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Differences in the capacity for radiationless energy dissipation in the photochemical apparatus of green and blue-green algal lichens associated with differences in carotenoid composition

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Abstract. Green algal lichens, which were able to form zeaxanthin rapidly via the de-epoxidation of violaxanthin, exhibited a high capacity to dissipate excess excitation energy nonradiatively in the antenna chlorophyll as indicated by the development of strong nonphotochemical quenching of chlorophyll fluorescence (F_M, the maximum yield of fluorescence induced by pulses of saturating light) and, to a lesser extent, F_O (the yield of instantaneous fluorescence). Blue-green algal lichens which did not contain any zeaxanthin were incapable of such radiationless energy dissipation and were unable to maintain the acceptor of photosystem II in a low reduction state upon exposure to excessive photon flux densities (PFD). Furthermore, following treatment of the thalli with an inhibitor of the violaxanthin de-epoxidase, dithiothreitol, the response of green algal lichens to light became very similar to that of the blue-green algal lichens. Conversely, blue-green algal lichens which had accumulated some zeaxanthin following long-term exposure to higher PFDs exhibited a response to light which was intermediate between that of zeaxanthin-free blue-green algal lichens and zeaxanthin-containing green algal lichens. Zeaxanthin can apparently be formed in blue-green algal lichens (which lack the xanthophyll epoxides, i.e. violaxanthin and antheraxanthin) as part of the normal biosynthetic pathway which leads to a variety of oxygenated derivatives of β-carotene during exposure to high light over several days. We conclude that the pronounced difference in the capacity for photoprotective energy dissipation in the antenna chlorophyll between (zeaxanthin-containing) green algal lichens and (zeaxanthin-free) blue-green algal lichens is related to the presence or absence of zeaxanthin, and that this difference can explain the greater susceptibility to high-light stress in lichens with blue-green phycobionts.

Key words: Carotenoids – Chlorophyll fluorescence – Chlorophyta – Cyanobacteria – Energy dissipation – Lichens – Light stress – Photoinhibition in phycobionts – Phycobiont – Zeaxanthin

Introduction

In the previous paper (Demmig-Adams et al. 1990) it was shown that, when in the fully hydrated state, lichens with blue-green phycobionts were more susceptible to sustained photoinhibition than were lichens with green phycobionts. This was not related to any intrinsic differences in the capacity to utilize, and thereby "dissipate", excitation energy via photosynthetic electron transport between the two groups as the rates of light- and CO_2-saturated photosynthetic O_2 evolution were similar in both. Thus the blue-green algal lichens are likely to possess a lower capacity to either prevent or repair "photoinhibitory damage".

A process which has the potential to prevent "photoinhibitory damage" is the removal of excitation energy, within the photochemical apparatus, which is in excess of that which can be used in photosynthesis. Such harmless (radiationless) energy dissipation is indicated by the (nonphotochemical) quenching of chlorophyll fluorescence in leaves and algae (Krause et al. 1982; Demmig and Björkman 1987; Weis and Berry 1987), and could, in theory, occur in the antenna chlorophyll and/or the reaction center of photosystem II (PSII; Butler and Kitajima 1975; Kitajima and Butler 1975). It has recently been suggested that energy dissipation in the antenna chlorophyll is mediated by the carotenoid zeaxanthin (Demmig et al. 1987, 1988; Demmig-Adams et al. 1989b) which can be formed rapidly from the di-epoxide

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Abbreviations and symbols: DTT = dithiothreitol; F_O = yield of instantaneous fluorescence; F_M = maximum yield of fluorescence induced by pulses of saturating light; F_V = variable yield of fluorescence; PFD = photon flux density (400–700 nm); PSII = photosystem II; Q = electron acceptor of PSII; q_{NP} = quenching coefficient for nonphotochemical fluorescence quenching; q_P (or $1-q_P$) = quenching coefficient for photochemical fluorescence quenching

violaxanthin (via the mono-epoxide antheraxanthin) as part of the xanthophyll cycle (Siefermann-Harms 1977; Yamamoto 1979; Hager 1980). Cyclic changes involving de-epoxidation of xanthophyll epoxides occur in higher plants and in all classes of algae, except those which contain phycobilisomes such as the blue-green algae (Hager and Stransky 1970; Stransky and Hager 1970; Goodwin 1980; Hager 1980). We have compared a variety of green algal lichens, which were able to rapidly form zeaxanthin through violaxanthin de-epoxidation, with a variety of zeaxanthin-free blue-green algal lichens, which were unable to form zeaxanthin rapidly from epoxides (xanthophyll cycle) or β-carotene (see e.g. McDermott et al. 1973; Demmig et al. 1987), in terms of their capacity to quench fluorescence nonphotochemically and maintain the acceptor of PSII in a low reduction state.

