Reversible Photoinhibition in Antarctic Moss during Freezing and Thawing[1]

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Tolerance of antarctic moss to freezing and thawing stress was investigated using chlorophyll *a* fluorescence. Freezing in darkness caused reductions in F_v/F_m (ratio of variable to maximum fluorescence) and F_o (initial fluorescence) that were reversible upon thawing. Reductions in F_v/F_m and F_o during freezing in darkness indicate a reduction in the potential efficiency of photosystem II that may be due to conformational changes in pigment-protein complexes due to desiccation associated with freezing. The absorption of light during freezing further reduced F_v/F_m and F_o but was also reversible. Using dithiothreitol (DTT), which inhibits the formation of the carotenoid zeaxanthin, we found reduced fluorescence quenching during freezing and reduced concentrations of zeaxanthin and antheraxanthin after freezing in DTT-treated moss. Reduced concentrations of zeaxanthin and antheraxanthin in DTT-treated moss were partially associated with reductions in nonphotochemical fluorescence quenching. The reversible photoinhibition observed in antarctic moss during freezing indicates the existence of processes that protect from photoinhibitory damage in environments where freezing temperatures occur in conjunction with high solar radiation levels. These processes may limit the need for repair cycles that require temperatures favorable for enzyme activity.

Physiological mechanisms that allow plants to freeze or desiccate without incurring tissue damage have allowed plants to survive in extremely cold and dry environments. Both freezing and desiccation in the light have been observed to cause irreversible reductions in the efficiency of PSII electron transport in plants that are intolerant of desiccation or freezing (Somersalo and Krause, 1990; Öquist and Huner, 1991). In contrast, freezing and desiccation lead to reversible reductions in the efficiency of PSII electron transport in plants acclimated to low temperatures (Hällgren et al., 1990; Post et al., 1990; Hurry and Huner, 1992) and/or desiccation (Muslin and Homann, 1992; Seel et al., 1992; Eickmeier et al., 1993).

Reversible photoinhibition is proposed to be indicative of regulatory processes that prevent the reduction of the electron transport chain to an extent to which damaging photooxidative reactions may occur (Öquist et al., 1993). Reduction of the electron transport chain such that the probability of photooxidative damage is increased most likely occurs when the light absorbed by plants is in excess of their capacity to utilize it in photosynthetic carbon or oxygen fixation or dissipate it safely as heat (Krause, 1988). This situation is created when the activity of enzymes of the photosynthetic carbon/oxygen reduction cycle are restricted by temperatures that are suboptimal for photosynthesis, as is likely in plants exposed to freezing (Krause, 1994), or because of desiccation, which is likely in plants exposed to water deficits (Eickmeier et al., 1993).

In the antarctic environment, moss can be exposed to freezing temperatures (below $-7°C$) in full sunlight (up to 2000 μmol quanta $m^{-2} s^{-1}$), particularly in the late summer months when snow cover has melted. To survive and grow in this environment, moss plants must have developed mechanisms that prevent the likelihood of photooxidative damage. In this study photoinhibition during freezing of the antarctic moss *Grimmia antarctici* Card. was examined.

The carotenoids zeaxanthin and antheraxanthin have been implicated in providing protection against photoinhibitory damage by facilitating the dissipation of excess energy absorbed by plants as heat (Demmig-Adams and Adams, 1992). This process is believed to occur within the LHCII, although the mechanism by which this occurs is still not fully understood (Osmond, 1994). In desiccation-tolerant *Selaginella*, zeaxanthin may help to stabilize the LHCII during desiccation and thus provide protection from excess light (Eickmeier et al., 1993). In cold-acclimated cereals, zeaxanthin was found in higher concentrations than in plants not acclimated to cold temperatures (Hurry and Huner, 1992). However, the protective role of zeaxanthin during cold-temperature treatments was not clearly established, since there were no differences observed in the

Abbreviations: $\Delta F/F_m'$, quantum efficiency of photochemistry; F_m, maximum fluorescence; F_m', maximum fluorescence after a saturating pulse of light while the moss was illuminated; F_o, initial fluorescence; F_s, steady-state fluorescence; F_v, variable fluorescence; LHCII, light-harvesting complexes of PSII; qN, nonphotochemical fluorescence quenching.

nonphotochemical energy dissipation of excess light energy between plants grown at different temperatures when exposed to cold treatments, despite the differences in the concentration of zeaxanthin. These findings led these researchers to conclude that excess light energy dissipation during cold treatments was not zeaxanthin dependent but was occurring within the PSII reaction centers. In contrast, recent experiments with broad-leafed freezing-tolerant plants have shown a strong correlation between qN and zeaxanthin and antheraxanthin concentrations during cold temperatures (Adams and Demmig-Adams, 1995).

