Lichens, *Lobaria pulmonaria*, *Lobaria scrobiculata*, screening, chlorophyll fluorescence, photoprotection, light harvesting antennae, dehydration

1. Introduction

Lichens are a symbiotic association in which a fungus, termed the mycobiont, derives organic carbon, and sometimes nitrogen, from a closely associated alga, cyanobacterium or both, termed the photobiont(s) (Honegger, 1991). Lichens are poikilohydric, with little control over their hydration status as compared to higher plants. Dehydration results in suspension of carbohydrate transfer between the photobiont and mycobiont (Honegger et al., 1996), as well as depression of the photosynthetic and respiratory activities of the symbionts (Nash et al., 1990). Lichens show remarkably rapid reversal of dehydration depression of physiology (Sass et al., 1996).

There is considerable literature on the effects of dehydration on overall CO_2 exchange in lichens, but less is known about effects of dehydration on light harvesting and electron transport in the photobionts (Sass et al., 1996). Photosystem II (PSII) is a pigment-protein complex that catalyses the initial step of photosynthesis, using light energy to oxidise water. Electrons freed by this photo-oxidation travel through an electron transport chain to provide reductant for carbon fixation and energy for ATP synthesis (Renger, 1993). In dehydrated lichen photobionts several mechanisms have been implicated in down-regulating PSII photochemistry, including state transitions, with diversion of excitation energy from PSII to PSI (Sigfridsson and Öquist, 1980; Chakir and Jensen, 1999; Jensen and Feige, 1991), functional decoupling of light harvesting antennae from PSII (Bilger et al., 1989; Chakir and Jensen, 1999) and interruption of charge separation within PSII (Sass et al., 1996).

Chlorophyll fluorescence is a sensitive, rapid and non-invasive indicator of PSII photochemistry and hence of photosynthetic electron transport through PSII. It has been used to study photosynthesis in lichen photobionts in the field (Bilger et al., 1995; Leisner et al., 1996; Green et al., 1998), during stress from hydration extremes (Calatayud et al., 1997; Chakir and Jensen, 1999; Jensen and Feige, 1991; Lange et al., 1996; Schroeter, 1994), light (Demmig and Björkman, 1987; Kappen et al., 1998; Manrique et al., 1993), heat (Jensen et al., 1997) and pesticides (Jensen et al., 1999). Pulse-amplitude modulated (PAM) chlorophyll fluorometry employs a weak, modulated probe beam to excite chlorophyll