In order to test further whether the observed differences in fluorescence quenching were indeed related to the presence or absence of zeaxanthin, or to other intrinsic properties of the two different photosynthetic systems, we have used an inhibitor of the violaxanthin de-epoxidase, dithiothreitol (DTT; Yamamoto and Kamite 1972), to prevent the formation of zeaxanthin in the green algal lichens. Furthermore, fluorescence quenching in thalli of zeaxanthin-free blue-green algal lichens was compared with that of thalli of the same species which had synthesized some zeaxanthin, apparently from β-carotene (see also McDermott et al. 1973; Goodwin 1980; Demmig et al. 1987; Demmig-Adams et al. 1989a, d), upon long-term exposure of the thalli to higher photon flux densities (PFDs).

Material and methods

Lichen material. The following species were examined in this study: the green algal lichens *Lobaria pulmonaria* (L.) Hoffm., *Peltigera aphthosa* (L.) Willd., *Pseudocyphellaria rufovirescens* (Church. Bab.) D. Galloway, *Ramalina maciformis* (Del.) Bory, *Sticta latifrons* A. Rich., and *Umbilicaria pustulata* (L.) Hoffm.; and the blue-green algal lichens *Parmeliella plumbea* (Lightf.) Müll. Arg., *Peltigera canina* (L.) Willd., *Peltigera polydactyla* (Neck.) Hoffm., *Peltigera rufescens* (Weiss) Hum., *Pseudocyphelleria dissimilis* (Nyl.) D. Galloway et P. James, *Pseudocyphellaria murrayi* D. Galloway, and *Sticta fuliginosa* (Huds.) Ach. The geographical origin and habitat description for each species is listed in Demmig-Adams et al. (1990). Most species were maintained in growth chambers under 10 μmol photons·m^{-2}·s^{-1} (12 h photoperiod) at 15° C. *Umbilicaria pustulata* and *Ramalina maciformis*, which grow in fairly exposed habitats, were maintained under 75 μmol photons·m^{-2}·s^{-1}. Thalli of the blue-green algal lichen *Peltigera rufescens* were maintained at either 10 or 120 μmol photons·m^{-2}·s^{-1} for two weeks. Those which were maintained at 120 μmol photons·m^{-2}·s^{-1} contained zeaxanthin and were transferred to 10 μmol photons·m^{-2}·s^{-1} for 3 h prior to measurements of fluorescence. Thalli of the blue-green algal lichen *Peltigera polydactyla* were either maintained under 10 μmol photons·m^{-2}·s^{-1} for a minimum of two weeks (zeaxanthin-free), or collected directly from the field in March/April 1989. Those collected directly from the field (containing zeaxanthin) were harvested in the evening, fully hydrated, and maintained in a growth chamber overnight (15° C, in darkness) prior to measurements of fluorescence. Pigment analyses showed that there was no decrease in zeaxanthin content overnight. Two groups of thalli of *Peltigera polydactyla* were collected directly from the field, one after several days of partially cloudy skies (thalli moist), and the second after several days of clear skies (thalli dry).

Pigment analyses and fluorescence measurements. Analyses of the carotenoids present in the green algal lichens were performed as described previously (Demmig et al. 1987), except that twice the normal amount of magnesium-hydroxide-carbonate was used during extraction of the pigments. The R_f values of the carotenoids in the blue-green algal lichens were compared with those of standards run on thin-layer chromatography (TLC) plates (see Czygan 1968a). β-Carotene also included traces of α-carotene in the green algal lichens but not in the blue-green algal lichens. In those blue-green algal lichens which contained zeaxanthin, a second band, which may be isozeaxanthin, was also present just above that identified as zeaxanthin (in the position which lutein normally occupies in analyses from leaves or green algae).

Exposure of submerged (in water) thalli to high light for 2 h was as described in Demmig-Adams et al. (1990). Some thalli were pretreated with DTT by submerging them in a solution of 3 mM DTT under 2 μmol photons·m^{-2}·s^{-1} at 15° C for a minimum of 1 h. This treatment did not affect the rates of photosynthetic O_2 exchange during the experiments which were subsequently performed (data not shown).

Chlorophyll fluorescence was measured with a PAM chlorophyll fluorometer (Walz, Effeltrich, FRG; Schreiber et al. 1986) as described previously (Demmig-Adams et al. 1989b). Photochemical quenching, q_P, is presented as $1\text{-}q_P$, an approximate measure of the reduction state of the acceptor Q of PSII. For the sake of simplicity, we have not considered a possible non-linearity between $1\text{-}q_P$ and the reduction level of Q caused by energy transfer between PSII units (Joliot and Joliot 1964). Thalli were fully hydrated and maintained at 15° C with 5% CO_2 in a water-jacketed chamber during the measurements of fluorescence.

