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Factors Associated with Depression of Photosynthetic Quantum Efficiency in Maize at Low Growth Temperature[1]

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The photosynthetic productivity of maize (*Zea mays*) in temperate regions is often limited by low temperatures. The factors responsible for the sensitivity of photosynthesis in maize to growth at suboptimal temperature were investigated by measuring (a) the quantum yields of CO_2 fixation and photosystem II (PSII) photochemistry, (b) the pigments of the xanthophyll cycle, (c) the concentrations of active and inactive PSII reaction centers, and (d) the synthesis of core components of PSII reaction centers. Measurements were made on fully expanded leaves grown at 14°C, both before and during the first 48 h after transfer of these plants to 25°C. Our findings indicate that zeaxanthin-related quenching of absorbed excitation energy at PSII is, quantitatively, the most important factor determining the depressed photosynthetic efficiency in 14°C-grown plants. Despite the photoprotection afforded by zeaxanthin-related quenching of absorbed excitation energy, a significant and more persistent depression of photosynthetic efficiency appears to result from low temperature-induced inhibition of the rate at which damaged PSII centers can be replaced.

In temperate regions, low temperatures during the early part of the growing season can severely restrict the photosynthetic performance and canopy development of maize (*Zea mays*) crops (Miedema, 1982; Baker and Nie, 1994). Depression of photosynthetic performance results from decreases in both photosynthetic capacity and the maximum quantum efficiency of CO_2 assimilation, ϕ_{CO_2} (Stirling et al., 1991; Nie et al., 1992).

There is substantial evidence to suggest that a large proportion of the decrease in ϕ_{CO_2} observed in leaves of plants grown at >22°C and subsequently exposed to low temperatures arises from temperature-related thermodynamic constraints on carbon metabolism. This inevitably reduces the maximum flux of electrons through the photosynthetic electron-transport chain, decreasing the rate at which absorbed excitation energy can be dissipated by PSII photochemistry (Ortiz-Lopez et al., 1990; Baker and Nie, 1994). One important consequence of this decreased capacity for electron transport is that the potential for photodamage to PSII reaction centers is greatly increased (Baker, 1994). Although carbon metabolism within 14°C-grown maize plants is subject to the same temperature-related thermodynamic constraints, depression of photosynthetic performance in these plants has also been linked to poor development of the photosynthetic apparatus (Nie et al., 1992), which results from a decreased accumulation of specific proteins within thylakoid membranes (Nie and Baker, 1991; Stirling et al., 1991).

It is becoming increasingly apparent that when the amount of light absorbed by PSII is in excess of that which can be utilized in photosynthesis, one or more photoprotective mechanisms can operate to minimize photodamage. For example, an increased proportion of absorbed excitation energy may be dissipated within the PSII antennas through nonradiative decay (lost as heat), which is associated with the conversion of violaxanthin to zeaxanthin (Demmig-Adams and Adams, 1992, 1994). This increased rate of de-excitation by nonphotochemical processes decreases the quantum efficiency of linear electron transport and ϕ_{CO_2} (Genty et al., 1989). If photodamage to the reaction centers does occur, as has been shown to be the case in maize leaves exposed to a combination of low temperature and high PPFD (Ortiz-Lopez et al., 1990), then this will contribute an additional, and more persistent, decrease in ϕ_{CO_2}.

It has been observed previously that ϕ_{CO_2} of mature maize leaves grown at 14°C increases rapidly (over several hours) following the transfer of plants to 25°C (Nie, 1991). Potentially, this response may provide important clues for

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Abbreviations: CP, core protein; ΔA_{518}, flash-induced absorption change at 518 nm; ΔA_{FL1} (ΔA_{FL2}), phase of ΔA_{518} induced by the first flash (second flash); F_m and F_m', maximum fluorescence yields in dark-adapted and light-adapted states; F_o and F_o', minimum fluorescence yields in dark-adapted and light-adapted states; F_s, steady-state fluorescence yield; F_v and F_v', variable fluorescence yields in dark-adapted and light-adapted states; LHCII, light-harvesting complex associated with PSII; ϕ_{CO_2}, maximum quantum yield of CO_2 assimilation; ϕ_{PSII}, quantum yield of PSII photochemistry.

determining the most important factors involved in the depression of ϕ_{CO_2} within maize leaves that have developed at low temperatures.