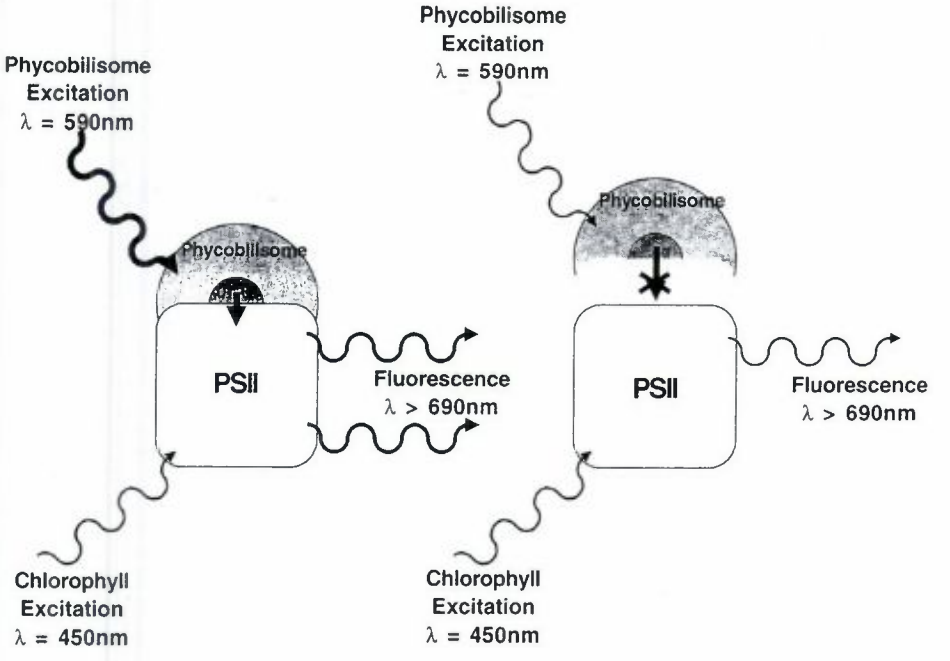

Figure 1. Model of dehydration-induced chlorophyll fluorescence depression in the cyanobacterial photobiont of *L. scrobiculata*. Associated phycobilisomes and PSII centres in hydrated cells would drive chlorophyll fluorescence from excitation by both amber (590 nm) and blue (450 nm) light. Decoupled phycobilisomes in dehydrated thalli would not transfer excitation energy from amber light, and remaining chlorophyll fluorescence only occurs from blue light excitation, so that $\phi 590/\phi 450$ would decrease.

fluorescence (Schreiber et al., 1986). The modulation frequency of the probe beam is specifically coupled with a far-red detector system for the fluorescence photons, so that the weak modulated fluorescence output is detectable even during intense or variable lighting conditions (Schreiber et al., 1994). The quantum yield of fluorescence varies greatly under different light and stress conditions, and can be used to assess physiological effects of those stressors upon the photosynthetic apparatus. The variable component of *in vivo* chlorophyll fluorescence is derived almost exclusively from PSII (Schreiber et al., 1994). The light energy exciting that fluorescence, however, is captured initially mostly by antenna pigments and then transferred into PSII. Chlorophyte light harvesting complexes are hydrophobic chlorophyll *a-b* protein complexes embedded within the thylakoid membranes, that strongly absorb blue and red light. In contrast, cyanobacterial phycobilisomes are hydrophylic complexes of phycobiliproteins anchored on the cytosolic surfaces of the thylakoid

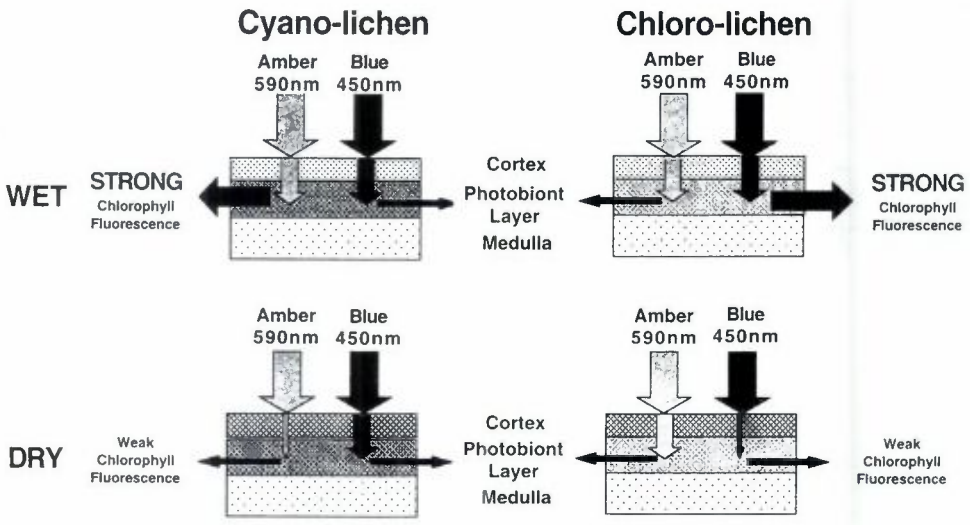

Figure 2. Model of dehydration-induced species-specific cortical screening of cyano- and phyco-lichen photobionts.

membranes, that absorb green, yellow and orange light between the blue and red absorbance peaks of chlorophyll. In cyanobacteria well resolved excitation of chlorophyll (photosystem cores) and phycobiliproteins (antennae) is thus possible by probing with blue (450 nm) and amber (590 nm) sources, respectively.