Results

Carotenoid composition in a variety of green and blue-green algal lichens. The green algal lichens possessed the complement of carotenoids which are typically found in green algae (Hager and Stransky 1970): β-carotene (with traces of α-carotene), lutein (as a derivative of α-carotene), neoxanthin, and the components of the xanthophyll cycle, violaxanthin, antheraxanthin, and zeaxanthin (Table 1). The total carotenoid content per chlorophyll was similar in all green algal lichens, and lutein was the major carotenoid component in all of these species. $\alpha+\beta$-Carotene was present in quantities which were 1.3- to 2.9-times smaller than lutein, and were similar to that of the total content of the three xanthophylls of the xanthophyll cycle. The zeaxanthin content was zero in all species examined under "control" conditions, i.e. the conditions under which the lichens were maintained in culture. Exposure of the green algal lichens to high PFD led to a strong increase in the zeaxanthin content of a similar magnitude (per chlorophyll) in all species. These increases in zeaxanthin content were accompanied by similar decreases in the content of violaxanthin and antheraxanthin.

The carotenoid compositions of a variety of blue-green algal lichens are shown in Table 2. It is noteworthy that the total carotenoid content per chlorophyll was very similar in the blue-green and green (Table 1) algal

Table 1. Carotenoid composition of lichens with green phycobionts before (LL = maintenance PFD of 10 or 75 μmol photons·m^{-2}·s^{-1}; see *Material and methods*) and after (HL) a 2-h exposure to 1800 μmol photons·m^{-2}·s^{-1}. Thallus temperatures were maintained at 15° C. All green algal lichens also contained neoxanthin, and trace amounts of β-cryptoxanthin were present in those species marked with[a]

Species and light conditions	Carotenoid content (mmol·(mol Chl)$^{-1}$)					Total carotenoids (mg·(g Chl)$^{-1}$)
	α+β-Carotene	Lutein	Xanthophyll cycle components			
			Zeaxanthin	Antheraxanthin	Violaxanthin	
Lobaria pulmonaria[a]						
LL	94	263	0	17.8	42.0	294
HL	79	224	44.0	0	22.1	295
Peltigera aphthosa						
LL	136	182	0	19.6	10.6	301
HL	145	189	41.0	0	0	287
Pseudocyphellaria rufovirescens[a]						
LL	139	246	0	7.8	35.6	290
HL	103	234	42.9	0	20.0	278
Ramalina maciformis						
LL	96	212	0	20.6	48.7	237
HL	106	214	47.7	12.9	10.4	248
Sticta latifrons[a]						
LL	91	222	0	7.9	60.5	288
HL	85	250	49.3	11.5	20.0	279
Umbilicaria pustulata[a]						
LL	224	292	0	23.9	25.4	336
HL	189	287	53.8	0	10.2	326

Table 2. Carotenoid composition of lichens with blue-green phycobionts before (LL = maintenance PFD of 10 μmol photons·m^{-2}·s^{-1}; see *Material and methods*) and after (HL) a 2-h exposure to 1800 μmol photons·m^{-2}·s^{-1}. Thallus temperatures were maintained at 15° C. Trace amounts of β-cryptoxanthin ([a]), and two other carotenoids which were probably caloxanthin ([b]), and nostoxanthin ([c]), were also present in those species so marked

Species and light conditions	Carotenoid content (mmol·(mol Chl)$^{-1}$)						Total carotenoids (mg·(g Chl)$^{-1}$)
	β-Carotene	Echinenone	Canthaxanthin	Xanthophyll cycle components			
				Zeaxanthin	Antheraxanthin	Violaxanthin	
Peltigera canina[a, b, c]							
LL	330	93	20	0	0	0	287
HL	322	92	15	0	0	0	253
Peltigera polydactyla[b, c]							
LL	296	111	0	0	0	0	285
HL	306	89	14.9	0	0	0	269
Pseudocyphellaria dissimilis[a, b]							
LL	403	71	22.6	0	0	0	311
HL	387	86	37.7	0	0	0	313
Pseudocyphellaria murrayi[b]							
LL	373	34	14.4	0	0	0	257
HL	373	56	23.6	0	0	0	280
Sticta fuliginosa[b, c]							
LL	283	145	47.8	4.3	0	0	309
HL	279	168	26.3	2.4	0	0	308

lichens, as was the chlorophyll content on an area basis (see Demmig-Adams et al. 1990). The blue-green algal lichens contained the carotenoids frequently found in blue-green algae (Stransky and Hager 1970), differing from leaves and green algae in that no derivatives of α-carotene (lutein) were present and that considerable amounts of the keto-carotenoids (derived from β-carotene) echinenone (with one keto group, 4-keto-β-carotene) and canthaxanthin (with two keto groups, 4,4'-diketo-β-carotene) were present (Table 2). No xanthophyll epoxides were present in any of the species. After maintenance at low PFD (LL), the thalli did not contain zeaxanthin, except for those of *Sticta fuliginosa* which had been collected in an exposed site and contained small amounts of zeaxanthin. Furthermore, small amounts of β-cryptoxanthin were present in some species, as well as small amounts of two additional carotenoids which were more polar than zeaxanthin. Previously, these two carotenoid fractions have been shown to contain three and four hydroxy groups, as determined from esterification with acetic anhydride (data not shown). Thus, all four of the hydroxylated derivatives of β-carotene were present in the various blue-green algal lichens, with one (β-cryptoxanthin), two (zeaxanthin in *Sticta fuliginosa*), three (presumably caloxanthin) and four (presumably nostoxanthin) hydroxy groups.