To determine the importance of zeaxanthin and antheraxanthin on photoinhibition in antarctic moss during freezing, we used DTT, an inhibitor of violaxanthin de-epoxidase, the enzyme that converts violaxanthin to antheraxanthin and zeaxanthin (Bilger et al., 1989), and Chl fluorescence techniques (Schreiber et al., 1986). We examined the formation of zeaxanthin and antheraxanthin and fluorescence quenching associated with the dissipation of excess light energy during freezing in antarctic moss. We also assessed how these processes influence the quantum efficiency of PSII.

MATERIALS AND METHODS

The experiments were conducted at Casey, an Australian antarctic station located in Wilkes Land, continental Antarctica (66°17′ S 110°32′ E). Specimens of *Grimmia antarctici* Card. used in these experiments were collected from moss beds close to the station. Two 10- × 10-cm sections of moss cushion were taken from similar microenvironments within the moss beds. Moss was then divided into 1-cm cores. Five cores were randomly assigned to each experimental treatment. Sexual reproduction in mosses has not been observed at Casey and thus the plant material used is likely to be genotypically similar.

Freezing and Thawing Treatments

Moss was kept in trays exposed to photon flux density of 20 μmol quanta m^{-2} s^{-1}, 5°C, and 100% RH for 1 d prior to experimental manipulation. Moss was frozen and thawed while fully hydrated. Exposing moss to freezing and thawing was done by placing cores of moss 1 cm diameter in a metal block drilled with holes (also 1 cm in diameter). The metal block was placed in a temperature-controlled water bath filled with a 60% ethylene glycol solution in a room maintained at 15°C. The temperature of the bath was manipulated such that the moss temperature at the beginning of the experiments was approximately 5°C (bath temperature approximately 4°C) and was reduced in five steps to −12°C (bath temperature of −16°C) during the next 6 h and then increased to 5°C during the next 6 h, creating a freezing rate of approximately 3°C h^{-1}. This rate of temperature change was chosen because it approximated the rate of decline in air temperatures during a freeze-thaw event in the field and was similar to rates of change of temperature previously measured at other antarctic locations (Seppelt and Ashton, 1978). After the 12-h freezing and thawing treatment moss was placed under 20 μmol

quanta m^{-2} s^{-1}, 5°C, and 100% RH for 12 h to monitor recovery.

To assess the influence of light on Chl fluorescence changes during freezing, moss was frozen either in darkness or at a photon flux density of 350 μmol quanta m^{-2} s^{-1}. Light was provided using a slide projector suspended above the water bath. The photon flux density was attenuated using neutral density filters (Baltzers, Liechtenstein). Moss surface temperatures were measured with a Grant thermistor (Grant Instruments, Cambridge, UK). Time rather than temperature was chosen as the independent variable in presenting the data because of hysteresis that made fluorescence against temperature trends difficult to see clearly. Temperatures over the replicate moss pieces at any measuring time varied less than 1°C.

To test the influence of the xanthophyll cycle pigments on changes in Chl fluorescence during freezing in the light, DTT, an inhibitor of the enzyme violaxanthin de-epoxidase that converts violaxanthin (V) to zeaxanthin (Z) via the intermediate antheraxanthin (A), was used (Bilger et al., 1989). Plant material was soaked in 3 M DTT or distilled water (controls) for 1 h in the dark prior to freezing. Three samples to determine pigment concentrations were taken before the experiment and after freezing and thawing. Only the top 2 mm of tissue were utilized for pigment analysis. Xanthophyll cycle pigments were quantified by HPLC using the method described by Gilmore and Yamamoto (1993). Because antheraxanthin and zeaxanthin have both been shown to correlate with qN (Gilmore and Yamamoto, 1993), the concentrations of xanthophyll cycle pigments likely to be actively contributing to qN is expressed as $(Z + A)/(V + A + Z)$.