We used saturation pulse chlorophyll fluorometry (reviewed by Schreiber et al., 1995) to measure the photochemical yield of PSII during dehydration of *Lobaria* thalli, concurrently with measures of thallus cortical albedo (reflectivity) and light transmission through the thalli. Changes in thallus colour and albedo during dehydration are notable in lichens (Gauslaa, 1984), and have been postulated as a protective mechanism against heating and excessive light in the photobiont layer (Kershaw, 1975). In other experiments, simultaneous measures of steady state and variable chlorophyll fluorescence excited via chlorophyll and phycobiliprotein absorbance were used to detect potential uncoupling of light harvesting antennae from reaction centres during progressive dehydration (Fig. 1), or wavelength-selective changes in the optical properties of the lichen cortex (Fig. 2).

Thallus albedo was thus monitored concurrently with chlorophyll fluorescence to explore its correlation to dehydration-induced changes in photochemical activity. Our goal was to investigate how changes in photosynthetic physiology and thallus optics contribute to metabolic suspension during dehydration.

2. Materials and Methods

Sample collection and preparation

Lichen material was collected from a mixed spruce-maple forest near Sackville, New Brunswick, Canada (ca. 45.94°N, 64.42°W) on 31 August 1999. The genus *Lobaria* is the most conspicuous member of the Lobarion association, a climax epiphytic community of deciduous woodlands dominated by large foliose lichens (Rose, 1988). The species sampled are locally common, but are generally restricted to dark, undisturbed maple forests (Gowan and Brodo, 1988). Intact individuals of *Lobaria pulmonaria* (L.) Hoffm. and *L. scrobiculata* (Scop.) DC. were removed from several large red maple (*Acer rubrum*) trees and transported to the laboratory in sealed plastic bags over ice in the dark.

In the laboratory, the specimens were air dried to completion at 25°C in the dark and then frozen at –20°C until required for experimentation. All experimentation was completed within three months of collection. Immediately prior to use, individual lichen thalli were sprayed with distilled water and placed between sheets of wet tissue paper to rehydrate. Thallus hydration was allowed to equilibrate in the dark at c. 25°C for about 2 hours, after which disks of 1.8 cm^2 were punched from each thallus.

Thallus optical properties during dehydration

Thallus albedo and transmission were quantified during dehydration by placing lichen disks in a stage suspended in front of the common end of a PAM-101 fibre optic bundle, and positioned above the weighing dish of a Denver Instruments XE-100 balance. Simultaneous measurements of chlorophyll fluorescence (see below), transmitted light and reflected light from the thallus were made continuously, while the stage was periodically lowered onto the balance to determine hydration status. The assembly was enclosed within a large black plastic pipe to shield the sample from variable light, temperature and humidity conditions. Temperature was controlled within the assembly by flow-through plumbing fitted to a water bath, and the samples were dehydrated by pumping dry air, drawn through a dessicator from outside the building, into the 750 ml enclosure pipe at about 350 ml min^{-1}.

Hydrated samples were blotted of excess water before loading into the assembly, where they were continuously illuminated at c. 10 μmol $m^{-2}s^{-1}$ after an initial dark acclimation period of 15 min. Saturating flashes to measure ϕ_{PSII} were made at 100 second intervals during the dehydration trials. Sample weight was measured at c. 5 minute intervals during dehydration until fluorescence and thallus albedo reached steady state, and variable fluorescence was undetectable (1 to 2 hours). Sample hydration was calculated as water

weight per sample dry weight. Sample dry weight was measured after baking the samples over a bed of Drierite® at 65°C for 24 hours.