Compared to the green algal lichens the blue-green algal lichens had much higher β-carotene contents. In contrast to the green algal lichens, none of the blue-green algal lichens was capable of zeaxanthin formation during a 2-h exposure to high PFD (HL in Table 2). The keto-carotenoid content appeared to be lower in lichens which had developed in deep shade (*Pseudocyphellaria murrayi* and *P. dissimilis*) and higher in those from the more exposed sites (*Sticta fuliginosa* from Portugal and *Peltigera polydactyla*). Furthermore, the keto-carotenoids appear to be limited to the phycobiont, since only those lobes of the *Pseudocyphellaria* phycosymbiodeme with the blue-green phycobiont contained these carotenoids (*P. murrayi* versus *P. rufovirescens*, with the same mycobiont). Despite the fact that the occurrence of the keto-carotenoids was limited to those lichens with blue-green phycobionts in the present study, green algae have also been shown to accumulate such carotenoids when cultured under nitrogen-deficient conditions (Czygan 1968a, b). It is noteworthy that, in contrast to a free-living alga (*Anacystis nidulans*, cultivated at very low PFD), several blue-green algal lichens (e.g. *Peltigera polydactyla*) exhibited extremely low ratios of phycobilins (in aqueous extracts) to chlorophyll *a* (in acetone extracts; data not shown).

Nonphotochemical and photochemical quenching of chlorophyll fluorescence in green and blue-green algal lichens. The original traces of chlorophyll fluorescence emission (at room temperature) from two *Pseudocyphellaria* species (with a green, *P. rufovirescens*, or a blue-green, *P. dissimilis*, phycobiont), subjected to successive increases in PFD, are shown in Figs. 1, 2. The (untreated) green algal lichens exhibited high maximum fluorescence yield

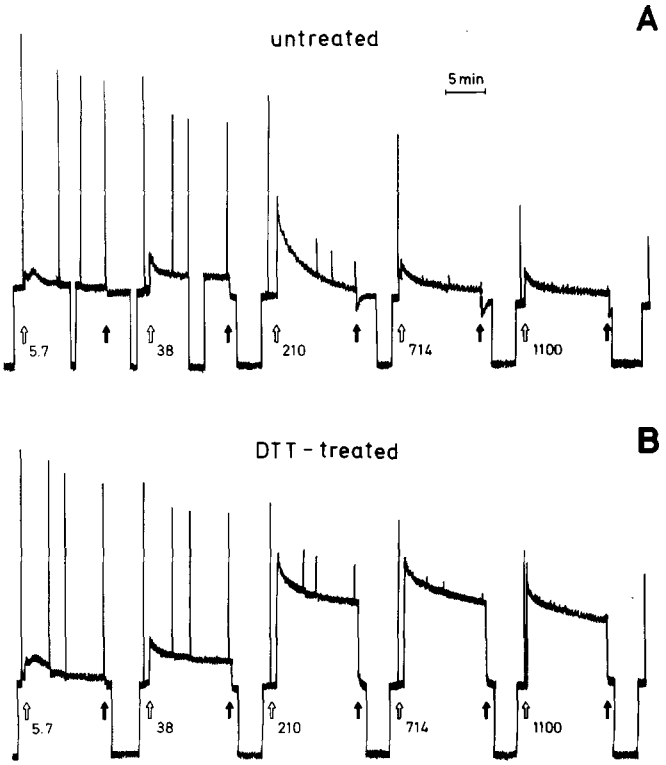

Fig. 1 A, B. Original traces of chlorophyll fluorescence from untreated (**A**) and DTT-treated (**B**) thalli of the green algal lichen *Pseudocyphellaria rufovirescens* upon exposure to successive increases in PFD. The fluorescence signal in darkness (excited by a weak measuring beam) is initial fluorescence, F_O, and the fluorescence signal during illumination is composed of F_O and various degrees of fluorescence from those PSII traps which are closed. Fluorescence in the saturating pulses is maximum fluorescence, F_M, when all PSII traps are closed. The values of F_O and F_M were first ascertained in darkness, followed by alternating periods of illumination (approx. 10 min) and darkness (approx. 5 min). *Open arrows* indicate the points at which the actinic light was switched on (numbers indicate the PFD, in μmol photons·m^{-2}·s^{-1}), and *closed arrows* indicate return to darkness. Saturating pulses of light were given during the exposure to each PFD, mostly after 3, 5 and 10 min of illumination, and after 5 min of darkness subsequent to each 10 min period of exposure. The measuring beam was switched off periodically to determine the base line

(at closed reaction centers) in darkness and at low PFDs (5.7 and 38 μmol photons·m^{-2}·s^{-1}), and a strong decrease in maximum fluorescence yield, i.e. an increase in nonphotochemical fluorescence quenching, at PFDs above 210 μmol photons·m^{-2}·s^{-1} (Fig. 1 A). Furthermore, the untreated green algal lichens exhibited a quenching of F_O, which was rapidly reversible upon darkening. A considerable extent of the quenching of F_M (or F_V) was also reversible within 5 min of darkening.