Chl Fluorescence

Chl *a* fluorescence was measured with a PAM-2000 Chl fluorescence measuring device (H. Walz, Effeltrich, Germany) at the following settings: measuring light intensity, 7; damping, 5; gain, 4; saturating pulse intensity, 7; duration of saturating pulse, 0.8 s. These settings ensured an F_o that ranged between 0.2 and 0.4 and F_m during the saturating pulse level of 0.7 to 1.3 mV in healthy moss tissue kept in darkness. Measurements were made by placing the end of the fiber optic cable close to the smooth surface of the densely packed gametophytes of the moss cores. The angle and distance of the end of the fiber optic cable from the moss core surface were kept constant throughout the experiment by using the metal spacer provided with the PAM-2000. The ratio of F_v/F_m, where $F_v = F_m - F_o$, was measured after moss had been in darkness for 10 min. F_s and $F_m′$ were measured at approximately hourly intervals during freezing and thawing. The $\Delta F/F_m′$ was estimated as $(F_m′ - F_s)/F_m′$ (Genty et al., 1989). Calculation of quenching of fluorescence due to nonphotochemical dissipation of absorbed light energy, qN $[(F_m - F_m′)/F_m′]$, which is directly proportional to the increase in the rate constant for energy dissipation as heat, was determined according to the method of Bilger and Björkman (1990).

RESULTS

Influence of Light on Chl Fluorescence during Freezing

Freezing resulted in decreases in both F_v/F_m (Fig. 1), which can be thought of as the potential yield of PSII (Schreiber et al., 1994), and F_o (Fig. 2) in both light and darkness. Decreases in both F_v/F_m and F_o during freezing were greater in the light than in darkness (Figs. 1 and 2), with F_v/F_m declining to approximately zero when frozen in the light. F_v/F_m values after freezing and thawing were lower in moss exposed to light compared with those frozen in darkness, although this difference was no longer apparent after a 12-h recovery period (Table I). F_o values after freezing and thawing were lower in moss exposed to light during freezing compared with those frozen in darkness. The lowering of F_o in moss frozen in the light relative to that frozen in darkness was no longer evident after a 12-h recovery period (Table I).

Influence of DTT on Chl Fluorescence and Xanthophyll Cycle Pigments during Freezing

The addition of DTT had little influence on either the potential quantum efficiency of PSII estimated by F_v/F_m (Fig. 3A) or the effective quantum efficiency of PSII ($\Delta F/F_m'$) (Fig. 3B) during freezing. Values of F_v/F_m after freezing and thawing were lower in plants treated with DTT compared with those not treated with DTT. However, after a 12-h recovery period these differences were small (Table I).

The addition of DTT greatly affected F_o during freezing and thawing (Fig. 4). F_o was reduced by approximately 50% when DTT was added compared with an 80% reduction in F_o observed in controls (Fig. 4). The substantial differences between the F_o values of DTT-treated and control moss and the small differences observed between the F_v/F_m of the control and DTT-treated moss indicate that the addition of DTT increased the total fluorescence yield relative to controls during freezing and thawing but did not substantially alter the ratio of F_v/F_m.
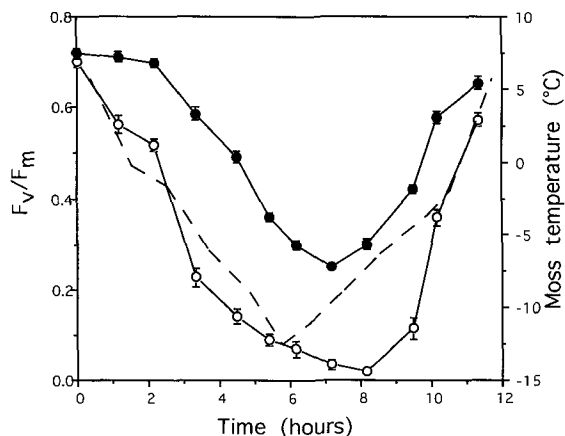

Figure 1. Changes in F_v/F_m during freezing of moss in darkness (●) or under 350 μmol quanta m^{-2} s^{-1} (O) and moss temperature (dashed line). Each point is the mean ± SE of five replicates.

