Plant Physiol. (1993) 103: 1413–1420

The Xanthophyll Cycle, Protein Turnover, and the High Light Tolerance of Sun-Acclimated Leaves[1]

Barbara Demmig-Adams* and William W. Adams III

Department of Environmental, Population, and Organismic Biology, University of Colorado, Boulder, Colorado 80309–0334

Changes in photosynthesis rate and photochemical characteristics in response to high irradiance, followed by recovery at low irradiance, were determined in four groups of sun-acclimated leaves of spinach (*Spinacia oleracea* L.). These four groups were untreated control leaves, leaves treated with either an inhibitor of energy dissipation associated with the xanthophyll cycle (dithiothreitol, DTT) or an inhibitor of chloroplast-encoded protein synthesis (chloramphenicol, CAP), as well as leaves treated with a combination of DTT + CAP. In these sun leaves, treatment with either CAP or DTT alone did not result in an inhibition of the recovery from high-light-induced decreases in photochemical efficiency. Only the treatment with a combination of CAP + DTT caused a strong and irreversible depression of photochemical efficiency. We suggest that in the presence of DTT (and in the absence of xanthophyll cycle-associated energy dissipation), protein turnover may be involved in the recovery process. We further suggest that the reversible depression of photochemical efficiency in CAP-treated sun leaves reflects xanthophyll cycle-associated energy dissipation. In the leaves treated with CAP + DTT a slowly developing decrease in the maximal yield of chlorophyll fluorescence in high light may indicate an alternative, xanthophyll cycle-independent dissipation process in the photochemical system. Moreover, CAP treatments did not cause any changes in the deepoxidation state of the xanthophyll cycle. However, CAP-treated leaves, but not those treated with CAP + DTT, exhibited some decrease in the pool size of the xanthophyll cycle during the exposure to high light.

Sun leaves possess a greater tolerance to high irradiance than shade leaves (Osmond, 1981; Powles, 1984; Demmig and Björkman, 1987; Öquist et al., 1992). This is in part related to an increased capacity of sun leaves to utilize absorbed light in photosynthesis (Björkman, 1981). More recently, it has been proposed that sun leaves possess additional mechanisms of averting adverse effects of high irradiance (see Ohad et al., 1990; Krause and Weis, 1991; Melis, 1991; Demmig-Adams and Adams, 1992b). Leaves grown in full sunlight have been shown to dissipate large amounts of excitation energy that cannot be used in photosynthesis via an energy dissipation process directly within the photochemical system (Björkman and Schäfer, 1989). This is the pH-dependent energy dissipation process described originally by Briantais et al. (1979; see also Krause and Weis, 1991). This energy dissipation process has been suggested to involve the xanthophyll cycle that consists of the interconvertible xanthophylls violaxanthin, antheraxanthin, and zeaxanthin (for reviews see Demmig-Adams, 1990; Demmig-Adams and Adams, 1992b). Zeaxanthin (and antheraxanthin; Gilmore and Yamamoto, 1993) have been implicated in photoprotective dissipation of excess excitation energy directly in the Chl pigment bed (Demmig-Adams and Adams, 1992b). Sun leaves possess much larger pools of the xanthophyll cycle pigments (Demmig-Adams et al., 1989; Thayer and Björkman, 1990; Demmig-Adams and Adams, 1992a) and a greater capacity for zeaxanthin formation as well as for energy dissipation in the Chl pigment bed than shade leaves (Demmig-Adams and Adams, 1993; Adams and Demmig-Adams, 1994).

It has also been suggested that a rapid turnover of proteins (particularly the D1 protein of the PSII core) may be involved in maintaining a functional photochemical system under high irradiance (Greer et al., 1986; Ohad et al., 1990; Greer et al., 1991; Melis, 1991; Barber and Andersson, 1992). Evidence for this stems largely from studies using CAP, an inhibitor of chloroplast-encoded protein synthesis. However, in the majority of these studies leaves or algae were exposed to a PFD several times greater than the growth PFD.

The purpose of our study was to assess the relative importance of xanthophyll cycle-associated energy dissipation versus protein turnover in preventing adverse effects of full sunlight on the photochemical system of sun-acclimated leaves. To accomplish this we determined the efficiency of photosynthetic energy conversion of sun leaves during and subsequent to exposure to full sunlight in untreated leaves and leaves treated with DTT, the inhibitor of xanthophyll cycle-associated energy dissipation process, with CAP, the inhibitor of chloroplast-encoded protein synthesis, or with a combination of both DTT and CAP.

MATERIALS AND METHODS

Spinach (*Spinacia oleracea* L. cv Giant Nobel) was grown in a naturally lit greenhouse in Colorado in March 1990 and

[1] This work was supported by the United States Department of Agriculture, Competitive Research Grants Office, award number 90–37130–5422, and a Faculty Development Award from the University of Colorado to W.W.A.

* Corresponding author; fax 1–303–492–8699.

Abbreviations: CAP, chloramphenicol; F, actual Chl fluorescence emission during illumination with PAR; F_o and F_m, minimal yield (at open PSII reaction centers) and maximal yield (at closed centers) of Chl fluorescence; F_o' and F_m', minimal and maximal yield of Chl fluorescence during illumination with PAR; F_v/F_m, intrinsic efficiency of PSII photochemistry; PFD, photon flux density.

