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Dissipation of Excess Excitation Energy by Drought-Induced Nonphotochemical Quenching in Two Species of Drought-Tolerant Moss: Desiccation-Induced Acceleration of Photosystem II Fluorescence Decay

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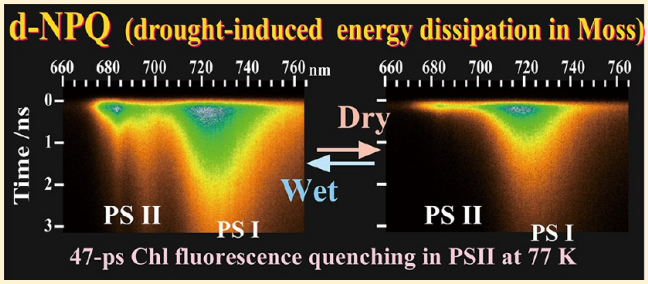
ABSTRACT: Drought-tolerant mosses survive with their green color intact even after long periods of dehydration that would kill ordinary plants. The mechanism of dissipation of excitation energy under drought stress was studied in two species of drought-tolerant moss, *Rhytidium rugosum* and *Ceratodon purpureus*. They showed severe quenching of photosystem II chlorophyll fluorescence (PSII) after being dehydrated in the dark. Quenching was induced by the acceleration of the fluorescence decay rate. This drought-induced nonphotochemical quenching (designated d-NPQ) was fully reversed by rehydration. Global analysis of fluorescence decay at 77 K indicated rapid 46 ps transfer of excitation energy from the 680−690 nm PSII bands to a 710 nm band, and to 740−760 nm bands. The latter bands decayed to the ground state with the same time constant showing the rapid dissipation of excitation energy into heat. The quenching by d-NPQ in dry moss was stronger than that by PSII charge separation or nonphotochemical quenching (NPQ), which operates under hydrating conditions. Drought-tolerant mosses, thus, dissipate excess excitation energy into heat. The d-NPQ mechanism in moss resembles that reported in lichens, suggesting their common origin.



Excess light energy above the capacity of plant photosynthesis triggers photoreactions to produce harmful oxygen compounds such as singlet oxygen, $^1O_2$, or other oxidants and destroys organisms.[1−3] A major mechanism for protection against the photodamage that occurs mainly on PSII has been known to be nonphotochemical quenching of excitation energy (NPQ). NPQ is induced by the light-induced xanthophyll cycle that is triggered by protonation of thylakoid proteins under strong illumination.[4−9] Movements of light-harvesting pigment−protein complexes on the thylakoid membranes from PSII to PSI induced by the state transition mechanism are also known to decrease the rate of influx of energy into PSII.[10] These two mechanisms mainly regulate the influx of excitation energy into PSII during illumination and optimize the yield of light reactions. However, NPQ and state transition do not operate under severe stresses such as those associated with drought, coldness, etc., that stop physiological reactions and promote photodamage. We here report a new mechanism of excitation energy dissipation found in dehydrated moss, which is drought-induced nonphotochemical quenching (d-NPQ) that suppresses photodamage even under fully dehydrating conditions. The mechanism resembles those recently identified and characterized in symbiont algae in two species of draft-tolerant lichens.[11−13]

Many species of moss (more than 15000 species in total) and most lichens (more than 13000 species) are known to be tolerant of desiccation.[14] They survive under direct sunlight even after being dehydrated. Recent studies[11−16] have revealed that symbiotic algae inside lichens activate a strong energy dissipation mechanism that activates an unknown fluorescence quencher in PSII to dissipate the excess excitation energy into heat under drought stress. The mechanism is activated by drying and is inactivated within a few minutes of the moss being made wet again.[13,17,18] The quenching was identified to be different from NPQ and state transition, which are induced by illumination under hydrating conditions and induce moderate or no acceleration of fluorescence decay, respectively. The d-NPQ mechanism in lichens dissipates light energy rapidly into heat in PSII. Interaction between symbiont algae and their environments provided by host fungi inside lichens seems to be important for the activation of d-NPQ because the ability is known to be lost after the growth of algae outside lichens.[15,16]

Recent studies revealed the drought-induced severe quenching of the PSII fluorescence in a drought-tolerant moss

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Rhytidium rugosum.[19,20] Illumination of hydrated *R. rugosum* induced quenching of PSII fluorescence because of the action of zeaxanthin-dependent energy dissipation (NPQ).[17] The effect was reversed gradually in the dark. More severe quenching caused by the d-NPQ mechanism, which is different from NPQ and state transition mechanisms, was detected after desiccation.[19] The fluorescence was decreased by the desiccation below the dark-adapted F_0 level when monitored by the pulse amplitude-modulated (PAM) fluorescence measurement, suggesting the activation of a fluorescence quencher stronger than the PSII reaction center trap.[19,20] The cycles of dehydration and hydration activated and inactivated the d-NPQ mechanism, respectively. When *R. rugosum* was dehydrated while being illuminated, slightly stronger quenching was detected, probably because of the preaccumulated NPQ or state transition effects in addition to d-NPQ quenching.[19]

In desiccated two-lichen species, picosecond fluorescence lifetime studies have revealed the fast migration of excitation energy from antenna pigments to the long-wavelength fluorescence-emitting pigments with time constants of 40 ps[12] or 23 ps[13] at 77 K (or 10 and 27 ps at 77 and 5 K, respectively, according to a more recent study[21]). The acceleration of decay was induced by the d-NPQ mechanism. It is, however, not yet clear whether the d-NPQ mechanism detected in these dehydrated lichens is similar to that observed in moss, which accelerated the fluorescence decay rate 5-fold at room temperature (RT).[19] The algal cells in lichens are known to be strongly affected by the specific environments provided by lichens,[15,16] while chloroplasts inside cells of moss are directly in contact with the moss cytoplasm.