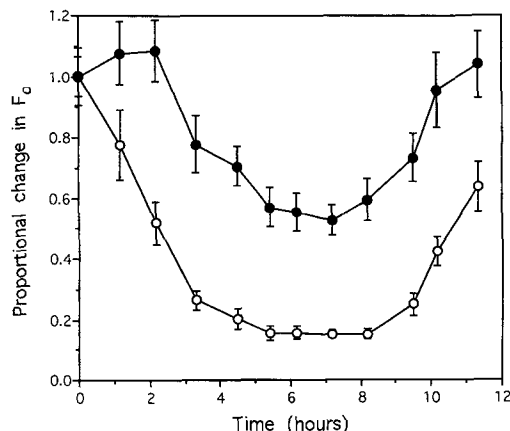**Figure 2.** Proportional changes in F_o during freezing of moss in darkness (●) or under 350 μmol quanta m^{-2} s^{-1} (O). Initial F_o was set to 1.0 to facilitate comparisons between treatments; initial values of F_o are shown in Table I. See Figure 1 for moss temperatures during freezing and thawing and the legend of Figure 1 for further details.

Chl fluorescence quenching due to qN was also influenced by temperature and the addition of DTT (Fig. 5). qN declined and remained low for both controls and DTT-treated moss during freezing but increased as temperatures increased above $-10°C$ for DTT-treated moss and $-8°C$ for control tissue. This was followed by a decline in qN as temperatures increased above $-5°C$. At the end of the experiment qN in DTT-treated moss was approximately 25% of the control values.

Half of the xanthophyll cycle pigments were present as zeaxanthin and antheraxanthin at the start of the experiment despite plant material having spent 24 h at low photon flux densities. During freezing in the dark the proportion of zeaxanthin and antheraxanthin decreased. In contrast, during freezing and thawing in the light the proportion of the xanthophyll cycle pigments present as zeaxanthin and antheraxanthin (i.e. [Z + A]/[V + A + Z]) was similar in the DTT-treated tissue, whereas it increased in the controls (Table I). The Chl a/b ratios and the concentration of xanthophyll cycle pigments with respect to Chl did not change during the course of the experiment (Table I).

DISCUSSION

Freezing-Induced Changes in Chl Fluorescence in the Dark

The potential quantum efficiency of PSII (F_v/F_m) and F_o were reduced by freezing in darkness (Figs. 1 and 2). These large changes, particularly in F_m and F_v, were reversible upon thawing (Figs. 1 and 2; Table I). During freezing water moves along a water potential gradient out of the cells toward ice nuclei that form extracellularly (Burke et al., 1976). This effectively dehydrates the tissue, although not to the same extent as does dehydration at higher temperatures (Crowe et al., 1990). The withdrawal of water during freezing results in changes in the conformation of biomolecules (Crowe et al., 1990). Changes in biomolecular conformation in photosynthetic organisms may alter the arrangement of proteins within thylakoid membranes,

Table I. *Changes in dark-adapted F_v/F_m; F_o; the proportion of the xanthophyll cycle carotenoids present as zeaxanthin and antheraxanthin, $(Z+A)/(Z+A+V)$; the ratio of the xanthophyll cycle pigments to the total Chl concentration (TChl); and the ratio of the concentration of Chl a to Chl b of the Antarctic moss G. antarctici, before and after a 12-h freezing and thawing treatment, and after a 12-h recovery period at 5°C and 20 μmol quanta m^{-2} s^{-1} once moss had thawed*

Moss was frozen either in darkness or under 350 μmol quanta m^{-2} s^{-1} having either been treated with DTT or in distilled water prior to freezing. Values are the means ± SE of five samples (fluorescence) or three samples (pigments).

Treatment	F_v/F_m	F_o	$(Z+A)/(Z+A+V)$	$(Z+A+V)/TChl$	Chl a/b
			$mol\ mol^{-1}$	$mol\ mol^{-1}$	
Before freezing					
Dark					
−DTT	0.719 ± 0.024	0.302 ± 0.045	0.474 ± 0.018	0.118 ± 0.014	2.50 ± 0.12
+DTT	0.707 ± 0.037	0.361 ± 0.038	0.519 ± 0.041	0.139 ± 0.013	2.42 ± 0.07
Light					
−DTT	0.701 ± 0.030	0.300 ± 0.062			
+DTT	0.683 ± 0.020	0.316 ± 0.026			
After freezing					
Dark					
−DTT	0.653 ± 0.031	0.312 ± 0.072	0.382 ± 0.069	0.102 ± 0.010	2.44 ± 0.04
+DTT	0.653 ± 0.045	0.382 ± 0.036	0.227 ± 0.089	0.084 ± 0.014	2.37 ± 0.04
Light					
−DTT	0.573 ± 0.032	0.192 ± 0.055	0.665 ± 0.011	0.121 ± 0.022	2.56 ± 0.11
+DTT	0.497 ± 0.016	0.398 ± 0.081	0.413 ± 0.083	0.116 ± 0.010	2.46 ± 0.05
12-h recovery					
Dark					
−DTT	0.709 ± 0.031	0.254 ± 0.047			
+DTT	0.720 ± 0.043	0.297 ± 0.037			
Light					
−DTT	0.695 ± 0.024	0.244 ± 0.047			
+DTT	0.667 ± 0.015	0.302 ± 0.054			

leading to reduction in the light-absorbing surface of the thylakoid membranes or increases in self-absorption of emitted fluorescence when frozen.