May 1992 with peak irradiance levels of 1500 μmol photons m^{-2} s^{-1}. Temperatures were approximately 19.5°C (night) and 22 to 26.5°C (day) in March and 26 to 30°C (day) in May. Daytime RH was approximately 30%.

Treatments with inhibitors followed the protocol described by Demmig-Adams et al. (1990). Leaves were excised and placed in vials containing water or solutions of DTT, CAP, or CAP + DTT and kept at a PFD of 40 μmol photons m^{-2} s^{-1} for 90 min. The CAP concentration used for all experiments shown here was 230 μmol L^{-1} CAP. The volume of solution taken up by the leaves over a 90-min period corresponded to 42 to 47% of the leaf water content resulting in final concentrations in the leaf bulk water of 97 to 108 μmol L^{-1} CAP. To establish that the presence of CAP in leaves had no direct effect on photosynthesis prior to the high-light treatments, rates of O_2 evolution were determined at 52 and 1850 μmol photons m^{-2} s^{-1} before and immediately after the 90-min exposure to 40 μmol photons m^{-2} s^{-1} (see below and Table I). Subsequent to the uptake periods, leaf discs of 10 cm^2 were transferred to an O_2 electrode where the high-light treatment (at 1850 μmol photons m^{-2} s^{-1}) and subsequent recovery (at 52 μmol photons m^{-2} s^{-1}) took place in an atmosphere of 5% CO_2 and 21% O_2 (balance N_2).

Measurements of photosynthesis (as O_2 exchange in a gas-phase leaf disc electrode; Hansatech, King's Lynn, UK) and Chl fluorescence (with an ambient temperature pulse modulation fluorometer; Walz, Effeltrich, Germany) (Schreiber et al., 1986) were performed simultaneously prior to and throughout the exposures to the high PFD followed by the low PFD (Adams et al., 1990; Demmig-Adams et al., 1990). Fluorescence measurements were made at 10 and 60 min during the exposure to high PFD and at 15, 45, and 105 min during the recovery at 52 μmol photons m^{-2} s^{-1}. Slopes of O_2 evolution were obtained at 8 to 10 and 58 to 60 min during the high-light treatment and at 13 to 17, 43 to 47, and 103 to 107 min during the recovery period.

Pigment collection and analyses were performed as outlined by Adams and Demmig-Adams (1992). Small discs (0.25 cm^2) were collected after 60 min at the high PFD, frozen immediately in liquid N_2, and analyzed by HPLC (Thayer and Björkman, 1990; Gilmore and Yamamoto, 1991; Adams and Demmig-Adams, 1992). These samples were obtained from treatments that corresponded to the treatments described above.

RESULTS

Determination of Appropriate Concentrations of CAP

We were interested in the effects of CAP that result from high-light treatments and are presumably due to the inhibition of chloroplast-encoded protein synthesis. However, it has been suggested that CAP can also directly inhibit photosynthesis at higher concentrations (Okado et al., 1991). To determine appropriate concentrations of CAP we established a concentration dependence of CAP effects on photosynthesis in leaves. At bulk leaf water concentrations of CAP of 140 μmol L^{-1} and above, an increasing direct inhibition of the rate of photosynthetic O_2 evolution was indeed observed (compared with control leaves), even in leaves that had not been exposed to high PFDs (not shown). In contrast, no direct effects on photosynthesis (at either limiting or saturating PFD for brief periods) were observed after an uptake period of 90 min in low light at CAP concentrations in the bulk leaf water below 140 μmol L^{-1}. We used CAP concentrations of 98 to 108 μmol L^{-1} in the bulk leaf water for our experiments. Table I shows that there were no consistent differences in the efficiency of overall photosynthetic energy conversion or in the photochemical efficiency of PSII between control leaves and leaves that had taken up CAP, DTT, or CAP + DTT prior to any extended treatments at high PFDs.

Effect of CAP, DTT, or a Combination of Both on the Recovery of Photosynthesis Subsequent to an Exposure to High Irradiance

A 1-h exposure of untreated sun leaves of spinach to a PFD equivalent to full sunlight resulted in a small transient decrease in the rate of photosynthesis at a low PFD, or the photon efficiency of photosynthesis, determined subsequent to the high-light treatment (Fig. 1). The photon efficiency of photosynthesis recovered almost completely over a period of 105 min at low PFD in these control leaves. Treatment of leaves with either CAP or DTT prior to the exposure to high PFD resulted in a more pronounced decrease in the rate of photosynthesis subsequent to the treatment at high PFD. There was substantial recovery of the photosynthesis rate in both cases over the same 105-min period at low PFD. In leaves treated with a combination of CAP and DTT, a greater depression of the rate of photosynthesis or the photon efficiency of photosynthesis was observed subsequent to the high-irradiance treatment and, in contrast to all other treatments, the leaves treated with both CAP and DTT did not exhibit any recovery over a period of 105 min at low PFD following the treatment at high PFD. The rates of recovery of the photon efficiency of photosynthesis are shown in Figure 2 in triplicate for the four treatments. Whereas the rates of recovery were similarly high in control leaves, leaves treated with DTT, and those treated with CAP, only the treatments with both inhibitors resulted in a complete inhibition of recovery over this time period.