The central focus of this study was to determine the extents to which nonradiative decay processes and the concentration of functional PSII reaction centers might be important in determining the quantum efficiency of PSII (ϕ_{PSII}) within the leaves of maize plants that have developed at 14°C. Our data suggest that a relatively large proportion of absorbed excitation energy is dissipated through nonradiative decay processes within light-adapted, fully expanded leaves of 14°C-grown plants and that the concentration of functional PSII centers within these leaves is substantially lower than within plants grown at >22°C. Following transfer of these plants to 25°C, the proportion of excitation energy dissipated as heat decreased substantially within the first 6 h, whereas the concentration of functional PSII centers increased between 12 and 48 h after transfer.

MATERIAL AND METHODS

Plant Material and Growth Conditions

Maize plants (*Zea mays* cv LG11, a cultivar developed for Northern Europe) were planted in a mixture (50:50, v/v) of perlite and potting compost (Levington No. 1; Fisons, Ipswich, UK) and watered daily with full-strength Hoagland medium. Seedlings were grown in a controlled-environment cabinet (Fitotron F600H, Fisons) on a 14/12°C day/night cycle (16-h day at a PPFD of 250 μmol m^{-2} s^{-1}). Once the second leaf had reached full expansion (between 35 and 40 d from sowing), the plants were transferred to a similar controlled-environment cabinet maintained at 25°C for the 16-h photoperiod (also at a PPFD of 250 μmol m^{-2} s^{-1}) and 22°C at night. A leaf-air water vapor pressure difference of 0.5 to 0.7 kPa was maintained within both cabinets.

Photosynthetic Activities

Simultaneous measurements of CO_2 assimilation and modulated Chl fluorescence yield were made using equipment and techniques described previously (Genty et al., 1989). Steady-state measurements were made at a PPFD of 250 μmol m^{-2} s^{-1}. Values of ϕ_{CO_2} were corrected for both leaf absorption (using a Taylor integrating sphere) and dark respiration. The quantum efficiency of PSII photochemistry at steady state (ϕ_{PSII}) was estimated from $(F_m' - F_s)/F_m'$ (Genty et al., 1989). The maximum quantum efficiency of PSII (when the primary electron accepting plastoquinone of PSII is fully oxidized) was estimated from the F_v/F_m values from dark-adapted leaves and the F_v'/F_m' values from light-adapted leaves, where $F_v = F_m - F_o$ and $F_v' = F_m' - F_o'$. F_m and F_m' were both taken as the maximum fluorescence yield attained during a 2-s saturating pulse (PPFD > 10,000 μmol m^{-2} s^{-1}), and F_o and F_o' were measured under weak, continuous illumination, as described previously (Genty et al., 1989).

Carotenoid Analyses

Leaf discs (1 cm diameter) were ground with a mortar and pestle in ice-cold ethanol (1 mL) for 30 s and then centrifuged at 15,000*g* for 4 min in a microfuge. The supernatants were collected and stored under a nitrogen atmosphere in the dark at −4°C. All procedures were carried out in dim light. Chromatography of the leaf pigments was performed using a modification of the method described by Gilmore and Yamamoto (1991). A Beckman Gold System equipped with a 5-μm particle diameter, 25-cm Spherisorb ODS-2 column (i.d. 4.6 mm) was used to separate pigments using a flow rate of 2 mL min^{-1} and injection volume of 20 μL. The mobile phase involved a two-solvent isocratic step. The first solvent consisted of acetonitrile: methanol:0.1 M Tris HCl buffer at pH 8.0 (72:8:2, v/v) and was run isocratically for 6 min. This was followed by a 10-min linear gradient to 100% of a second solvent consisting of methanol:ethyl acetate (68:32, v/v). Pigments were detected by their A_{440}. The concentrations of the separated pigments from leaf samples were determined from the integrated area under pigment peaks. Calibration of peak areas was from chromatography of known amounts of the individual pigments previously isolated, whose concentrations had been determined spectroscopically using previously reported extinction coefficients (Davies, 1976).