Changes in protein conformation during freezing may also interrupt energy transfer between LHCII and PSII, thus possibly reducing the number of functional PSII centers. It has been demonstrated in the green algal symbionts of lichens that desiccation results in an interruption of energy transfer between the light-harvesting Chl a/b pigment-protein complexes of LHCII and PSII (Bilger et al., 1989). Alternatively, PSII and LHCII may still be connected during freezing with freezing resulting in increased quenching in the reaction centers of PSII and/or highly competitive quenching in the LHCII complexes. Low temperatures after exposure to light may cause a sustained pH gradient between the stroma and lumen that would facilitate fluorescence quenching in darkness (Gilmore and Björkman, 1992). It should also be noted that during freezing fluorescence signals become very small and thus prone to error.

Light-Induced Changes in Chl Fluorescence during Freezing

Absorption of light during freezing resulted in lower F_v/F_m ratios than those observed in control plants frozen in darkness. The effective quantum yield of PSII ($\Delta F/F_m'$) also underwent a similar decrease during freezing. Havaux and Davaud (1994) and Terashima et al. (1994) recently suggested that cold-induced photoinhibition occurs in PSI rather than in PSII. Photoinhibition of PSI rather than PSII

should be reflected in reduced effective yield of PSII ($\Delta F/F_m'$) compared to potential yields of PSII (F_v/F_m). In these experiments both effective and potential quantum yields of PSII were decreased proportionally, indicating that freezing-induced photoinhibition observed in moss occurs at PSII.

In studies of photoinhibition of the desiccation-tolerant fern *Selaginella*, exposure to increasing photon flux densities during desiccation resulted in decreased F_v/F_m values (Eickmeier et al., 1993). In addition, exposure of *Plagiomnium* moss species to light during freezing also resulted in reductions in F_v/F_m compared to moss frozen in darkness (Rutten and Santarius, 1992). The observed light-induced reductions in potential and effective quantum efficiency of PSII during freezing and desiccation could be due to (a) dissipation of light energy in LHCII, possibly facilitated by zeaxanthin and antheraxanthin; (b) light-enhanced disconnection of LHCII from PSII (discussed above); or (c) increases in the number of PSII centers that become photosynthetically nonfunctional.

To test whether light-dependent reductions in F_v/F_m and $\Delta F/F_m'$ during freezing were associated with the presence of zeaxanthin and antheraxanthin, DTT was used to prevent formation of zeaxanthin and antheraxanthin in the light. Neubauer (1993) recently showed that concentrations of DTT in isolated chloroplasts above 2 mmol can also reduce the activity of ascorbate peroxidase, which reduces the Mehler-ascorbate peroxidase reaction and prevents lumen acidification. DTT concentrations used in these experiments were 3 mmol and thus may have inhibited ascorbate
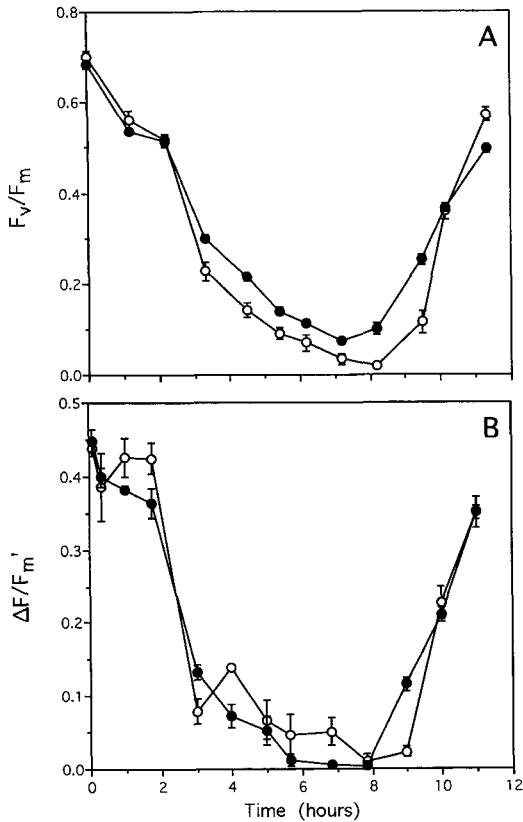
Figure 3. Changes in F_v/F_m (A) and $\Delta F/F_m'$ (B) during freezing of moss exposed to 350 μmol quanta m^{-2} s^{-1}. Moss was either pretreated with DTT (●) or distilled water (O) prior to freezing. See Figure 1 for moss temperatures during freezing and thawing and the legend of Figure 1 for further details.
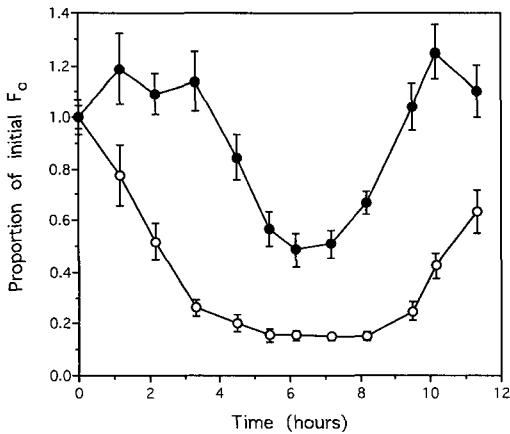