In this study, we carefully analyzed fluorescence decay kinetics of two desiccation-tolerant moss species (sun-adapted *R. rugosum* and *Ceratodon purpureus*), which are in different orders and phylogenetically rather distant from each other, at RT and 77 K to analyze fluorescence responses. The analysis identified the operation of the d-NPQ mechanism in both species that is apparently comparable to that found in unicellular green algae in lichens.

MATERIALS AND METHODS

A desiccation-tolerant moss species, *R. rugosum* (Ehrh.) Kindb. (family Rhytidiaceae in order Hypnobryales), was collected from a sun-exposed location on calcareous soil near Leinach, 25 km from Würzburg, Bavaria, Germany, as reported previously.[19] Another desiccation-tolerant sun-adapted moss species, *C. purpureus* (family Ditrichaceae in order Diccranales), was collected from a sun-exposed location on a campus of Nagoya University in central Japan. After collection, prolonged dark adaptation (usually 36 or 48 h) of hydrated thalli before dehydration was intended to decrease levels of zeaxanthin, which is known to be converted to violaxanthin in the dark or at a low light intensity.[6] Each hydrated moss was, then, slowly dried in the dark or under dim light and stored in the dark at a relative humidity of <65% before being used for experiments.

The absorption spectrum of a single thallus of each moss species was measured with a home-built spectrometer, in which a light guide from a fiber monochromator (BLK-CXR-SR, StellarNet, Tampa, FL) was placed on a microscope (BH-2, Olympus, Tokyo, Japan) in place of an eyepiece lens to collect the light that passed through a target focusing point.

The flash-induced yield of Chl fluorescence at wavelengths above 700 nm, which was excited by a 650 nm LED flash light, was monitored by a pulse amplitude modulation fluorometer (PAM 101, Walz, Effeltrich, Germany) at RT[22] to monitor the sample conditions before the fluorescence lifetime measurement as described previously.[19]

Fluorescence lifetimes were measured as reported previously.[23,24] A 430 nm laser pulse from a Ti:sapphire laser (Mai Tai, Spectra-Physics in Newport Corp., Irvine, CA) was passed through a 430 nm interference filter and excited the fluorescence of dry and wet thalli of *R. rugosum*. The frequency-doubled light at 430 nm was generated by a type I BBO crystal from an 860 nm laser flash with a flash duration of 150 fs and a repetition rate of 80 MHz. The intensity of the laser at the focusing point was attenuated to be 100 μW. In the measurement of *C. purpureus*, a 405 nm laser diode with a pulse duration of 50 ps was used at a 1 MHz repetition rate (M4734-32, Hamamatsu Photonics, Hamamatsu, Japan) instead of the Ti:sapphire laser. The intensity of the excitation laser at the focusing point was set at 1.0 μW to give an intensity of each pulse that was slightly lower than that of the 430 nm pulse described above. Fluorescence excited by either type of laser was focused onto the entrance slit of a 50 cm polychromator, dispersed in a streak camera, and detected with a charge capacitance detector (Chromex 2501-S, 100 g/mm monochromator, and Streak Scope C4334, Hamamatsu Photonics). The streak camera system was operated in photon-counting mode to give two-dimensional (2D) images in (640 pixels as for wavelength) × (480 pixels as for delay time with respect to the flash peak time) as seen in Figure 3. Each image covered photon counts over the 636−778 nm fluorescence emission range with a 1 nm spectral resolution (*x*-axis), and for the full scan time ranges of 1100 and 5350 ps (*y*-axis). Photon counts were accumulated for approximately 1 h in each measurement, and obtained images were analyzed with home-built software as reported previously.[23,24] Dark-dehydrated or rehydrated moss thalli were set in a plastic holder under dim light and then adapted to the dark for more than 30 min before the start of measurements to prevent the effects of preillumination.

RESULTS

Effects of Dehydration on the Shapes of Thalli and Absorption Spectra of Two Moss Species. Figure 1 shows photographic images of dehydrated (dry) and rehydrated (wet) thalli of drought-tolerant mosses, *R. rugosum* and *C. purpureus*, which has shapes smaller than those of *R. rugosum* and is phylogenetically distant from *R. rugosum*. They were dried on a laboratory desk under dim light at RT for more than 1 day to diminish the effects of NPQ and state shift and rehydrated again via addition of water as described in Materials and Methods. No gross changes in the appearance, except for deeper green colors, due to larger overlap and stronger light scattering, of thalli were detected upon dehydration. Absorption spectra of selected single thalli were measured by a fiber monochromator connected to a microscope (see Materials and Methods) to minimize the effects of light scattering. The two absorption spectra indicated peaks of Chl *a* around 435 and 670 nm, shoulders caused by Chl *b* at 480 and 650 nm, and carotenoid peaks around 500 nm under both hydrated and dehydrating conditions (only the spectra in the red range are shown in Figure 1). As seen in Figure 1, spectral shapes were almost unaffected by the dehydration treatment. The results indicate that both the dry and wet mosses show almost unaltered pigment absorption peaks and absorb solar