Flash-Induced Absorption Change at 518 nm

Attached, fully expanded second leaves were enclosed within a custom-built chamber. A laboratory-built, kinetic spectrophotometer, similar to that described elsewhere (Wise and Ort, 1989), was used to measure ΔA_{518} induced by saturating, single-turnover flashes. Red, actinic flashes were provided by a Xenon flash lamp (FX-200; EG&G, Salem, MA), filtered by a Melles-Griot (Irvine, CA) heat-reflecting mirror (03 MHG 007) and a red glass filter (CS 2–58; Corning, Inc., Corning, NY). The measuring light and actinic flashes were delivered to the adaxial surface of the leaf through a bifurcated light guide. Inactive PSII centers were quantified by applying two saturating, single-turnover flashes spaced 50 ms apart.

The fast (<1 μs) phase of ΔA_{518} induced by the first flash (ΔA_{FL1}) reflects charge separation at all reaction centers and is directly proportional to the number of centers that undergo charge separation (Chylla and Whitmarsh, 1989). During the 50-ms interval before the second flash, the primary, stable electron acceptors of active PSII centers and PSI centers are re-oxidized. Since inactive PSII centers are re-oxidized with a half-time of 1.5 to 2 s (Chylla and Whitmarsh, 1989), most of these centers are "closed" at the time of the second flash. Consequently, the amplitude of the fast phase of ΔA_{518} induced by the second flash (ΔA_{FL2}) represents charge separation only at active PSII centers plus PSI centers. The difference between the amplitudes of ΔA_{FL1} and ΔA_{FL2} can therefore be attributed to charge separation at inactive PSII centers and the proportion of PSII centers that are inactive can be calculated as:

$$(\Delta A_{FL1} - \Delta A_{FL2})/\Delta A_{FL1}$$

Labeling of Proteins in Leaves

Incorporation of [^{35}S]Met into leaf proteins was performed using a previously described modification (Nie and Baker, 1991) of the method of Cooper and Ort (1988). The upper surfaces of leaves attached to the plant in the growth cabinet were abraded with carborundum paper, and aliquots of [^{35}S]Met (400 μCi mL^{-1}, specific activity >800 Ci mmol^{-1}) in 0.4% (v/v) Tween 20 were evenly applied using a cotton swab. Treated leaves were kept in the ambient growth conditions for 3 h and then rinsed in ice-cold, nonradioactive 1 mM Met before isolation of the thylakoids.

Isolation of Thylakoids and Analyses of Polypeptides

Thylakoids were isolated from mesophyll cells essentially as described previously (Hayden and Hopkins, 1976) using an isolation and resuspension medium containing 330 mm sorbitol, 10 mm NaCl, 5 mM $MgCl_2$, and 50 mM Tricine at pH 8.0; the wash medium was similar except that sorbitol was omitted.

Solubilization and electrophoretic separation of thylakoid polypeptides were performed essentially as described previously (Nie and Baker, 1991). Thylakoid proteins were solubilized in 4% (w/v) SDS, 60 mM DTT, and 8% (w/v) Suc using an SDS:Chl ratio of 32:1 (w/w). A resolving gradient gel of 10 to 18% (w/v) acrylamide and 4 to 8% (w/v) Suc was used for the electrophoretic separation. Staining of the gels with Coomassie brilliant blue and fluorography to detect [^{35}S]Met were performed as described previously (Nie and Baker, 1991).

Capacity of Thylakoids to Bind Atrazine

The binding of ^{14}C[atrazine] to isolated thylakoid membranes was used to determine the concentration of plastoquinone binding sites, i.e. the concentration of PSII complexes potentially capable of plastoquinone reduction, according to the principles first described by Tischer and Strottman (1977). The experimental procedures for assay of atrazine-binding capacity were essentially similar to those described previously (Bradbury and Baker, 1986), except that thylakoid preparations were diluted to 45 nM Chl in 1 mM Na_2EDTA, 10 mM $MgCl_2$, 150 mM NaCl, and 40 mM Hepes (pH 7.6) and then incubated at 25°C in the dark for 10 min with a concentration range (20–300 mM) of [*ethyl*-1-^{14}C]atrazine having a specific activity of 25 Ci mol^{-1}.