Chlorophyll fluorescence measures

Simultaneous measurement of chlorophyll fluorescence driven by both chlorophyll absorbance and phycobiliprotein absorbance was accomplished using simultaneous excitation of samples by two chlorophyll fluorometers. A PAM-101 (Walz, Germany) was configured with a modulated amber LED (peak emission 590 nm; modulated at 100 kHz) to preferentially excite chlorophyll fluorescence through light absorbance by the phycobiliprotein antennae of the cyanobacterial photobiont of *L. scrobiculata*. In the phyco-lichen, the 590 nm source weakly excited chlorophyll fluorescence, presumably through slight absorbance by chlorophyll and accessory carotenoids. Although the phyco-lichen *L. pulmonaria* contains cyanobacterial cells in scattered cephalodia, these are not the primary photobionts and they contributed little to the 590 nm-excited fluorescence. An OS1-FL (OptiSciences, USA) was used with a modulated blue LED (peak emission 450 nm; modulated at 20 kHz) to preferentially excite chlorophyll fluorescence through absorbance by chlorophyll itself (Fig. 1). Each modulated probe source was directed upon the same lichen sample through different fibre-optic cables placed at c. 45° angles to the sample. The two fluorescence-exciting beams were phase-locked to their own detectors and individually amplified by the respective fluorometers. The detection regions of the two units were similar, at >700 nm and >690 nm, respectively. By calculating ratios of steady state fluorescence yield excited by 590 nm and by 450 nm modulated beams (ϕ_{590}/ϕ_{450}), we could investigate potential decoupling of phycobilisomes within the cyanobacterial symbiont of *L. scrobiculata* (Fig. 1), or differential light screening by the thallus cortex in both species (Fig. 2). For presentation, the initial hydrated control ϕ_{590}/ϕ_{450} ratio was normalised to 1.

Actinic light was provided to the samples by a DC-powered white-light bulb (Phillips, USA) at c. 10 μmol $m^{-2}s^{-1}$ through a branch of the PAM-101 fibre-optic bundle. Another branch of the PAM-101 fibre-optic bundle conducted saturating white-light pulses to the thallus, while another branch carried reflected light from the thallus to a S1133 silicon photodiode (Hamamatsu, Japan) housed in a commercially available light sensor (Vernier Software, USA). Saturation pulses of c. 1500 μmol $m^{-2}s^{-1}$ and 1 s duration were controlled by a PAM-103 unit. This comparatively low saturation intensity was used to minimise light-induced damage which could bias ϕ_{PSII} measures later in each trial (Schreiber et al., 1995). This flash intensity was, however, sufficient to drive maximal fluorescence (data not shown).

The photochemical yield of PSII electron transport was measured during the dehydration-rehydration cycles. The photochemical yield of PSII electron transport under a given condition can be assessed by the difference between steady-state and maximal chlorophyll fluorescence, as:

$$\phi_{PSII} = \frac{F'_m - F_s}{F'_m}$$

(Genty et al., 1989). This equation represents the effective or realised (typically illuminated) yield, with nomenclature following van Kooten and Snel (1990). Fluorescence, actinic and reflected light intensities were recorded at a rate of 10 Hz and displayed real-time by a Universal Lab Interface® and LoggerPro® software (Vernier Software, USA), running on a Macintosh G3 computer (model M4787, Apple Computer, Inc., USA). ϕ_{PSII} displayed in the results was calculated using fluorescence excited by the 590 nm measuring beam, but ϕ_{PSII} calculated from the 450 nm measuring beam were similar.

Dry air was pumped across the initially hydrated thallus at c. 0.3 ms^{-1}, which dried it to completion in c. 45 minutes. The thalli were then wet with liquid water and supported by saturated blotting paper while hydrated air was pumped across it at the same rate. The treatment cycle was repeated with three separate samples of each species. The method of measuring thallus weight (and hence hydration) during the dehydration trials (see above) was not compatible with the dual-wavelength fluorescence trials, so during these trials time of dehydration was measured rather than hydration itself.

3. Results

Our results showed that cortex albedo rose in both species during dehydration, consistent with data from other tested lichens (Kershaw, 1975; Gauslaa, 1984). Cortical albedo was similar in the two species at 5–7% in hydrated thalli, increasing to about 10–12% in dehydrated thalli (Fig. 3a). Transmission of incident light through the thallus was more species-specific, being about 0.45% in *L. pulmonaria* and 0.07% in *L. scrobiculata* when hydrated. Transmission in both species dropped with dehydration, *L. pulmonaria* to 0.15–0.2% and *L. scrobiculata* to 0.03–0.04% of incident light (Fig. 3b). Although the absolute levels of transmission were low, the large drop during dehydration indicates that light travelling through the thallus (including the photobiont cells within it) decreased substantially during dehydration. Dehydration also caused a marked depression of the ϕ_{PSII} in both lichens, although the kinetics of ϕ_{PSII} decline with dehydration were different between the two species