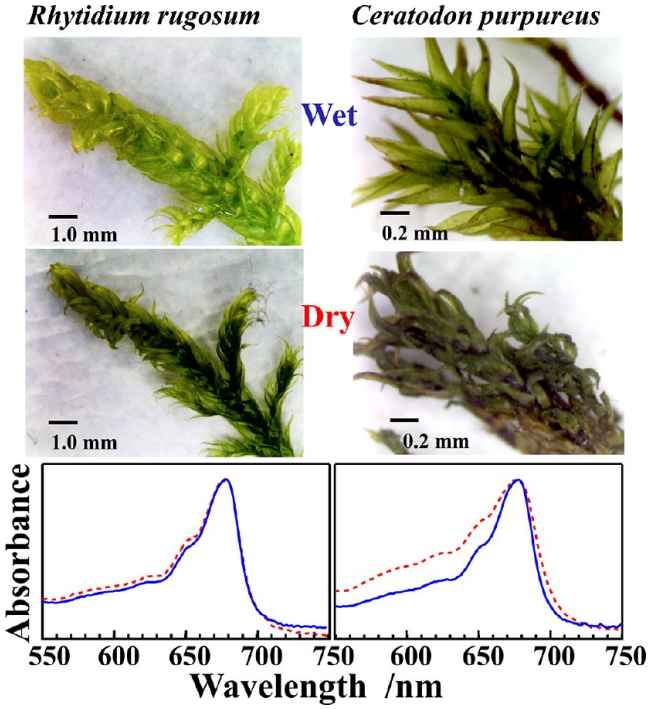

Figure 1. Photographs of hydrated (wet) and dehydrated (dry) *R. rugosum* and *C. purpureus* and their absorption spectra. Blue and red lines represent absorption spectra measured under wet and dry conditions, respectively, at room temperature.

energy to similar extents. This type of dry−wet cycle seems to be experienced by the organisms under natural conditions.

Effects of Dehydration on Fluorescence Yield and Emission Spectra. Figure 2 shows fluorescence spectra of *R. rugosum* and *C. purpureus*. The spectra measured at RT showed peaks and shoulders around 684 and 710−740 nm, respectively. The PSII fluorescence intensity is known to be decreased by NPQ, state transition, or radical quenching mechanisms[19] under strong continuous illumination, so that the moss thalli were dehydrated in the dark to prevent these effects. The spectra in Figure 2 at RT were measured with a weak measuring light that did not cause such quenching. Dehydration decreased the magnitude of the 684 nm peaks specifically to the levels almost comparable to the heights of 710−740 nm shoulder bands in both species (Figure 2a,c). Fluorescence yields of dehydrated mosses were already very low from the beginning of the measurement at a level somewhat lower than the F_0 level and were almost constant during the measurement.[19] The dehydration effects were fully reversed if the mosses were rehydrated. The spectra in Figure 2 were measured under the dehydrating conditions first and then 0.5−1 h after the rehydration to measure spectra almost in the same samples to ensure a better quantitative comparison. It is clear that dehydration decreased fluorescence yields of both species, more significantly in *R. rugosum*.

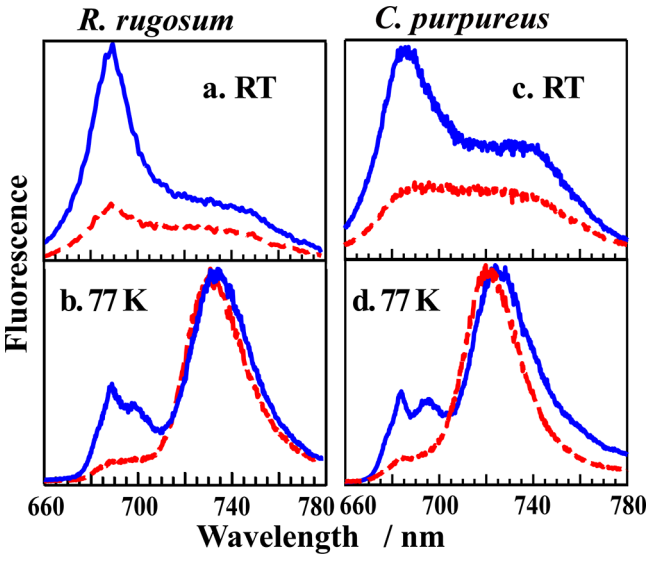

Figure 2. Fluorescence emission spectra of *R. rugosum* and *C. purpureus* measured at RT and 77 K. (a and b) *R. rugosum* at RT and 77 K, respectively, and (c and d) *C. purpureus* at RT and 77 K, respectively. Blue and red lines represent spectra measured under wet and dry conditions, respectively. These steady state spectra were calculated as the spectra of fluorescence integrated 0−5 ns after flash excitation from the measurements with the streak camera system similar to those in Figure 3 as described in Materials and Methods.

We also measured the fluorescence of moss thalli at 77 K (Figure 2b,d). The spectrum at 77 K of wet thalli of *R. rugosum* showed a high PSI peak at 733 nm together with the PSII bands at 686 and 697 nm. The frozen dehydrated thalli also showed a large PSI peak at 732 nm, with the significantly suppressed PSII peaks, consistent with the results at RT. The spectrum at 77 K of wet *C. purpureus* showed PSII peaks at 682 and 694 nm and a PSI peak at 724 nm. Upon dehydration, the PSII peaks were significantly depressed, and the PSI peak was slightly blue-shifted to 721 nm. These results indicate the severe depression of PSII fluorescence bands upon desiccation, with a small effect on the PSI fluorescence band.