RESULTS

Changes in ϕ_{CO_2} and ϕ_{PSII} following the Transfer of 14°C-Grown Plants to 25°C

The ϕ_{CO_2} within fully expanded second leaves of 14°C-grown maize plants was depressed by nearly 75%, when compared to plants grown at 25°C (0.015 compared to 0.057). Following transfer of 14°C-grown plants to 25°C, ϕ_{CO_2} recovered to 0.045 within 48 h, with approximately 70% of this recovery occurring within the first 6 h (Fig. 1).

Transfer of 14°C-grown plants to 25°C also induced changes in the maximum ϕ_{PSII} (F_v/F_m). These changes were complex, consisting of at least three distinct phases (Fig. 2). During the first 14 min, a rapid increase in F_v/F_m was observed (Fig. 2, inset). This was followed by a slower increase in F_v/F_m, which was interrupted by a significant decrease between 4 and 6 h. The pattern of these changes in F_v/F_m was matched by somewhat larger changes in ϕ_{PSII} and F_v'/F_m' (also shown in Fig. 2), measured from leaves adapted to a PPFD of 250 μmol m^{-2} s^{-1}. Since the fluorescence parameter ϕ_{PSII} is the product of F_v'/F_m' and the proportion of "open" PSII centers (Genty et al., 1989), the similar values for ϕ_{PSII} and F_v'/F_m' reflect the maintenance of a highly oxidized primary electron accepting plastoquinone of PSII pool at ambient PPFD, which was demonstrated by the measured values of the photochemical quenching coefficient of above 0.9 (data not shown).

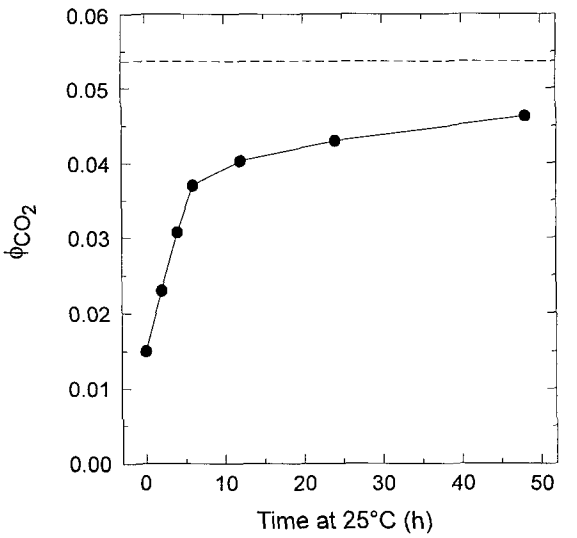

Figure 1. Time course for the recovery in ϕ_{CO_2} of 14°C-grown maize leaves on transfer to 25°C. Measurements were made from leaves at steady-state photosynthesis at the ambient growth PPFD of 250 μmol m^{-2} s^{-1}. The dashed line indicates ϕ_{CO_2} of leaves grown at 25°C. SEs were calculated for a minimum of five replicates and in all cases were less than 10% of the mean.

The data presented in Figure 3 show the relationship between ϕ_{CO_2} (from Fig. 1) and ϕ_{PSII} (from Fig. 2) immediately before and at various times within the first 48 h after transfer of 14°C-grown plants to 25°C. If a constant proportion of the electrons derived from charge separation at PSII were used for CO_2 fixation, a linear relationship would exist between these two parameters, as has been shown to be the case for maize leaves over a range of physiological and environmental conditions (Edwards and Baker, 1993). Extrapolating between any two of the points between 0 and 6 h produces an intercept along the ϕ_{PSII} axis, indicating that an increasing proportion of the electron equivalents derived from charge separation at PSII are being used for CO_2 fixation. Conversely, extrapolating through the 6 and 48 h points produces an intercept along the ϕ_{CO_2} axis, indicating that the proportion of electrons derived from charge separation going to CO_2 is decreasing during this period.