Table I. *Photon efficiency of photosynthetic O_2 evolution (ϕ_i) and PSII photochemical efficiency (F_v/F_m) after a 90-min uptake period at 40 μmol photons m^{-2} s^{-1} in untreated (control) and treated (with CAP, DTT, or CAP + DTT) spinach leaves*

Values are means from three independent experiments (± SD). All measurements were made at 25°C and in an atmosphere containing 5% CO_2. ϕ_i was calculated from the rate of O_2 evolution at 52 μmol photons m^{-2} s^{-1} incident light, which is in the linear range of the O_2 evolution/PFD relationship

	ϕ_i	F_v/F_m
	mol O_2 evolved mol^{-1} photons incident	
Control	0.067 ± 0.003	0.804 ± 0.017
CAP	0.069 ± 0.007	0.786 ± 0.010
DTT	0.073 ± 0.001	0.786 ± 0.004
CAP + DTT	0.073 ± 0.009	0.776 ± 0.007

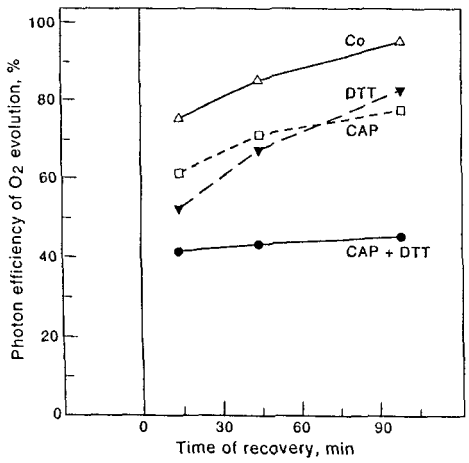

Figure 1. Recovery of the photon efficiency of photosynthetic oxygen evolution at 52 μmol photons m^{-2} s^{-1} subsequent to a 60-min treatment at 1850 μmol photons m^{-2} s^{-1} in control leaves (Co) and leaves treated with DTT, CAP, or a combination of CAP and DTT. The photon efficiency is the rate of O_2 evolution at 52 μmol photons m^{-2} s^{-1} divided by this PFD. All values are expressed as a percent of those values (Table I) prior to the exposure to the high PFD. All measurements and exposures were made at 25°C in an atmosphere containing 5% CO_2. One typical set of experiments is shown here. Similar results were obtained in two comparable sets of experiments (not shown; but see Fig. 2).

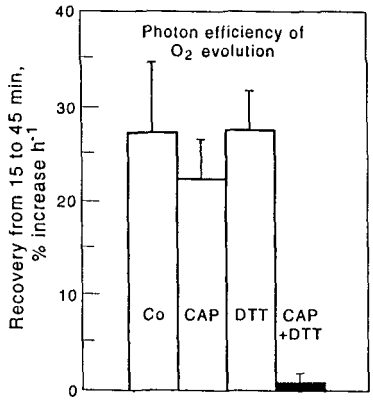

Figure 2. Rapidity of the recovery of the photon efficiency of photosynthetic oxygen evolution at 52 μmol photons m^{-2} s^{-1} subsequent to exposure to 1850 μmol photons m^{-2} s^{-1} in control leaves (Co) and leaves treated with DTT, CAP, or a combination of CAP and DTT. These values are the means (SD indicated by error bars) of three independent experiments. Recovery was calculated as change in the rate of O_2 evolution between 15 and 45 min at low PFD (see Fig. 1) expressed per h and as percent of the rate determined prior to the exposure to the high PFD. All measurements and exposures were made at 25°C in an atmosphere containing 5% CO_2.

Effect of CAP + DTT on the Photosynthesis Rate at Various PFDs Determined Subsequent to an Exposure to High Irradiance

PFD response curves of photosynthesis were determined for leaves treated with CAP + DTT and subjected to a 1-h treatment at high irradiance followed by a 105-min exposure to a low PFD. The rate of photosynthesis was much more strongly depressed at low PFDs, with an inhibition at low PFD of almost 70% compared with only approximately 20% at high PFD, immediately subsequent to the exposure to high PFD (Fig. 3). As illustrated also in Figures 1 and 2, there was no recovery of the rate of photosynthesis at the low PFD over a 105-min period, but there was recovery of the small depression of the rate of photosynthesis at high PFD (Fig. 3). Thus, there was no difference in the light-saturated rate of photosynthesis before and after the entire (high light plus 105-min recovery) treatment; in contrast, there was a pronounced difference in the light-limited rate of photosynthesis. This indicates that treatment with CAP + DTT adversely affected the ability of the leaf to convert light into photochemistry at low PFDs but did not prevent high rates of photosynthesis at high PFDs.