Effects of Dehydration on the Fluorescence Lifetime. The decay of fluorescence was measured in hydrated (wet) and dark dehydrated (dry) moss thalli. Both samples were adapted to the dark for ∼1 day before measurements were taken. Photons emitted as fluorescence were dispersed as for wavelength in a monochromator first and then as for the arrival time of photons in a streak camera and recorded on a CCD. The positions of photons on CCD images were counted in single-photon counting mode as described in Materials and Methods. Each dot on the images in Figure 3 represents a trace of photon displaced as for wavelength on the *x*-axis and as for arrival time, which was expressed as the delay from the peak time of the excitation laser, on the *y*-axis as reported previously.[23,24]

A fluorescence decay image of *R. rugosum* was measured at RT (Figure 3a). It showed a wide dispersion along *x*-axis at 660−785 nm with a peak at 684 nm, which showed a long tail along the *y*-axis indicating the slow decay of PSII fluorescence. In the desiccated conditions, *y*-axis tail was significantly shortened (Figure 3b). It is, therefore, clear that the desiccation accelerated fluorescence decay significantly as reported previously.[19] Figure 3c shows an image at 77 K of wet *R. rugosum*. It shows long *y*-axis tails of PSII bands at 686 and 697 nm, together with a longer tail of the wide PSI band at 733 nm. Figure 3d shows the image of the dry thalli. It also shows a large slowly decaying PSI band as seen in the wet thalli. On the other hand, PSII fluorescence bands showed very short *y*-axis tails, indicating the specific decay acceleration.

A fluorescence decay image was also measured for *C. purpureus* (Figure 3) with the 405 nm excitation diode laser pulse. At RT, wet thalli showed a long tail of PSII fluorescence

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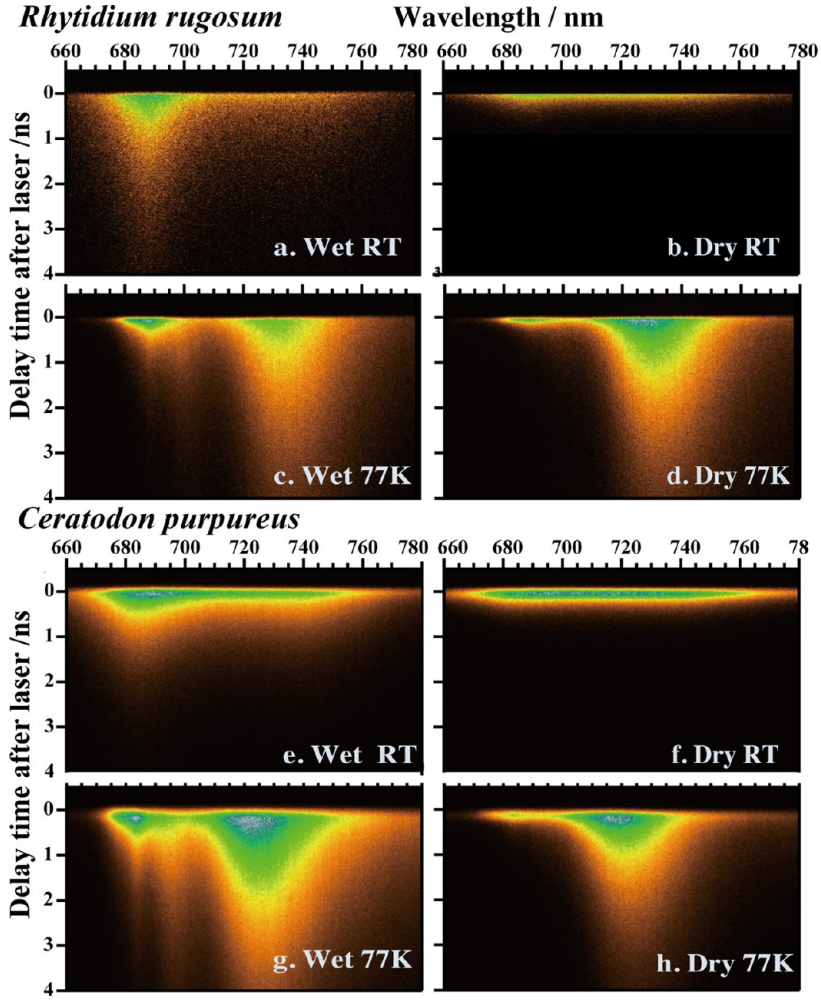

Figure 3. Wavelength (*x*-axis)−decay time (*y*-axis) 2D images of the fluorescence of wet and dry *C. purpureus* and *R. rugosum* at RT and 77 K. (a and b) Fluorescence images of wet and dry *R. rugosum*, respectively, measured at RT. (c and d) Like panels a and b, respectively, but at 77 K. (e and f) In wet and dry *R. rugosum*, respectively, at RT. (g and h) Like panels e and f, respectively, but at 77 K.

while dry thalli showed a very short tail indicating the acceleration of decay (Figure 3e,f). At 77 K, wet thalli showed clearly separated PSII bands at 682 and 694 nm and a strong 724 nm PSI band, which has a wide and long tail (Figure 3g). Significant accelerations of PSII bands similar to those in *R. rugosum* were induced by desiccation (Figure 3h). The PSI band, on the other hand, was accelerated slightly.

Figure 3 indicates that desiccation specifically accelerates the PSII fluorescence, with a weak effect on PSI fluorescence at both RT and 77 K. The results indicate that the drought-induced depression of PSII fluorescence intensity in Figure 2 occurred by the severe acceleration of fluorescence decay. The 2D images are examined in more detail below.

Fluorescence Decay Kinetics. Figure 4 (left) shows the decay kinetics of fluorescence at 77 K of PSII bands at 685 and 695 nm and of the PSI band at 740 nm calculated from the 2D images of *R. rugosum* in panels c and d of Figure 3. The decay of PSII bands was accelerated by desiccation. The decay of the 740 nm PSI peak was only slightly affected by desiccation.