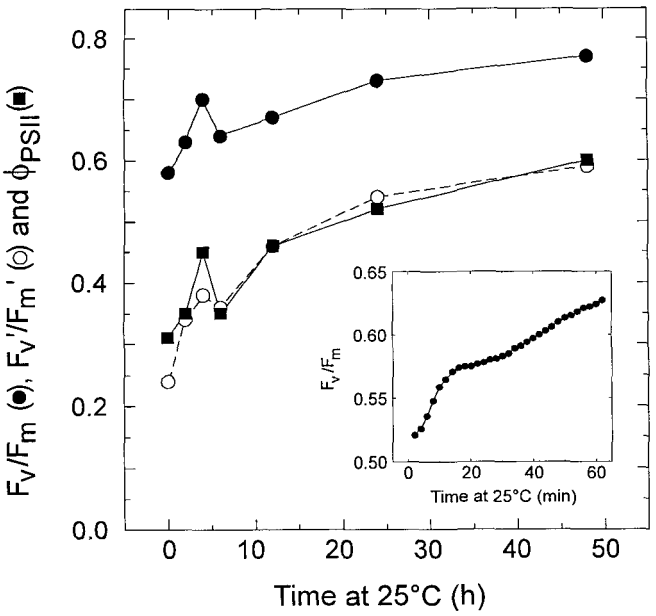

Figure 2. Time course for the recovery in the F_v/F_m (●), F_v'/F_m' (○), and ϕ_{PSII} (■) on transfer of 14°C-grown leaves to 25°C. F_v'/F_m' and ϕ_{PSII} were measured at steady-state photosynthesis at the ambient growth PPFD of 250 μmol m^{-2} s^{-1}. SEs were calculated for a minimum of five replicates and in all cases were less than 10% of the mean. The inset shows the changes in F_v/F_m for a single leaf during the first 60 min after transfer to 25°C.

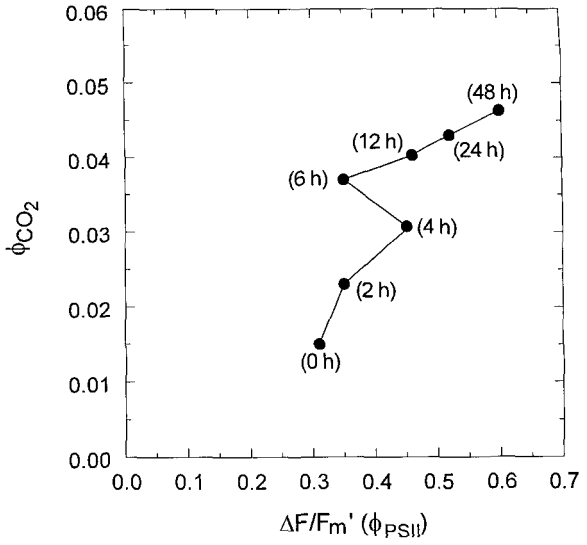

Figure 3. The relationship between ϕ_{CO_2} and ϕ_{PSII} on transfer of 14°C-grown leaves to 25°C. The number of hours elapsed after transfer to 25°C is shown in parentheses.

The Contribution of Zeaxanthin-Dependent Quenching to the Depression of Photosynthetic Performance within 14°C-Grown Plants

Studies of a wide range of species, in a variety of environmental situations, have demonstrated a strong correlation between the stimulation of nonphotochemical quenching of Chl fluorescence (which reflects an increase in nonradiative decay), induced by the absorption of light in excess of that required for photosynthesis, and the accumulation of zeaxanthin within the thylakoid membrane (Demmig-Adams, 1990; Demmig-Adams and Adams, 1992). Accumulation of the de-epoxide, zeaxanthin, results from de-epoxydation of the di-expoxide, violaxanthin, via the mono-epoxide, antheraxanthin. To evaluate whether zeaxanthin quenching of excitation energy might contribute to the inefficiency of photosynthesis within 14°C-grown plants, the xanthophyll cycle pigment contents (violaxanthin, antheraxanthin, and zeaxanthin) of leaves adapted to a PPFD of 250 μmol m^{-2} s^{-1} were analyzed before and for 48 h after transfer to 25°C. These data (Fig. 4) demonstrate that most of the xanthophyll pool within 14°C-grown leaves exists as zeaxanthin but that this pool is quickly epoxidized to violaxanthin after transfer to 25°C, with most of the conversion occurring within the first 10 h. It would therefore seem that relaxation of down-regulation, reflected in the decrease in nonphotochemical quenching associated with decreasing levels of zeaxanthin within the thylakoid membrane, is largely responsible for the increased F_v'/F_m' during the first 10 h after transfer of 14°C-grown leaves to 25°C. Since ϕ_{PSII} is primarily defined by F_v'/F_m' during this period (Fig. 2), it follows that a high level of nonradiative decay, within LHCII and associated with the presence of zeaxanthin, contributes significantly to the depressed photosynthetic performance of 14°C-grown maize plants.