Figure 4. Proportional changes in F_o during freezing of moss exposed to 350 μmol quanta m^{-2} s^{-1}. Moss was either pretreated with DTT (●) or distilled water (O) prior to freezing. Initial F_o was set to 1.0 to facilitate comparisons between treatments; initial values of F_o are shown in Table I. See Figure 1 for moss temperatures during freezing and thawing and the legend of Figure 1 for further details.
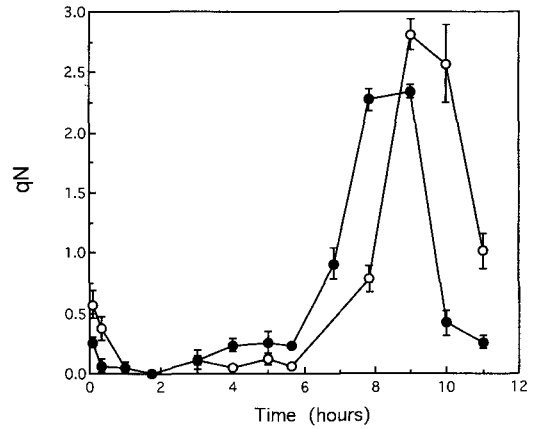

Figure 5. Changes in qN during freezing of moss exposed to 350 μmol quanta m^{-2} s^{-1}. Moss was either pretreated with DTT (●) or distilled water (O) prior to freezing. See Figure 1 for moss temperatures during freezing and thawing and the legend of Figure 1 for further details.

peroxidase and influenced the development of the proton gradient between the lumen and the stroma, as well as inhibiting violaxanthin de-epoxidase.

Half of the xanthophyll cycle pigments were present as zeaxanthin and antheraxanthin in the moss tissue prior to the freezing treatment despite being under low light levels and moderate temperatures (compared to the natural environment) 24 h prior to the experiment. This may indicate slow reconversion of zeaxanthin to violaxanthin in antarctic moss as has been shown in other plants at low temperatures (Bilger and Björkman, 1991; Adams and Demmig-Adams, 1995). The constant presence of zeaxanthin and antheraxanthin may be important for rapid development of qN, which may protect against photooxidative damage in cold environments where the activities of enzymes of the photosynthetic carbon reduction cycles are restricted by cold temperatures (Adams and Demmig-Adams, 1995). In a somewhat analogous situation, zeaxanthin was present during desiccation of the resurrection fern *Selaginella* and possibly contributed to protecting PSII when photosynthetic carbon fixation was restricted because of desiccation (Eickmeier et al., 1993).

In addition to the presence of zeaxanthin in moss tissue under low light conditions, F_v/F_m ratios were also low compared to an average F_v/F_m ratio of approximately 0.83 measured for other C_3 species (Demmig and Björkman, 1987). Maximum F_v/F_m ratios measured for mosses were approximately 0.72. Thus, even under favorable conditions for photosynthesis quantum efficiencies of PSII in moss were depressed. This may be associated with the continuous presence of zeaxanthin and antheraxanthin within the moss.