The decay kinetics of fluorescence of *C. purpureus* at 77 K calculated from the images in Figure 3 (right) were almost similar to those of *R. rugosum*, although the accelerations were less marked. It is noted that the PSI fluorescence decay at 740 nm was only slightly accelerated in this case. The slower

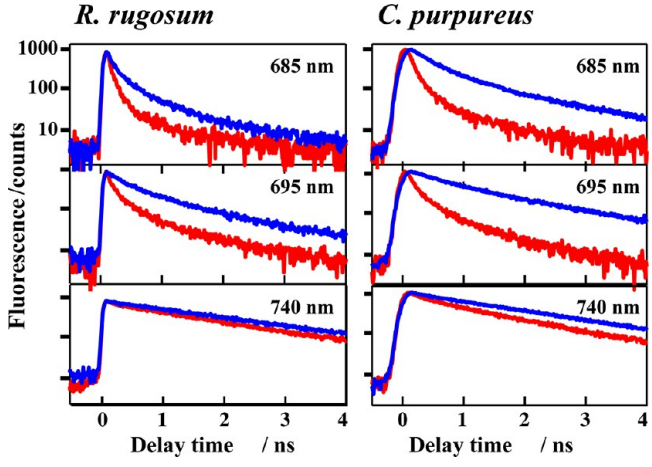

Figure 4. Fluorescence decay time courses of wet and dry *R. rugosum* and *C. purpureus* at 77 K. Fluorescence decay time courses at 685 and 695 nm (mainly PSII) and 750 nm (mainly PSI) were calculated from the 2D images in Figure 3 for (left) *R. rugosum* and (right) *C. purpureus*. Time courses in the wet and dry samples are colored blue and red, respectively. Each time course was calculated from the image in Figure 3 as a sum of the intensity in the wavelength range within 5 nm with a center at the indicated wavelength.

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increases in *C. purpureus* fluorescence simply came from the longer pulse duration of the excitation diode laser used in this measurement.

Apparent (average) decay times (1/*e* times) at 77 K calculated for PSII bands at 695 nm were 312 and 92 ps for wet and dry *R. rugosum*, respectively, and 686 and 161 ps for wet and dry *C. purpureus*, respectively, indicating ~3−4-fold accelerations by dehydration (Table 1). Such remarkable accelerations upon dehydration are similar to those reported in two species of lichens[12,13] and different from those in spinach PSII particles that showed no acceleration upon desiccation.[13] The PSI fluorescence at 740 nm was only slightly accelerated in both organisms. The extent of acceleration of the PSI decay in *C. purpureus* was slightly larger compared to that in *R. rugosum*.

Table 1. Time Constants of Fluorescence Decay at RT and 77 K in Dry and Wet *R. rugosum* and *C. purpureus*[a]

	t_c (ps)		
	wet	dry	wet/dry acceleration ratio
F685 at RT			
R. rugosum	372	72	5.3
C. purpureus	368	127	2.9
F695 at 77 K			
R. rugosum	312	92	3.4
C. purpureus	686	161	4.3

[a]Each time constants of fluorescence decay at 685 nm at RT (F685) or at 695 nm at 77 K (F695) was calculated by deconvolution of time courses similar to those shown in Figure 4.

Time-Resolved Fluorescence Spectra. Time-resolved fluorescence spectra were calculated from the image data in panels a and d of Figure 3 at 77 K for *R. rugosum* as shown in Figure 5. Wet thalli showed a large florescence band around 680 nm 5−20 ps after excitation. The peak, then, shifted to 685−695 nm, showing the migration of energy from the shorter-wavelength bands of antenna Chls to the so-called F684 and F695 bands on the PSII core. The PSI band was low compared to the PSII bands at the beginning and then decayed slowly, showing a small red shift of the peak from 730 to 735 nm. The PSI band became dominant after 340 ps.

In the dry thalli, a peak of bulk antenna emission at 680 nm was smaller and was almost comparable to that of PSI at 5 and 20 ps. The contribution of the 700−710 nm intensity was relatively larger at 20 ps. The peak shifted to the longer wavelengths of 685 and 695 nm, as seen in the wet thalli, at 200−300 ps. The intensities of these bands decreased very rapidly. The fast decay rate of PSII fluorescence suggests the activation of the quenching process by desiccation. PSI fluorescence bands were similar to that detected in the wet thalli, although the peak wavelength finally attained at 870 ps was 730 nm. A slightly shorter PSI peak wavelength in the dry thalli suggests a slight modification of PSI Chls by desiccation.

Estimation of the Energy Transfer Process by Global Analysis. We further analyzed the fluorescence decay at 77 K of *R. rugosum* by a global analysis program and calculated decay-associated spectra (DAS) as shown in Figure 6. In the figure, decays and increases in fluorescence with the same time constant (t_c) are shown as one spectrum giving positive and negative peaks, respectively. DAS, therefore, suggests the flow of energy from the positive band to the negative band with calculated time constant t_c.