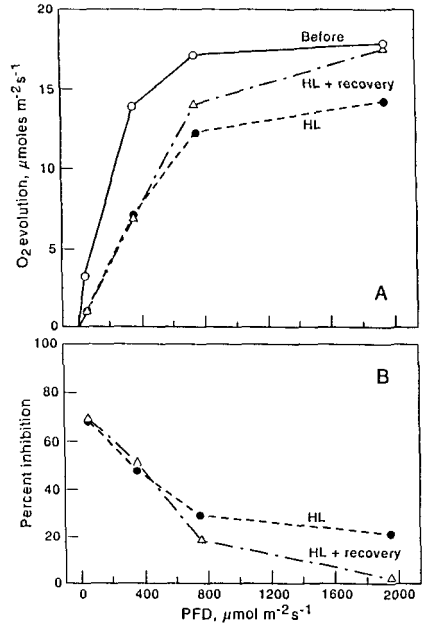

Figure 3. PFD response of photosynthetic O_2 evolution (A) in leaves treated with CAP + DTT before and immediately after 60 min at 1850 μmol photons m^{-2} s^{-1} (HL) as well as after 105 min of recovery at 52 μmol photons m^{-2} s^{-1} (HL + recovery). Response curves were established rapidly in a treatment corresponding to that in Figure 1. B, The rates of O_2 evolution at the various PFDs after HL and HL + recovery were expressed as a percent of the respective rates prior to HL (set to 100%), and the percent inhibition is shown as 100 minus each calculated percentage. All measurements and exposures were made at 25°C in an atmosphere containing 5% CO_2. One typical experiment is shown here. In other comparable sets of experiments (see Fig. 1), similar differences in the percent inhibition of O_2 evolution at 52 μmol photons m^{-2} s^{-1} versus high PFD were observed.

Effect of CAP, DTT, or a Combination of Both on the Recovery of PSII Photochemical Efficiency Subsequent to an Exposure to High Irradiance

The four different treatments had effects on the photochemical efficiency of PSII, determined as the ratio F_v/F_m (Fig. 4), that were similar to the effects on the efficiency of the overall photosynthesis process. Although all leaves showed a decrease in photochemical efficiency immediately subsequent to the high-light treatment, untreated leaves, as well as those treated with DTT or with CAP alone, all showed substantial recovery of F_v/F_m. The only treatment that was characterized by a sustained strong depression of F_v/F_m, and thus a failure to show any recovery, was that with a combination of CAP + DTT.

Figure 5 shows the corresponding changes in the yield of minimal fluorescence immediately subsequent to the high-light period and over the 105-min recovery at low PFD. In both the control leaves and those treated with CAP, a strong decrease in F_o was observed immediately subsequent to the high-light treatment. This decrease in F_o is indicative of energy dissipation in the Chl pigment bed (for reviews, see Björkman, 1987; Krause, 1988; Demmig-Adams, 1990), which thus does not seem to be affected by CAP (see also below). As expected (Bilger and Björkman, 1990; Demmig-Adams et al., 1990), DTT, the inhibitor of xanthophyll cycle-associated energy dissipation, prevented this decrease in F_o, both when given alone or in combination with CAP.

Figure 5 illustrates that the untreated leaves as well as those treated with DTT or CAP alone exhibited similar levels of F_o after the recovery period, whereas the level of F_o clearly increased much beyond that in the other treatments in the leaves treated with the combination of CAP + DTT. Such increases in F_o have been associated with some form of "damage" to PSII or perhaps a functional disconnection of PSII and its light-collecting pigment system (Demmig and Björkman, 1987; for reviews, see Krause, 1988; Demmig-Adams, 1990). The only treatment that caused strong and irreversible changes of this nature is the treatment in which both xanthophyll cycle-associated energy dissipation and protein turnover were inhibited, resulting in a strong and irreversible depression in PSII photochemical efficiency and the photon efficiency of photosynthesis.

Table II shows changes in the yield of F_m' (see van Kooten and Snel, 1990, for fluorescence nomenclature), expressed as changes in nonphotochemical fluorescence quenching ($F_m/F_m' - 1$) (Bilger and Björkman, 1990; Demmig-Adams, 1990), during and subsequent to the high-light treatment. After 10 min at the high PFD a strong decrease in F_m', or an increase in nonphotochemical quenching, was observed in the control and the CAP-treated leaves, whereas only a small increase in nonphotochemical quenching was apparent in the DTT-treated (or the DTT + CAP-treated) leaves. Such a rapid and DTT-sensitive decrease in F_m', as well as F_o' (Fig. 5), is indicative of xanthophyll cycle-associated energy dissipation (Bilger and Björkman, 1990; Demmig-Adams et al., 1990).

It should be noted that extended exposure to the high PFD

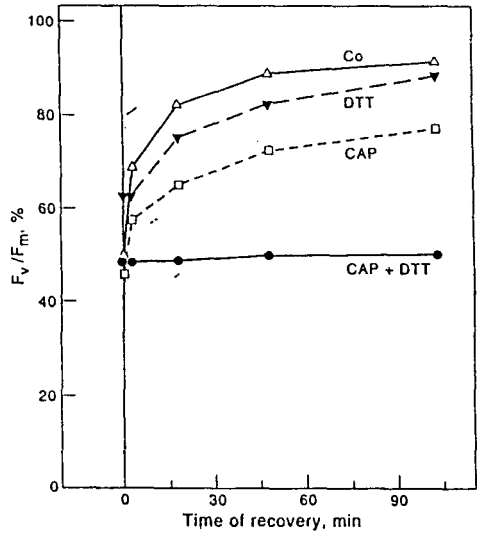

Figure 4. Recovery of PSII photochemical efficiency (determined as the fluorescence ratio F_v/F_m) at 52 μmol photons m^{-2} s^{-1} subsequent to a 60-min treatment at 1850 μmol photons m^{-2} s^{-1}. These measurements were performed on the same leaf disc and simultaneous to those described in Figure 1. The values of F_o and F_m used to calculate F_v/F_m were obtained after 3 min under far-red illumination at the time points indicated. All exposures were at 25°C in an atmosphere containing 5% CO_2. We obtained a value of 100% from each leaf prior to exposure to high light. One typical set of experiments is shown here. Similar results were obtained in two comparable sets (not shown).