The addition of DTT to moss prior to freezing prevented any further production of zeaxanthin in moss throughout the experiment (Table I). The lower concentrations of zeaxanthin in the DTT-treated tissues compared with the controls were associated with lower qN values during the initial cooling period (Fig. 5). Despite increasing qN during

thawing, qN was lower in the DTT-treated moss compared to controls and was approximately 25% of control values at the completion of the experiment. Increases in qN in both DTT-treated and control moss during thawing occurred at temperatures close to the freezing point of the tissue (Melick and Seppelt, 1992), with increases in qN in DTT-treated moss occurring at lower temperatures than control moss. This may be due to DTT decreasing the freezing point of the tissue because of increased concentrations of solutes within the tissue. The decline in qN during the last hour of thawing may be due to induction of photosynthesis.

Differences in qN between DTT-treated and control moss did not quantitatively correlate with the difference in the zeaxanthin and antheraxanthin concentrations between the control and DTT-treated tissue. This has been shown for other plant species (Demmig-Adams et al., 1990; Bilger and Björkman, 1991; Eickmeier et al., 1993; Gilmore and Yamamoto, 1993; Adams and Demmig-Adams, 1995). Despite the analysis of pigments being restricted to the top layer (2 mm) of moss tissue, it is possible that there were gradients in light penetration into the tissue that influenced the formation of zeaxanthin and antheraxanthin. The comparison of fluorescence measurements, which are representative of the upper layer of cells, with an average concentration of zeaxanthin and antheraxanthin obtained from tissue in which light penetration may be variable (Robinson et al., 1993), may account for the quantitative discrepancy observed between qN and the concentration of zeaxanthin and antheraxanthin within the moss tissue.

Decreases in F_o are thought to be indicative of photoprotective processes, whereas sustained increases in F_o are associated with photoinhibitory damage (Cleland et al., 1986). In the experiments described here, F_o was reduced by approximately 50% during freezing in the dark, possibly because of physical changes during freezing (Fig. 2). The absorption of light during freezing reduced F_o by a further 30%. The addition of DTT and lower concentrations of zeaxanthin in the DTT-treated tissue resulted in a smaller quenching of F_o during freezing compared with the controls, such that F_o in DTT-treated tissue was similar to that observed in tissue frozen in darkness (cf. Figs. 2 and 4). A similar relative increase in F_o was also observed during desiccation of DTT-treated *Selaginella* (Eickmeier et al., 1993). These observations are consistent with the hypothesis that zeaxanthin is involved in reversible dissipation of light energy in LHCII, which is reflected by reductions in F_o. It has been proposed that zeaxanthin is associated with a conformational change of the LHCII (Ruban et al., 1993; Bilger and Björkman, 1994), which has been specifically proposed to be due to an aggregation of LHCII (Ruban et al., 1993), possibly facilitated by increased membrane fluidity that is associated with zeaxanthin formation (Gruszecki and Strzalka, 1991).

By comparing changes in qN with the potential or effective quantum efficiencies of PSII during freezing and thawing, it is evident that DTT had only a small influence on the potential and effective quantum efficiencies of PSII, whereas it influenced qN substantially, particularly during thawing. Thus, during freezing and thawing the quantum efficiency of PSII appears to be largely independent of the dissipation of excess light energy measured as qN. These data suggest that there may be multiple mechanisms leading to fluorescence quenching during freezing. It has been proposed that reversible photoinhibition during cold temperatures is due to changes within PSII centers that lead to dissipation of energy within the reaction center of PSII (Hurry and Huner, 1992; van Wijk and van Hasselt, 1993), a proposal that supports the reaction center quenching model of Weis and Berry (1987). A proposed mechanism of PSII deactivation is through the loss of calcium ions (Krieger and Weis, 1993).

The results described in this study of freezing-tolerant moss do not enable us to determine the mechanisms responsible for the reversible light-induced reductions in the quantum efficiency of PSII during freezing. However, reductions in the efficiency of PSII observed during freezing may reflect processes that reduce the likelihood of photooxidative damage to PSII and the subsequent formation of damaged PSII centers. This may be a particularly important protective process in environments where enzymic activity necessary for repair cycles (Aro et al., 1993) are restricted because of low temperatures.

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