In the wet thalli (Figure 6A), the fastest phase with a t_c of 83 ps showed a positive peak at 680 nm and negative peaks at 695 and 728 nm. These peaks indicate the transfer of excitation energy from the shorter-wavelength antenna Chls emitting at 680 nm to either the PSII core bands around 690 nm or the

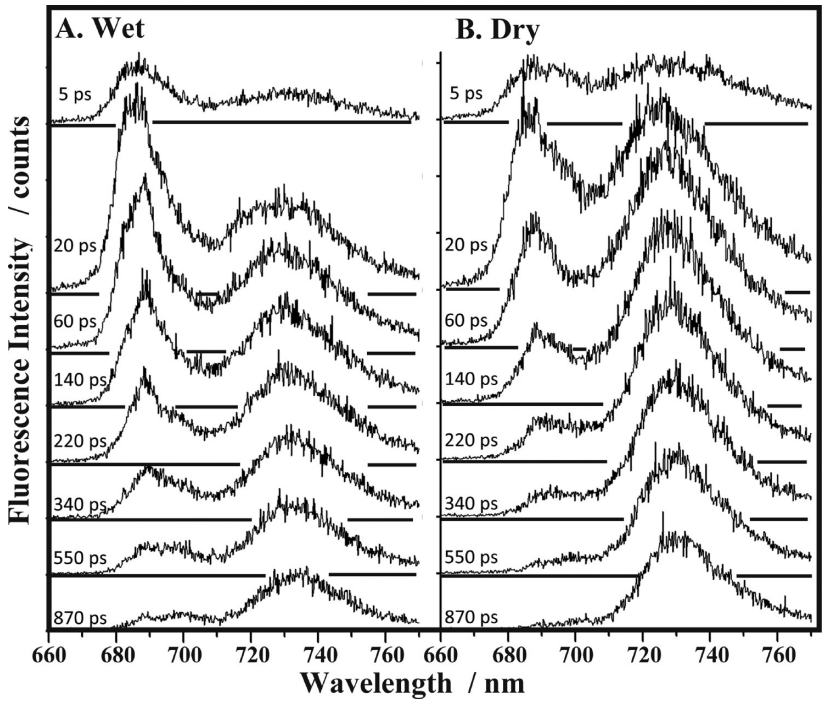

Figure 5. Time-resolved fluorescence spectra in wet (A) and dry (B) *R. rugosum* at 77 K. Each time-resolved spectrum was calculated from the image data in Figure 3 by selecting the time range centered at the indicated time without normalization. Note that the ordinate scale in panel B is expanded to twice that in panel A.

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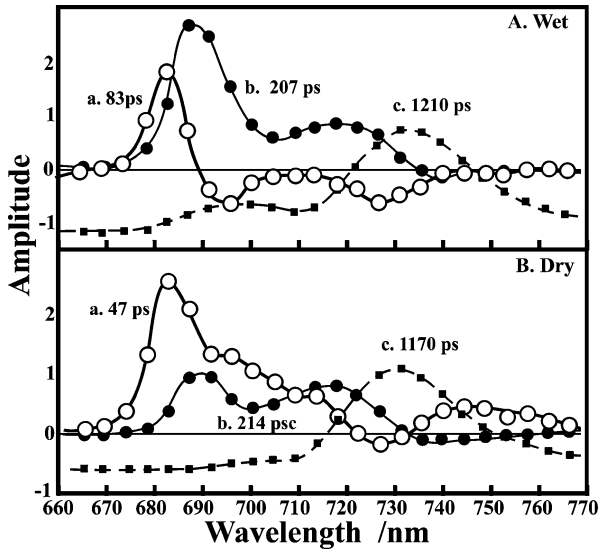

Figure 6. Global analysis of fluorescence kinetics in wet (A) and dry (B) *R. rugosum* at 77 K. Decay (positive peaks) and rise (negative peaks) components developed with the same time constant as indicated are given in a single DAS curve. Note that the baselines for the longest components (trace c in panels A and B) are displaced in the negative direction because of the large systematic error in the estimation of the baseline. Data in Figure 3 were analyzed as described in Materials and Methods.

longer-wavelength PSI band around 730 nm. The DAS curve with an intermediate t_c of 207 ps showed positive peaks around 690 and 720 nm, corresponding to the fluorescence emission from the energy-accepted PSII and PSI bands, respectively. The slowest phase with a long t_c of 1210 ps indicated the positive band (emission) of PSI at 732 nm. DAS analysis, thus, indicates the transfer of excitation energy from the shorter antenna Chls to F684 and F695 bands of the PSII core, and to the F730 band of PSI, and the dissipation of excitation energy as the fluorescence emission from these bands as is typical for green plants.

DAS analysis of the data of the dry thalli (Figure 6B) gave the shortest t_c of 47 ps with a 680 nm positive peak, a positive shoulder around 695−715 nm, and changes in the longer-wavelength region with a wide positive peak at 740−760 nm. A shallow negative band might also be present around 725 nm. A DAS curve with an intermediate t_c of 214 ps indicates positive peaks around 690 and 720 nm. The latter band is almost the same that seen under the wet conditions and seems to indicate the decay of the PSI intermediate band that gives energy to the band around 732 nm. The peak around 690 nm in the 214 ps DAS, on the other hand, was significantly smaller than that in the 207 ps DAS of wet thalli, suggesting that most of the excitation energy in PSII has been transferred to the 740−760 nm band, probably through the 710 nm band, already in the 47 ps t_c phase. The DAS curve with a long t_c of 1170 ps in desiccated thalli was similar to the 1210 ps curve in the wet thalli. It indicates the weak effect of dehydration on the slowly decaying PSI band.

Comparison of DAS spectra under the wet and dry conditions indicates the acceleration of the decay around 680 nm as well as the faster decays of F684 and F690 bands with a t_c of 47 ps. The positive band at 740−760 nm with the t_c of 47 ps indicates the appearance of the rapidly decaying emission caused by desiccation. A small 710 nm band in this DAS may also be included in this transfer process. This process seems to depress the 200 ps intermediate phase of the PSII band under the dry conditions. On the other hand, the long 1200 ps decay of the PSI band at 732 nm and the 200 ps decay around 720 nm were similar between the DAS spectra under the dry and wet conditions, indicating little effect on the rise and decay of PSI fluorescence. The DAS results, thus, indicate the drought-induced fast energy transfer from the PSII 680−690 nm band to the newly appearing 740−760 nm band that decays rapidly with a t_c of 46 ps. This seems to be induced by the specific acceleration−quenching process in PSII. Similar DAS curves were obtained for *C. purpureus* (not shown).