In contrast, there was much less quenching of F_M in the blue-green algal lichens with increases in PFD, and steady-state fluorescence rose strongly (Fig. 2A). Thus there was little nonphotochemical fluorescence quenching (or radiationless energy dissipation) and, consequently, the reduction state of the acceptor Q of PSII became very high in the blue-green algal lichens upon

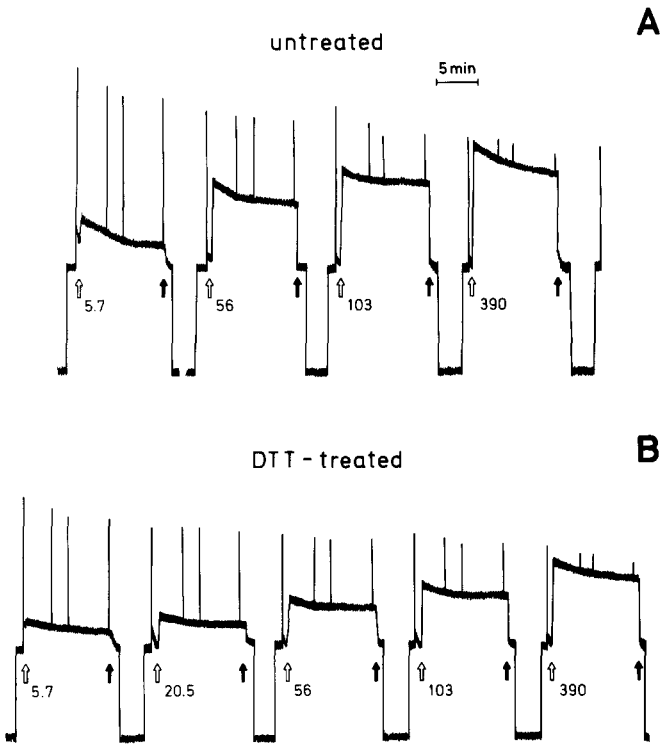

Fig. 2A, B. Original traces of chlorophyll fluorescence from untreated (**A**) and DTT-treated (**B**) thalli of the blue-green algal lichen *Pseudocyphellaria dissimilis* upon exposure to successive increases in PFD. See legend of Fig. 1 for further details

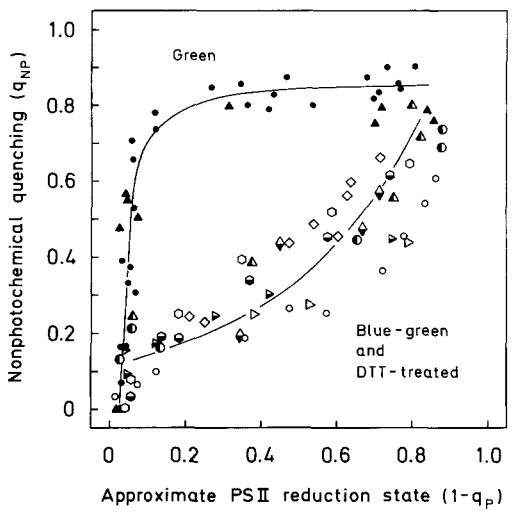

Fig. 3. Relationship between nonphotochemical fluorescence quenching (q_{NP}) and the approximate reduction state of PSII ($1-q_P$) in two green algal lichens *Lobaria pulmonaria* (▲, △) and *Pseudocyphellaria rufovirescens* (●, ◐), and in the blue-green algal lichens *Sticta fuliginosa* (◇, ◈), *Pseudocyphellaria murrayi* (○, ◒), *Pseudocyphellaria dissimilis* (▷, ▶), and *Peltigera canina* (⬡, ⬙). *Half-closed symbols* indicate that the thalli were pretreated with 3 mM dithiothreitol (DTT) under 2 μmol photons·m^{-2}·s^{-1} at 15° C for a minimum of 1 h. The relationships were established from the quenching of chlorophyll fluorescence observed in response to different PFDs in the presence of 5% CO_2 at a temperature of 15° C. The highest obtained value of F_V (observed in darkness in all green algal lichens and the blue-green algal lichens *Pseudocyphellaria dissimilis* and *P. murrayi*, or under 5.7 μmol photons·m^{-2}·s^{-1} in all other blue-green algal lichens) was used as the control value for all calculations of q_{NP}

exposure to excessive light. Furthermore, there was no quenching of F_O in the blue-green algal lichens, and the small degree of quenching of F_M (or F_V) was not reversible within 5 min of darkening.