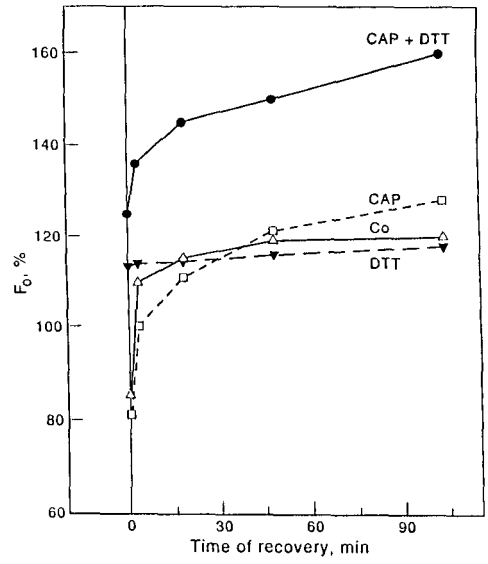

Figure 5. Recovery of F_o at 52 μmol photons m^{-2} s^{-1} subsequent to a 60-min treatment at 1850 μmol photons m^{-2} s^{-1}. F_o was determined as the lowest yield of Chl fluorescence in the absence of PAR but with far-red illumination. These measurements were performed on the same leaf disc and simultaneous to those described in Figures 1 and 4. All exposures were at 25°C in an atmosphere containing 5% CO_2. We obtained a value of 100% from each leaf prior to exposure to high light. One typical set of experiments is shown here. Similar results were obtained in two comparable sets (not shown).

Table II. *Nonphotochemical fluorescence quenching and PSII reduction state in untreated (control) and treated (with CAP, DTT, or CAP + DTT) spinach leaves at various times during a 60-min exposure to 1850 mol photons m^{-2} s^{-1} (HL), followed by a 105-min exposure to 52 mol photons m^{-2} s^{-1} (LL)*

All measurements were made at 25°C and in an atmosphere containing 5% CO_2. Nonphotochemical fluorescence quenching is expressed as $F_m/F_m' - 1$. The PSII reduction state is estimated from Chl fluorescence as $(F - F_o')/(F_m' - F_o')$, where F_o' is the minimal fluorescence yield (at open PSII reaction centers) and F is the actual fluorescence emission during illumination with PAR

	$F_m/F_m' - 1$			PSII Reduction State	
	10′ HL	60′ HL	105′ LL	10′ HL	60′ HL
Control	1.96	2.09	0.01	0.46	0.40
CAP	2.12	2.34	0.25	0.39	0.36
DTT	0.44	0.80	0.00	0.75	0.52
CAP + DTT	0.43	1.20	0.65	0.74	0.49

led to a slow, further decrease in F_m', between 10 and 60 min at the high PFD, and a corresponding increase in the parameter $F_m/F_m' - 1$ in the leaves treated with CAP, DTT, or with CAP + DTT, but not in the controls (Table II). Subsequent to the 105-min recovery period at low PFD, F_m' had fully recovered in both control and DTT-treated leaves. However, there was a residual depression of F_m' in the leaves treated with CAP and particularly those treated with CAP + DTT. The fact that there was some difference between the control leaves and the CAP-treated leaves with respect to sustained depressions in F_m' suggests that chloroplast protein synthesis prevents such effects in control leaves under our treatment conditions. We cannot conclude, however, that this must also occur under natural conditions at peak irradiance, since the treatment PFD (1850 μmol photons m^{-2} s^{-1}) exceeded the peak growth PFD (1500 μmol photons m^{-2} s^{-1}) somewhat and since CAP may also exacerbate oxidative damage (see Okado et al., 1991).

Table II also shows the reduction state of PSII after 10 and 60 min at the high PFD. After 10 min, at which time xanthophyll cycle-associated energy dissipation was presumably fully developed in the control and CAP-treated leaves, these two treatments exhibited reduction states of about 40%, which remained at these levels throughout the 60-min treatment at high PFD. In contrast, the treatments with DTT or DTT + CAP, which prevent xanthophyll cycle-associated energy dissipation, resulted in much higher reduction states of about 75% initially (after 10 min). However, these reduction states decreased to levels of about 50% after 60 min of exposure to the high PFD. Such a slow, further decrease in the PSII reduction state, as well as the slow decrease in F_m', would be consistent with the development of an additional, xanthophyll cycle-independent process that dissipates excess energy in PSII.