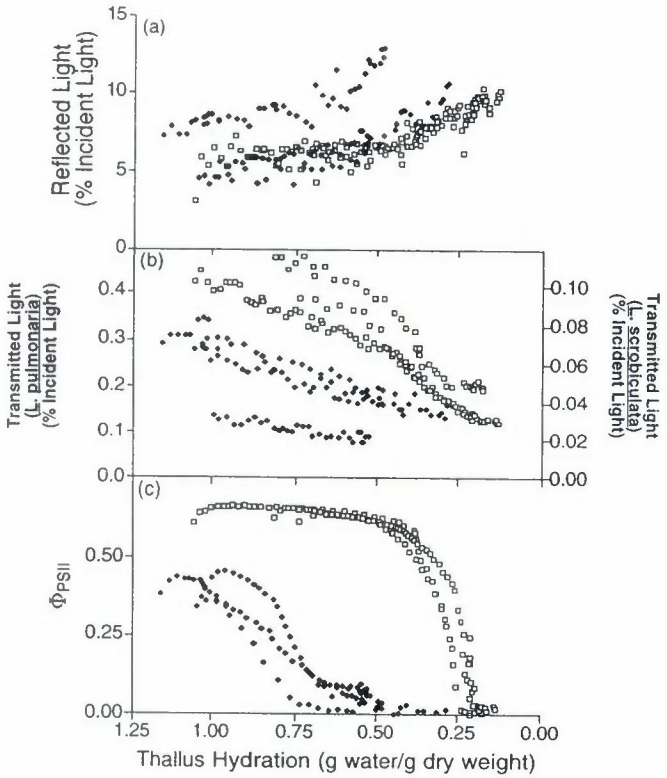

Figure 3. (a) % reflection of incident light from thallus (cortex albedo), (b) % transmission of incident light through thallus (note scale difference between *L. pulmonaria* and *L. scrobiculata*), and (c) ϕPSII during dehydration at 10 µmol photons $m^{-2}s^{-1}$ white light. Reflected light values are corrected for diffuse back-scattering. (*L. scrobiculata* [closed diamonds] and *L. pulmonaria* [open squares]) Data for all three trials for each of the species are shown.

(Fig. 3c). Depression of ϕPSII with dehydration is well known from other lichens (Honegger et al., 1996), as are the characteristic differences between phyco- and cyano-lichen ϕPSII depression during dehydration (Lange et al., 1996).

F_s excited by both amber and blue light declined during dehydration in both species. Thalli of the cyano-lichen *L. scrobiculata* showed a larger decline in F_s excited by amber light relative to blue light, with a 56% decrease in $\phi 590/\phi 450$ from the initial level (Fig. 4). $\phi 590/\phi 450$ quickly recovered to control levels upon rehydration. The drop in $\phi 590/\phi 450$ coincided with the rise in thallus albedo during dehydration more closely than with the somewhat earlier decline in