Following treatment of the lichens with DTT, which completely inhibited the formation of zeaxanthin throughout the duration of the experiment, the response of the green algal lichen to increases in PFD was very similar to that of the blue-green algal lichen (compare Figs. 1B, 2). The DTT-treated green algal lichen exhibited very high levels of steady-state fluorescence and only small decreases in the maximum fluorescence yield, i.e. little nonphotochemical fluorescence quenching and a high reduction state of Q. The quenching of F_O in the green algal lichen was also inhibited by DTT. The blue-green algal lichen treated with DTT exhibited fluorescence quenching in response to PFD which was similar to that of the untreated blue-green algal lichen (Fig. 2).

The approximate reduction state of the acceptor Q of PSII ($1-q_P$) was plotted against nonphotochemical fluorescence quenching (q_{NP}) for untreated and DTT-treated thalli of several green and blue-green algal lichen species (Fig. 3). To calculate q_{NP}, the maximum yield of variable fluorescence was determined for each lichen. In the green algal lichens and in some of the blue-green algal lichens (*Pseudocyphellaria dissimilis* and *P. murrayi*) the highest value of F_V (and F_V/F_M) was obtained in darkness, as is the case in leaves of higher plants (see Björkman and Demmig 1987). In other blue-green algal lichen species, however, values of F_V/F_M determined after 12 h of darkness were as low as 0.5, and increased considerably to values between 0.6 and 0.68 during illumination at a low PFD (5.7 μmol photons·m^{-2}·s^{-1}; data not shown). In these cases the maximum yield of fluorescence was determined with a pulse of saturating light given during the illumination with 5.7 μmol photons·m^{-2}·s^{-1}. All calculations of q_{NP} were based on the highest determined value of F_V, whether this was obtained in darkness or in low light, resulting in the highest possible values of q_{NP}. This conservatively leads to an underestimation of the difference between the green and blue-green algal lichens compared to any lower values of F_V determined in darkness.

As was apparent from the original traces of fluorescence (Fig. 1), nonphotochemical fluorescence quenching reached its maximum extent while the reduction state of PSII was maintained below 0.2 in the untreated green algal lichens (Fig. 3). In contrast, DTT-treated green algal lichens as well as untreated and DTT-treated blue-green algal lichens all exhibited a strong increase in the reduction state of PSII and only little nonphotochemical quenching of fluorescence for values of $1-q_P$ between 0 and 0.4. Only at very high values of $1-q_P$ did q_{NP} increase in the thalli which did not contain any zeaxanthin.

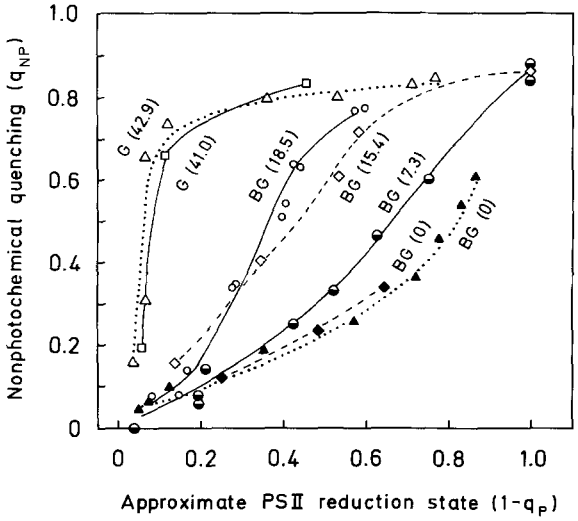

Fig. 4. Relationship between nonphotochemical fluorescence quenching and the approximate reduction state of PSII in two green (G) algal lichens *Pseudocyphellaria rufovirescens* (△) and *Peltigera aphthosa* (□) and in the blue-green (BG) algal lichens *Pseudocyphellaria murrayi* (▲), *Peltigera polydactyla* (○, ◒) and *Peltigera rufescens* (◇, ◆) containing different amounts of zeaxanthin. The zeaxanthin content, in mmol zeaxanthin·(mol Chl)$^{-1}$, is indicated in *brackets* along each line. The relationships were established from the quenching of chlorophyll fluorescence observed in response to different PFDs in the presence of 5% CO_2 at a temperature of 15° C. The highest obtained value of F_V (observed in darkness in all green algal lichens and in the blue-green algal lichen *Pseudocyphellaria murrayi* and zeaxanthin-containing thalli of the blue-green algal lichens *Peltigera polydactyla* and *P. rufescens*, or under 5.7 μmol photons·m^{-2}·s^{-1} in thalli of *P. rufescens* which did not contain any zeaxanthin) was used as the control value for all calculations of q_{NP}. These control values of F_V for zeaxanthin-containing and zeaxanthin-free blue-green algal lichens were very similar