Effect of CAP, DTT, or a Combination of Both on the Carotenoid Composition of Sun Leaves under High Irradiance

Table III shows that the 90-min uptake period at low PFD had a negligible effect on the levels of zeaxanthin and an-

Table III. *Composition of the xanthophyll cycle in spinach leaves prior to (after LL) and following exposure to (HL) 1850 μmol photons m^{-2} s^{-1} at 25°C for 60 min in an atmosphere containing 5% CO_2*

Leaves were either untreated (control) or treated with CAP, DTT, or CAP + DTT. For the CAP treatments, values are given in LL before and after the 90-min uptake period. Each value represents a mean of two separate samples taken from the same leaf. V, Violaxanthin; A, antheraxanthin; Z, zeaxanthin

	Z	A	V	Z/(V + A + Z)	(A + Z)/(V + A + Z)
	mmol mol^{-1} Chl a + b				
Control					
After LL	1.5	4.4	88.2	0.016	0.063
HL	61.1	11.1	16.9	0.686	0.810
CAP					
Before LL	2.3	7.6	95.1	0.021	0.094
After LL	1.3	5.5	99.2	0.012	0.064
HL	58.6	6.0	15.9	0.728	0.803
DTT					
After LL	1.2	1.5	92.3	0.013	0.028
HL	2.4	4.6	85.1	0.026	0.076
CAP + DTT					
Before LL	3.6	7.4	100	0.032	0.099
After LL	2.1	1.9	98.0	0.021	0.039
HL	2.1	5.4	94.5	0.021	0.074

theraxanthin in the case of CAP treatments and caused some decrease in the levels of these two xanthophylls in the case of treatments with DTT + CAP. As had also been reported previously (Bilger and Björkman, 1990; Demmig-Adams et al., 1990), DTT inhibited the high-light-induced formation of zeaxanthin (Table III). In contrast to the effect of DTT in isolated chloroplasts (Gilmore and Yamamoto, 1992), antheraxanthin formation was also largely inhibited to the extent that the levels of zeaxanthin + antheraxanthin were similarly low after 60 min at the high PFD in leaves treated with DTT or DTT + CAP as those in control or CAP-treated leaves prior to the high-light exposure. There was neither inhibition of violaxanthin deepoxidation nor an increase in zeaxanthin + antheraxanthin content in CAP-treated leaves after 60 min at high PFD compared with the high levels found in the control leaves exposed to high PFD.

Table IV shows the levels of all carotenoids and Chls *a* and *b* before and after the 1-h treatment at the high PFD. We did not identify any consistent differences in the changes of the levels of lutein, neoxanthin, β-carotene, or Chl *a* and *b* among the various treatments subjected to high-light exposure. In contrast, a high-light-induced decrease of 24% in the size of the xanthophyll cycle pool was observed in the CAP-treated leaves compared with only small changes in this parameter in all other treatments, including that with CAP + DTT.

DISCUSSION

There are numerous reports that CAP or other inhibitors of chloroplast-encoded protein synthesis have a strong inhibitory effect on the recovery from "photoinhibition," i.e. high-light-induced depressions in photochemical efficiency (for reviews, see Krause, 1988; Ohad et al., 1990; Demmig-Adams and Adams, 1992). There is some difference of opinion as to whether these results reflect the importance of continuous protein turnover or an actual damage/repair cycle for the maintenance of a functional photochemical system. In the majority of these studies leaves or algae were exposed to PFDs several times greater than their growth PFD. We were interested in determining the effect of CAP on sun leaves during an exposure to PFDs equivalent to full sunlight, i.e. a level of light stress to which they are acclimated.

Our results with sun leaves differ from the previous reports in that CAP alone does not cause irreversible depressions in photochemical efficiency over a 60-min exposure to high irradiance. However, subsequent to the same exposure, a much greater level of depression and a complete lack of recovery was observed when these leaves were treated with a combination of CAP + DTT. DTT inhibited deepoxidation of the xanthophyll cycle as well as the xanthophyll cycle-associated energy dissipation process in the pigment bed. In addition, treatment with DTT alone did not result in irreversible depressions of photochemical efficiency in these sun leaves. Therefore, our results indicate that inhibition of either CAP- or DTT-sensitive processes alone does not prevent a relatively rapid return to a high photochemical efficiency in sun leaves subsequent to an exposure to high irradiance.

In other words, these sun leaves did indeed show a response to CAP similar to that of shade leaves (Greer et al., 1986), provided that DTT-sensitive processes (e.g. xanthophyll cycle-associated energy dissipation) were not allowed to occur. We speculate that the difference in the size of the

Table IV. *Pigment content of untreated (control) and treated (with CAP, DTT, or CAP + DTT) spinach leaves prior to (after LL) and after (HL) a 60-min exposure to 1850 μmol photons m^{-2} s^{-1} at 25°C in an atmosphere containing 5% CO_2*

For the treatments with CAP and CAP + DTT, values are given in LL before and after the 90-min uptake period. Each value represents a mean of two separate samples taken from the same leaf. The percent change was calculated from the change between prior to (after LL) and after (HL) the high-light exposure in each case. V, Violaxanthin; A, antheraxanthin; Z, zeaxanthin; β-C, β-carotene; L, lutein; N, neoxanthin; ΣCar, the sum of all carotenoids