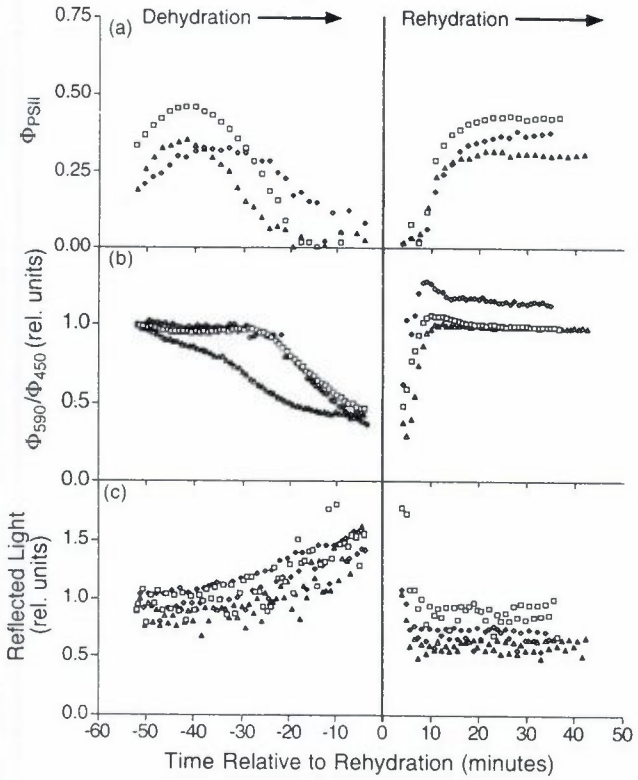

Figure 4. (a) ϕPSII, (b) $\phi 590/\phi 450$, and (c) relative thallus albedo of *L. scrobiculata* samples during a dual-excitation dehydration-rehydration cycle. Different symbols represent three individual trials. Trials were aligned by setting the point of rehydration at time zero, with initial hydration of about 1.1 g water/g dry weight.

ϕPSII during dehydration. Also, upon rehydration $\phi 590/\phi 450$ recovered coincidentally with thallus albedo, and more quickly than the slower recovery of ϕPSII. This strongly suggests the measured change in $\phi 590/\phi 450$ resulted from changes in thallus optics (Fig. 2), rather than from uncoupling of the phycobilisomes from PSII centres (Fig. 1).

The phyco-lichen *L. pulmonaria* also showed changes in the ratio of F_s excited by amber and blue light during dehydration. *L. pulmonaria* showed an average rise of 50% in $\phi 590/\phi 450$ during dehydration (Fig. 5), which quickly declined again upon rehydration. The rise in $\phi 590/\phi 450$ was largely coincident with both an increase in thallus albedo and with a decline of ϕPSII.

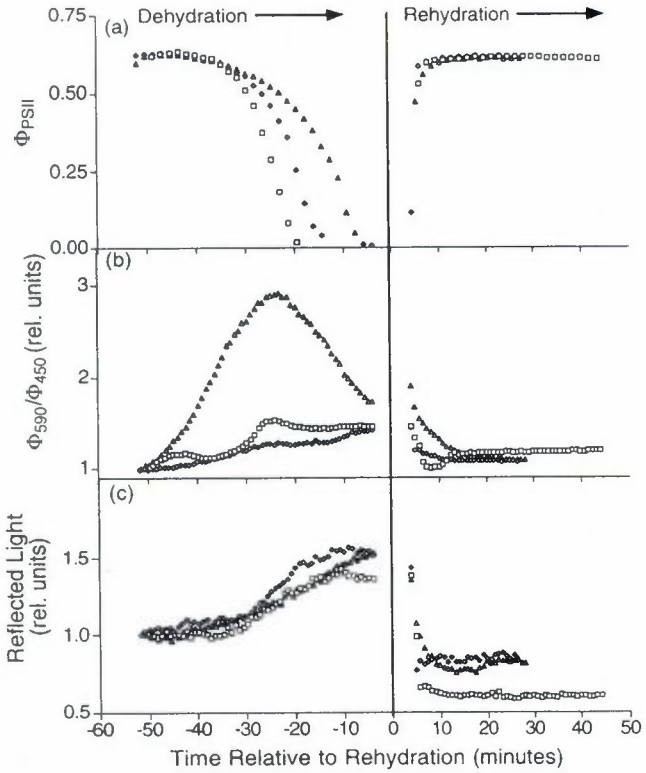

Figure 5. (a) ϕPSII, (b) $\phi 590/\phi 450$, and (c) relative thallus albedo of *L. pulmonaria* samples during a dual-excitation dehydration-rehydration cycle. Different symbols represent three individual trials. Trials were aligned by setting the point of rehydration at time zero, with initial hydration of about 1.05 g water/g dry weight.