In the previously described analyses of fluorescence quenching in blue-green algal lichens, only zeaxanthin-free thalli which had been maintained at a low PFD in a growth chamber for a minimum of several days were used. Thalli of the blue-green algal lichen *Peltigera polydactyla* taken directly from a natural site after a period of sunny days, and those of *Peltigera rufescens* maintained at 120 μmol photons·m^{-2}·s^{-1}, however, did contain zeaxanthin (but no epoxides). These zeaxanthin-containing blue-green algal lichens also exhibited a different pattern of chlorophyll fluorescence quenching in response to PFD from that observed in zeaxanthin-free blue-green algal lichens. With increasing zeaxanthin content the relationship between nonphotochemical fluorescence quenching and the (approximate) reduction state of the acceptor Q of PSII in these two blue-green algal lichens became increasingly similar to that of the zeaxanthin containing green algal lichens, such as *Peltigera apthosa* (Fig. 4). For comparison, the relationship between q_{NP} and $1-q_P$ for the green and blue-green thalli of the *Pseudocyphellaria* phycosymbiodeme are also indicated (dotted lines) in the same figure. It is noteworthy that the thalli of *Peltigera polydactyla* and *Peltigera rufescens* which contained zeaxanthin also contained small amounts of an additional carotenoid with an R_f value similar to that of zeaxanthin.

Discussion

Nonphotochemical fluorescence quenching in zeaxanthin-free and zeaxanthin-containing lichens. Upon exposure to excessive PFDs, lichens with green phycobionts, which have the capacity to form zeaxanthin in the xanthophyll cycle within a few minutes, exhibited strong nonphotochemical quenching of fluorescence accompanied by a quenching of F_O indicative of radiationless energy dissipation, similar to that observed in leaves (Weis and Berry 1987; Demmig-Adams et al. 1989b). Furthermore, the increase in radiationless energy dissipation, indicated by the increase in q_{NP}, facilitated the maintenance of the acceptor of PSII in a low reduction state, which is thought to be important for the avoidance of photoinhibitory damage (Weis and Berry 1987). In contrast, lichens with blue-green phycobionts, which do not possess the xanthophyll cycle and did not contain zeaxanthin, did not possess this capacity for a reversible increase in radiationless energy dissipation and were unable to maintain PSII in a low reduction state upon these short-term exposures to increasing PFDs (Fig. 3).

These findings are consistent with the suggested function of zeaxanthin as a "fluorescence quencher" which mediates the dissipation of excess excitation energy (Demmig et al. 1987, 1988; Demmig-Adams et al. 1989b). Since the zeaxanthin-associated component of nonphotochemical fluorescence quenching relaxed rapidly upon darkening of the thalli, we suggest that the zeaxanthin-associated dissipation process is responsible for (at least part of) the so-called "high-energy-state" quenching. Under different conditions the same process can apparently be more slowly reversible (Demmig et al. 1988). That the different responses of the two algal groups are indeed related to the presence or absence of zeaxanthin and not to other differences between these two photosynthetic systems is indicated by the following two pieces of evidence. The response of the green algal lichens becomes similar to that of the blue-green algal lichens when the former are treated with the inhibitor of the violaxanthin de-epoxidase, dithiothreitol, which prevents the formation of zeaxanthin as well as the development of the dissipation process in the antenna chlorophyll (Fig. 3). Conversely, blue-green algal lichens which did contain zeaxanthin (apparently formed from β-carotene during long-term exposure to higher PFDs) exhibited an increase in nonphotochemical fluorescence quenching (i.e. radiationless energy dissipation) upon exposure to excessive PFDs such that their response was more similar to that of the green algal lichens which accumulated zeaxanthin (Fig. 4). Thus the presence or absence of zeaxanthin appears to determine whether or not the lichen thalli have the capacity to dissipate excessive energy nonradiatively. Due to the small amounts of phycobilins present in these blue-green algal lichens (data not shown), chlorophyll *a* must largely exercise

the light-harvesting function in these organisms and the zeaxanthin-associated dissipation process probably occurs in the antenna chlorophyll as has been suggested for green algal lichens and leaves of higher plants. The fact that zeaxanthin-containing blue-green algal lichens exhibited a high fluorescence yield in darkness or at low PFD and considerable nonphotochemical fluorescence quenching at excessive PFDs further indicates that an (unknown) activation mechanism, related to the "high-energy-state" of the photosynthetic membranes, renders zeaxanthin effective as a fluorescence quencher only when light becomes excessive (see also Demmig-Adams et al. 1989b, c).