The features of the desiccation-induced changes in the fluorescence decay in two moss species indicated the rapid transfer of excitation energy into the 740−760 nm band in PSII. The features are similar to those found in lichens,[12,13,21] although the analysis described above is rather rough because of the simplification of the complex decay processes, which consist of larger numbers of bands and decay components in both PSII and PSI as discussed in higher plants.[24]

DISCUSSION

Drought-Induced Changes in the Fluorescence of Moss. Moss occupies a broad spectrum of ecological niches. Many of them are tolerant to dehydration and resistant to repeated dehydration−hydration cycles in sunlight. Desiccated moss thalli were not damaged even under very high photon fluxes.[17,19]

Previous studies of a desiccation-tolerant moss species *R. rugosum* indicated multiple energy quenching mechanisms. Strong illumination under hydrating conditions at RT decreased the F_M level by inducing NPQ or state transition.[17,19] Under dehydrating conditions, the fluorescence yield was already low, below the F_0 level at the start of measurement light, and did not change, further indicating no action of NPQ or the state transition mechanism caused by illumination. It was concluded that the d-NPQ mechanism, which quenches the excitation energy in PSII strongly by accelerating the fluorescence decay, abolished all the physiological reactions, including charge separation. The d-NPQ process, thus, is most effective in energy dissipation compared to the other energy dissipation mechanisms. However, it has been identified in only two naturally collected species of lichens at RT and 73−77 K,[12,13,21] one species of moss at RT,[19] and not other organisms. Plants with developed transpiration systems are usually easily subjected to drought stress and lack d-NPQ as typically shown for spinach PSII particles, which did not change the fluorescence decay rate even under severe dehydration.[13] The mechanism of d-NPQ newly characterized in two moss species in this study is discussed below.

Change in the Fluorescence Decay at RT by Desiccation. PSII RC increases the fluorescence yield from a low level (F_0) to a high level (F_M) during illumination. The high F_M level is depressed by the effects of NPQ and state transition under strong illumination. Thus, the PSII fluorescence spectra measured by the weak excitation light in the hydrated moss in Figure 2 were measured at the F_M level with only small effects of NPQ or state transition. On the other hand, the fluorescence decay was measured between the F_0 and F_M levels in Figure 3 judging from the coexisting fast (200 ps) and slow (around 1 ns) decay phases, which are emitted from the closed and open PSII, respectively. Therefore, it is not obvious whether the decrease in the fluorescence intensity at

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RT can be fully explained mainly by the acceleration of the fluorescence decay by d-NPQ. However, the decay rates of PSII fluorescence at RT in two drought-tolerant mosses were accelerated 3−5-fold and were much faster than the PSII trapping rate under desiccated conditions (Table 1 and Figure 3). The acceleration causes the depression of the fluorescence yield to around 30% in Figure 2, consistent with the previous observation at RT in *R. rugosum*.[19] Therefore, we concluded that the drought-induced acceleration of the fluorescence decay is the major cause of the decrease in the fluorescence yield.

Although NPQ and state transition mechanisms are not induced by the illumination under dehydrating conditions, the effects that were preaccumulated before the dehydration treatment may affect the fluorescence yield under dehydrating conditions, too. It was reported in *R. rugosum* that the F_0 level in the light-dehydrated thalli was slightly lower compared to that of the dark-dehydrated thalli, which was already very low compared that of wet thalli.[19] The d-NPQ mechanism, therefore, seems to be more efficient than other energy dissipating mechanisms. The relation between d-NPQ and the other energy dissipation mechanisms under drought conditions seems to be important in the clarification of the mechanism of d-NPQ. It remains to be studied.

Change in the Fluorescence Decay at 77 K by Desiccation. We measured the yield and decay of fluorescence in the dark-dehydrated thalli at 77 K to focus on the mechanism of d-NPQ. At 77 K, dry *R. rugosum* and *C. purpureus* showed accelerated decay of F684 and F695 fluorescence bands. It is, therefore, clear that the excitation energy absorbed by antenna Chls migrated to F684- and F695-emitting Chls on the PSII core rapidly and then to the quencher, which dissipates the excitation energy into heat at a rate fast enough to compete against the trapping by the PSII reaction center (RC) in the open state. The efficiency of energy dissipation seems to be higher than those of NPQ and state transition mechanisms.

DAS analysis indicated the fast energy migration from F684 and F695 bands to 710 and 740−760 nm bands with a t_c of 47 ps in dry *R. rugosum*. The excited state on the 740−760 nm band seems to be dissipated with a similar t_c. DAS with this fast t_c, which was not present under wet conditions, suggests the rapid dissipation of excitation energy into heat from the 740−760 nm band. These results indicate the drought-induced formation of a new band at 740−760 nm that also functions as a quencher. The energy transfer time of 47 ps is shorter than the average energy trapping time of ∼200 ps by the open PSII RC. The situation resembles that in lichens, in which a similar d-NPQ process occurred with a t_c of 10−40 ps at 77 K.[12,13,21] Steady state fluorescence yields were suppressed to 1/3.4 and 1/5.3 at 77 K and RT, respectively, by desiccation based on the results in Figure 4. Therefore, most of the excitation energy was converted into heat in dry moss. This is evidence of the strong drought tolerance of moss even in direct sunlight. The mechanism appears to be similar to that proposed for lichens.[12,13,21] On the other hand, the decay kinetics of PSI red Chl bands at 734 and 723 nm in the two moss species were not modified much (1.3−1.7-fold) by dehydration. It is slightly different from the observation in lichens that showed appreciable acceleration of PSI decay after desiccation.[12,13,21]

We conclude that most of the excitation energy in PSII is transferred to the drought-activated quencher, which fluoresces at 740−760 nm and dissipates excitation energy into heat rapidly in both lichens and mosses.