	V + A + Z	β-C	L	N	ΣCar	Chl *a* + *b*	Chl *a*/*b*
	mmol mol^{-1} Chl a + b					*μmol m^{-2}*	
Control							
After LL	94.1	78.3	139	39.4	350	318	2.37
HL	89.1	76.2	138	40.4	344	278	2.27
Percent change	−5.3	−2.7	−0.7	+3.0	−1.7	−12.6	−4.2
CAP							
Before LL	105	79.9	136	38.2	359	244	2.39
After LL	106	84.1	135	36.8	363	260	2.42
HL	80.5	75.0	130	37.6	323	228	2.34
Percent change	−24.1	−10.8	−3.7	+2.0	−11.0	−12.3	−3.3
DTT							
After LL	95.0	78.0	134	38.4	345	279	2.36
HL	92.1	75.4	128	38.8	334	248	2.30
Percent change	−3.0	−3.3	−4.5	+1.0	−3.2	−11.1	−2.5
CAP + DTT							
Before LL	111	80.8	137	41.7	371	240	2.29
After LL	102	80.6	131	38.3	352	255	2.25
HL	102	73.4	126	39.1	341	239	2.24
Percent change	0	−8.9	−3.8	+2.1	−3.1	−6.3	−0.4

xanthophyll cycle pool between sun and shade leaves is an important factor for their difference in the response to CAP. We also conclude that a greater capacity for xanthophyll cycle-associated energy dissipation is likely to be an important factor for the greater tolerance of sun leaves to high irradiance. A similar conclusion was drawn by Öquist et al. (1992). It is interesting to note that the fact that the sun leaves had high rates of photosynthesis did not prevent the strong irreversible depressions in photochemical efficiency in the presence of a combination of CAP + DTT.

The responses described in this study also indicate that depressions of the photon efficiency of photosynthesis can have different underlying mechanisms. We speculate that the reversible decrease in photochemical efficiency in CAP-treated leaves is related to xanthophyll cycle-associated energy dissipation and that the recovery in DTT-treated leaves may be associated with protein turnover. If the recovery in DTT-treated leaves is indeed due to protein turnover, one would have to conclude that this protein turnover is very rapid, considerably more rapid than in shade leaves of bean, for example (Greer et al., 1986). This conclusion has also been drawn by Öquist et al. (1992) from a comparison of leaves grown at different PFDs.

Interestingly, the rather drastic changes in the efficiency of converting photons into photochemistry in leaves treated with both CAP + DTT had only a very minor or no effect on the high rates of photosynthesis at saturating PFDs (Fig. 3). This could be the result of a process that (also) dissipates a certain percentage of excitation energy at the level of the photochemical reactions. A xanthophyll cycle-independent energy dissipation process apparently develops during high PFD and persists upon return to low PFD in leaves treated with CAP + DTT. This conclusion is based on the slow decreases in F_m' and changes in the reduction state in leaves treated with CAP + DTT (Table II). This may be the same phenomenon that has been addressed as "energy dissipation by photoinhibited PSII reaction centers" (see Cleland et al., 1986; Somersalo and Krause, 1990; Krause and Weis, 1991; Ottander and Öquist, 1991), and may occur not only when xanthophyll cycle-associated energy dissipation is inhibited but perhaps also when the capacity of this latter process is exceeded. Such a modification of PSII may also involve changes that are responsible for the sustained large increase in F_o in CAP + DTT-treated leaves (Fig. 5; see also Osmond et al., 1993). In conclusion, our results may suggest that in the absence of xanthophyll cycle-associated energy dissipation an alternative process develops that is characterized by slowly developing (xanthophyll cycle-independent) energy dissipation and, possibly, a functional dissociation between PSII reaction centers and antennae (as indicated by the increase in F_o) that is irreversible in the presence of CAP, and thus presumably the absence of protein synthesis. This modification strongly depresses the photon efficiency of photosynthesis but does not interfere with a high photosynthetic activity under saturating PFDs.

Finally, the effect of CAP, but not CAP + DTT, in causing a decrease in the pool size of the xanthophyll cycle may be interpreted in several ways. It is possible that a chloroplast-encoded protein with an extremely rapid turnover is involved in maintaining a large xanthophyll cycle pool under high irradiance when large amounts of zeaxanthin and antheraxanthin are formed and become involved in energy dissipation. One may assume that zeaxanthin and antheraxanthin become degraded in high light since they are involved in energy dissipation and are continuously replaced. This would explain the observation of a decrease in the xanthophyll cycle pool in CAP-treated leaves when zeaxanthin and antheraxanthin are allowed to be formed but not when their formation is inhibited by DTT. Alternatively, the presence of CAP could give rise to active oxygen species, as suggested by Okado et al. (1991), that may also lead to a degradation of xanthophylls. Such oxygen species might possibly become reduced and inactivated by DTT. Irrespective of the underlying cause, any effects of CAP on the xanthophyll cycle pool must be considered in longer-term treatments with CAP.

Received April 27, 1993; accepted August 26, 1993.

LITERATURE CITED

Adams WW III, Demmig-Adams B (1992) Operation of the xanthophyll cycle in higher plants in response to diurnal changes in incident sunlight. Planta **186:** 390–398

Adams WW III, Demmig-Adams B (1994) Energy dissipation and the xanthophyll cycle in CAM plants. *In* K Winter, AP Smith, JAC Smith, eds, Crassulacean Acid Metabolism. Biochemistry, Ecophysiology, and Evolution. Springer, Berlin (in press)

Adams WW III, Demmig-Adams B, Winter K, Schreiber U (1990) The ratio of variable to maximum chlorophyll fluorescence from photosystem II, measured in leaves at ambient temperature and at 77K, as an indicator of the photon yield of photosynthesis. Planta **180:** 166–174

Barber J, Andersson B (1992) Too much of a good thing: light can be bad for photosynthesis. Trends Biochem Sci **17:** 61–66

Bilger W, Björkman O (1990) Role of the xanthophyll cycle in photoprotection elucidated by measurements of light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynth Res **25:** 173–185