These differences in the ability for radiationless energy dissipation indicate that the greater resistance to light stress in green algal lichens in the short-term (see Demmig-Adams et al. 1990) is indeed related to the ability of green algal lichens to rapidly form zeaxanthin from violaxanthin in the xanthophyll cycle. However, our results also indicate that blue-green algal lichens may acquire the capacity for photoprotective energy dissipation upon long-term exposure to excessive PFD. In this context it is interesting that some blue-green algal lichens can be found in desert habitats (Kappen 1988).

We should like to stress that the fluorescence characteristics of the blue-green algal lichens were, except for the inability to dissipate energy nonradiatively, remarkably similar to those of the green algal lichens such that our comparisons between the two were justified. A relatively low ratio between F_M and F_O in blue-green algae has been reported by many (e.g. Lange et al. 1989; Samuelsson et al. 1985), whereas in some studies (Mullineaux and Allen 1988; Melis et al. 1989) this ratio has been high and similar to that of green algae or leaves. In the present study, the maximum values of F_V/F_M in the blue-green algal lichens (0.68) were only slightly less than those in green algal lichens and, furthermore, were similar at room temperature and at 77K (Demmig-Adams et al. 1990). We conclude that the chlorophyll fluorescence which we detected emanated primarily from PSII. Furthermore, decreases in PSII photochemical efficiency, F_V/F_M, induced by exposure to high light in both lichen groups were quantitatively related to decreases in the photon yield of photosynthesis (Demmig-Adams et al. 1990). In contrast to the green algal lichens, some but not all of the blue-green algal lichens exhibited maximum fluorescence yields in low PFD rather than in darkness. It is interesting that this response was observed in zeaxanthin-free thalli of *Peltigera rufescens* but not in zeaxanthin-containing thalli of the same species.

Carotenoid composition in green and blue-green algal lichens. The analyses of carotenoid composition showed that the green algal lichens did possess the xanthophyll cycle and that the blue-green algal lichens did not contain the epoxides of the xanthophyll cycle under any conditions, as had been reported previously for green versus blue-green algae (Hager and Stransky 1970; Stransky and Hager 1970). After having been maintained at a low PFD for several days, all lichens contained either no zeaxanthin or only trace amounts of zeaxanthin (*Sticta fuliginosa*). Upon short-term exposure to excessive light, the thalli with green phycobionts were able to rapidly increase their zeaxanthin content, whereas those with blue-green phycobionts were not.

It is noteworthy that the green algal lichens, even those which had developed in the shade, contained less $\alpha+\beta$-carotene and much more of the xanthophyll-cycle components than shade leaves of higher plants (Demmig et al. 1987; Demmig-Adams et al. 1989d). Rather, the sum of the three xanthophylls of the xanthophyll cycle in green algal lichens was similar to that found in leaves which receive high levels of radiation in the field (Demmig-Adams et al. 1989a).

Whereas, in the short-term, blue-green algal lichens were unable to form zeaxanthin, thalli of the blue-green algal lichens *Peltigera polydactyla* and *P. rufescens* which were growing in exposed sites or maintained at a higher PFD were found to have up to half of the zeaxanthin content (per chlorophyll) which was observed in green algal lichens following a short-term exposure to high light. It is likely that the zeaxanthin in these blue-green algal lichens was formed, over longer periods, in the (normal) biosynthetic pathway from β-carotene (McDermott et al. 1973). The presence of zeaxanthin in the thalli of some blue-green algal lichens but not in others is consistent with previous reports that blue-green algae may contain no zeaxanthin, or zeaxanthin which represents up to 50% of the total carotenoid content (e.g. Healey 1968). It is interesting that the blue-green algal lichens possessed, in addition to β-carotene, exclusively the oxygenated derivatives of β-carotene, and that these derivatives were generally present in greater amounts in those thalli which had been growing in sites which received higher PFDs. Whenever zeaxanthin (3,3′-dihydroxy-β-carotene) was present in blue-green algal lichens there was also a carotenoid with a very similar R_f value present. It is possible that this was isozeaxanthin (4,4′-dihydroxy-β-carotene), which has been suggested to be the precursor of canthaxanthin (4,4′-diketo-β-carotene; see Goodwin 1980).

In view of the results presented here, the acquirement of the xanthophyll cycle by green algae and higher plants allows for the rapid formation of zeaxanthin upon exposure to excessive light levels and the rapid removal of zeaxanthin upon return to non-excessive light levels. Since the increase in radiationless energy dissipation associated with zeaxanthin (formed from either violaxanthin or β-carotene) apparently results in a decrease in photochemical efficiency (Demmig et al. 1988), the capability for a rapid removal of zeaxanthin is perhaps the most important aspect of the xanthophyll cycle, as it may facilitate a more rapid increase in the efficiency of photochemical energy conversion upon return to conditions under which light is no longer excessive. In habitats in which irradiance is constantly excessive, as e.g. in the desert (Kappen 1988), the absence of the xanthophyll cycle may not be disadvantageous for blue-green algal lichens.

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