4. Discussion

Decline in uptake and fixation of CO_2 is the net effect of photosynthetic depression during dehydration, but there is debate whether the limitation occurs primarily in the Calvin Cycle or in light capture and electron transport. Several authors suggest that rising cortical albedo is a protective mechanism to lower absorbed light (Büdel and Lange, 1994) and heating (Sancho et al., 1994) of the photobionts during periods of dehydration. Sancho et al. (1994) further suggest that cortical albedo is a valuable indicator for photosynthetic activity in Umbilicariaceae. Our study confirms that, with dehydration, cortical albedo rises in *Lobaria*. The potential of the rise in cortical albedo to increase

light screening of the photobionts is, however, limited by the low absolute levels of cortical albedo, ranging between 5 and 12% of incident light.

Our dual-fluorescence data do nevertheless show evidence of wavelength-selective light screening of the photobionts during dehydration. As expected, the $\phi 590/\phi 450$ ratio dropped with dehydration in the cyano-lichen, and recovered with rehydration. This shows that during dehydration the excitation of PSII via phycobilisome absorbance declines in the cyanobacterial photobiont of *L. scrobiculata*. The hypothesis that wavelength-dependent screening causes the $\phi 590/\phi 450$ ratio change in *L. scrobiculata* is supported by the distinct pattern of changes observed in the phyco-lichen *L. pulmonaria*. *L. pulmonaria* showed a rise in the $\phi 590/\phi 450$ ratio upon dehydration, which then dropped with rehydration. We hypothesised that decoupling of the antennae in the phyco-lichen would not significantly change $\phi 590/\phi 450$ because the absorption of 450 nm and 590 nm is not separated in the phyco-lichens as in the cyano-lichens. Indeed, any change would be expected to drive the ratio down because of a slight bias in absorption of 590 nm light in the antenna of the phyco-lichen by carotenoids and chlorophyll *b*. A rise in $\phi 590/\phi 450$ indicates declining excitation from blue light during dehydration in *L. pulmonaria*, whereas the declining $\phi 590/\phi 450$ ratio from *L. scrobiculata* indicates declining excitation from amber light during dehydration. Preferential screening of the photobiont from excess light during dehydration would be most important at those wavelengths absorbed by the principal light harvesting pigments of the antennae. The chlorophyll-based light harvesting antennae of *L. pulmonaria* absorbs strongly in blue light, while the phycobiliprotein antennae of *L. scrobiculata* absorbs strongly in amber light, so that in each species the drop in excitation was largest at the wavelengths absorbed strongly by the particular antennae in each species. Our results do not exclude the possibility of phycobilisome decoupling in the cyano-lichen during dessication as suggested by Bilger et al. (1989) and others. Also, our observations were conducted during and immediately after acute dehydration, not after several days of extreme dessication (as in Bilger et al., 1989) during which time the postulated decoupling might occur.

We did not differentiate whether reflection or absorption by the thallus cortex caused the light screening. Although the absolute level of reflected light was low in *Lobaria* at between 5 and 12% of incident light, it did increase by 40–80% during dehydration while transmission of light through the thallus dropped 33–71%. A proportional drop in the light transmitted through the thallus cortex to the photobionts would strongly lower their illumination. Our measures, however, only described changes in light reflected from the cortex and transmitted through the full thickness of the thallus, and could not measure light transmitted through the cortex to the photobionts alone.

Regardless of whether the observed changes in $\phi 590/\phi 450$ are due to observed wavelength-dependent reflection (Gauslaa, 1984) or transmission of light to the photobionts, the selective screening likely results from changes in the fungal cortex.

The capacity to withstand frequent dehydration is a great ecophysiological pressure upon lichen photobionts. This study provides evidence for wavelength-dependent changes in the transmission of light to the photobionts of two *Lobaria* species, with proportionately greater screening coinciding with the absorbance peaks of the specific light harvesting antennae. Changes in cortical reflection and transmission are unlikely to be solely responsible for photoprotection during dehydration and high light exposure. Nevertheless, the targeted selectivity of the decline in cortical transmission lends support to the symbiotic concept of the lichen association, with the coevolution of light capture and screening between the symbionts (see Honegger, 1991 for review).

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