Molecular Mechanisms of Energy Dissipation in d-NPQ. In higher plants, energy dissipation by NPQ occurs in major light-harvesting complex LHCII[25] or in the minor antenna proteins such as CP24 or CP29.[26] It has been proposed that the aggregation of LHCII proteins[25−27] or modification of energy transfer from Chl to low-lying electronic states of xanthophylls such as zeaxanthin or lutein[28,29] changes the PSII fluorescence yield in the NPQ mechanism. Other proposals suggest that the rapid charge transfer from Chl to xanthophyll,[30−32] or to another Chl,[33] is followed by energy-dissipating charge recombination reactions. The analysis in this study indicates that the d-NPQ mechanism in moss resembles that proposed in lichens[12,13,21] and is different from the NPQ or state transition mechanism. In d-NPQ, excitation energy accumulates in F684 and F695 bands rapidly as in the wet moss and then is transferred to the bands at 710 and 740−760 nm with a t_c of 47 ps, and it is dissipated into heat. We, therefore, assume that the PSII RC remains almost intact with F684 and F695 species and antenna outside the PSII core are associated with the 710 and 740−760 nm species as shown in a model in Figure 7. Although we have no firm evidence of the location of

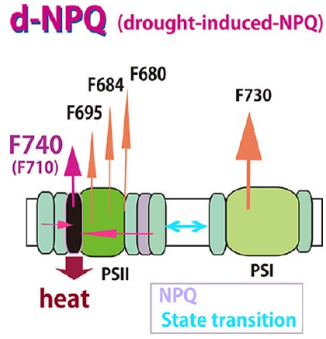

Figure 7. Scheme for energy transfer in d-NPQ in PSII of dry moss. The d-NPQ quencher (F740−760 that is probably associated with F710) is activated by desiccation, accepts excitation energy from PSII, and dissipates it into heat. See the text for details.

newly appearing quenchers, they are formed only after dehydration in limited species of moss, so that we assume they are on the LHC as shown in Figure 7. Slavov et al.[34] proposed that in addition to the d-NPQ mechanism described above spillover of excitation energy from PSII to PSI also works in a desiccated lichen by assuming $P700^+$ is the dominant quencher. However, we detected no specific fluorescence quenching effect by $P700^+$ in a desiccated moss *R. rugosum*.[19] The results in this study indicate that the extent of drought-induced acceleration of fluorescence decay almost quantitatively interprets the extent of the decrease in fluorescence at both RT and 77 K. However, it is not known at present whether the energy dissipation mechanisms other than d-NPQ might contribute to the drought tolerance of other species of lichens and mosses, collected from different environments. Further studies are required.

As for the molecular mechanism of d-NPQ, we can assume drought-induced change in Chls. Modified interaction between Chls may form the aggregated long-wavelength Chls with shorter fluorescence lifetimes. The situation, however, is different from that of F730 in PSI that shows a long lifetime of around 1100 ps at 77 K (see Figure 3). If the 740−760 nm radiation-emitting pigment represents the charge transfer state stabilized by Chl aggregation, it should be more strongly

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coupled to the ground state, as proposed for aggregated LHCII by Müller et al.,[33] to realize the fast energy dissipation rate. This idea suggests F740−760 originates from Chl *a* aggregates and F710 is the counterpart in the CT band. Another possibility is the modified interaction between Chl and carotenoids, which dissipate excitation energy from F740−760 species and quench excitation energy at the close contact, during dehydration.[29] Studies of the wider variety of mosses and lichens will contribute to the elucidation of the molecular mechanism of d-NPQ by showing the location of F740−760 species. Further studies of other species of mosses and lichens under different conditions might give a greater variety of d-NPQ mechanisms.

Drought Tolerance of Mosses and Lichens. In natural environments, we often find lichens in the higher dry locations and mosses in the lower, wetter locations. Drought tolerance is distributed in a majority of lichen species while being associated only with some moss species,[19,20] and none in the other plants, at present. It is also reported that the photobiont algae lost their drought resistance when they were cultured outside lichens[15,35] and regained it if mixed with a certain sugar molecule like arabitol during dehydration.[36] Therefore, d-NPQ in lichen seems to be activated by the interaction of photobiont algae with host fungi by unknown mechanisms. Drought tolerance is expressed constitutively in some moss species and temporally in others[19,20] and is absent from the majority of mosses. It is not yet clear whether the drought tolerance in lichens and mosses comes from single or multiple mechanisms or whether there is a similar mechanism in terrestrial plants. The discovery of d-NPQ in two moss species indicated that d-NPQ is no more unique for lichens. We will be able to study the d-NPQ mechanism in genetically and biochemically well-defined moss species, too. This type of material has been unavailable in lichens and will allow us to work on artificial manipulation and transfer of the d-NPQ ability to other plants. The study of d-NPQ in mosses may also aid in improving our understanding of the evolution mechanism of the transpiration system in plants.

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Notes

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ABBREVIATIONS

Chl, chlorophyll; d-NPQ, drought-induced nonphotochemical quenching of fluorescence; F684, F695, and F720, fluorescence bands at 684, 695, and 720 nm, respectively; LHCII, light-harvesting complex II; NPQ, nonphotochemical quenching of fluorescence; PAM, pulse amplitude-modulated fluorescence measurement; PSI and PSII, photosystems I and II, respectively; RC, reaction center; RT, room temperature.

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