Björkman O (1981) Responses to different quantum flux densities. *In* OL Lange, PS Nobel, CB Osmond, H Ziegler, eds, Physiological Plant Ecology I: Encyclopedia of Plant Physiology. New Series, Vol 12A. Springer, Berlin, pp 57–107

Björkman O (1987) Low-temperature chlorophyll fluorescence in leaves and its relationship to photon yield of photosynthesis in photoinhibition. *In* DJ Kyle, CB Osmond, CJ Arntzen, eds, Photoinhibition. Elsevier, Amsterdam, pp 123–144

Björkman O, Schäfer C (1989) A gas exchange-fluorescence analysis of photosynthetic performance of a cotton crop under high-irradiance stress (extended abstract). Phil Trans R Soc Lond B **323:** 309–311

Briantais J-M, Vernotte C, Picaud M, Krause GH, Weis E (1979) A quantitative study of the slow decline of chlorophyll a fluorescence in isolated chloroplasts. Biochim Biophys Acta **548:** 128–138

Cleland RE, Melis A, Neale PJ (1986) Mechanism of photoinhibition: photochemical reaction center inactivation in system II of chloroplasts. Photosynth Res **9:** 79–88

Demmig B, Björkman O (1987) Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O_2 evolution in leaves of higher plants. Planta **171:** 171–184

Demmig-Adams B (1990) Carotenoids and photoprotection in plants. A role for the xanthophyll zeaxanthin. Biochim Biophys Acta **1020:** 1–24

Demmig-Adams B, Adams WW III (1992a) Carotenoid composition in sun and shade leaves of plants with different life forms. Plant Cell Environ **15:** 411–419

Demmig-Adams B, Adams WW III (1992b) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol **43:** 599–626

Demmig-Adams B, Adams WW III (1993) The xanthophyll cycle. *In* RG Alscher, JL Hess, eds, Antioxidants in Higher Plants. CRC Press, Boca Raton, FL, pp 91–110

Demmig-Adams B, Adams WW III, Heber U, Neimanis S, Winter K, Krüger A, Czygan F-C, Bilger W, Björkman O (1990) Inhibition of zeaxanthin formation and of rapid changes in radiationless energy dissipation by dithiothreitol in spinach leaves and chloroplasts. Plant Physiol **92:** 293–301

Demmig-Adams B, Winter K, Winkelmann E, Krüger A, Czygan F-C (1989) Photosynthetic characteristics and the ratios of chlorophyll, β-carotene, and the components of the xanthophyll cycle upon a sudden increase in growth light regime in several plant species. Bot Acta **102:** 319–325

Gilmore AM, Yamamoto HY (1991) Resolution of lutein and zeaxanthin using a non-endcapped, lightly carbon-loaded C-18 high-performance liquid chromatographic column. J Chromatogr **543:** 137–145

Gilmore AM, Yamamoto HY (1993) Linear models relating xanthophylls and lumen acidity to non-photochemical fluorescence quenching. Evidence that antheraxanthin explains zeaxanthin-independent quenching. Photosynth Res **35:** 67–78

Greer DH, Berry JA, Björkman O (1986) Photoinhibition of photosynthesis in intact bean leaves: role of light, temperature and requirement for chloroplast-protein synthesis during recovery. Planta **168:** 253–260

Greer DH, Ottander C, Öquist G (1991) Photoinhibition and recovery of photosynthesis in intact barley leaves at 5 and 20°C. Physiol Plant **81:** 203–210

Krause GH (1988) Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. Physiol Plant **74:** 566–574

Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol **42:** 313–349

Melis A (1991) Dynamics of photosynthetic membrane composition and function. Biochim Biophys Acta **1058:** 87–106

Ohad I, Adir N, Hiroyuki K, Kyle D, Inoue Y (1990) Mechanism of photoinhibition in vivo. A reversible light-induced conformational change of reaction center II is related to an irreversible modification of the D1 protein. J Biol Chem **265:** 972–979

Okado K, Satoh K, Katoh S (1991) Chloramphenicol is an inhibitor of photosynthesis. FEBS Lett **295:** 155–158

Öquist G, Anderson JM, McCaffery S, Chow WS (1992) Mechanistic differences in photoinhibition of sun and shade plants. Planta **188:** 422–431

Osmond CB (1981) Photorespiration and photoinhibition. Some implications for the energetics of photosynthesis. Biochim Biophys Acta **639:** 77–98

Osmond CB, Ramus J, Levavasseur G, Franklin LA, Henley WJ (1993) Fluorescence quenching during photosynthesis and photoinhibition of *Ulva rotundata* Blid. Planta **190:** 91–106

Ottander C, Öquist G (1991) Recovery of photosynthesis in winter-stressed Scots pine. Plant Cell Environ **14:** 345–349

Powles S (1984) Photoinhibition of photosynthesis induced by visible light. Annu Rev Plant Physiol **35:** 15–44

Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res **10:** 51–62

Somersalo S, Krause GH (1990) Reversible photoinhibition of unhardened and cold-acclimated spinach leaves at chilling temperatures. Planta **180:** 181–187

Thayer SS, Björkman O (1990) Leaf xanthophyll content and composition in sun and shade determined by HPLC. Photosynth Res **23:** 331–343

van Kooten O, Snel JFH (1990) The use of fluorescence nomenclature in plant stress physiology. Photosynth Res **25:** 147–150