Changes in the Concentrations of Active and Inactive PSII Centers following the Transfer of 14°C-Grown Plants to 25°C

The possibility that the transfer of 14°C-grown plants to 25°C might produce an increase in the total number of photochemically competent PSII complexes was examined through measurements of the capacity of isolated thyla-

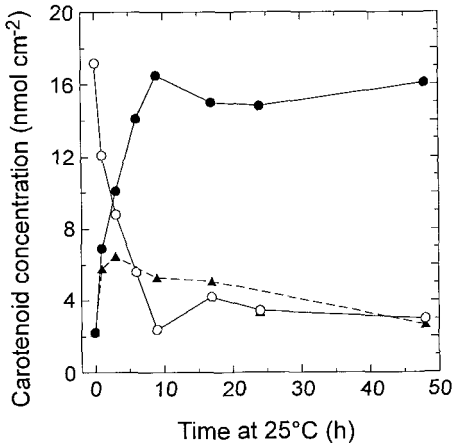

Figure 4. Time course for the recovery in the content of antheraxanthin (▲), violaxanthin (●), and zeaxanthin (○) in 14°C-grown leaves after transfer to 25°C. SEs were calculated for a minimum of five replicates and in all cases were less than 10% of the mean. Concentrations of the various carotenoids are expressed on the basis of leaf area.

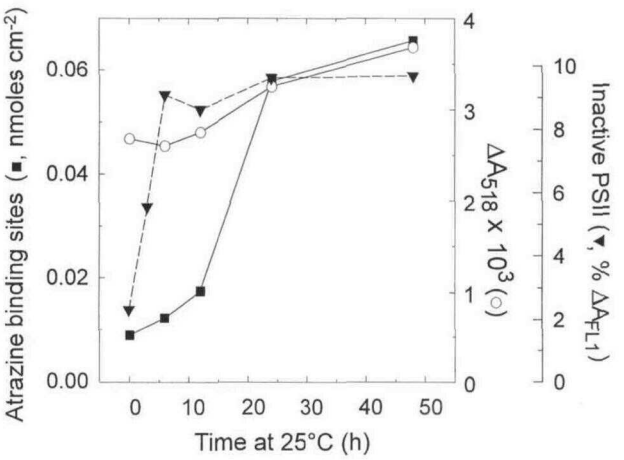

Figure 5. Time course for the recovery in the number of atrazine-binding sites in isolated maize mesophyll thylakoids (■), ΔA_{518} from leaves (○), and the estimated content of inactive PSII reaction centers (▼) on transfer of 14°C-grown leaves to 25°C. The content of inactive PSII reaction centers was estimated as described in "Materials and Methods" from flash-induced absorption changes at 518 nm. SEs were calculated for a minimum of five replicates and in all cases were less than 10% of the mean.

koids to bind ^{14}C[atrazine] and from the amplitude of ΔA_{FL1}. The contribution of inactive PSII centers to ΔA_{518} was also quantified (see "Materials and Methods"). Only a small increase in the capacity of isolated mesophyll thylakoid membranes to bind atrazine occurred during the first 12 h after transfer of 14°C-grown leaves to 25°C (Fig. 5). Similarly, there was no significant change in the amplitude of ΔA_{FL1} formation during this period. Between 12 and 24 h after transfer to 25°C, an approximately 600% increase in atrazine-binding was observed, after which the number of atrazine-binding sites continued to increase but at a much slower rate. The amplitude of ΔA_{518} also increased after the 12-h "lag." However, this increase was very much smaller (approximately 28%) than the increase in the number of atrazine-binding sites. The large increase in atrazine binding between 12 and 24 h after transfer to 25°C indicates an increase in the number of PSII centers capable of binding plastoquinone. This would be expected to cause a parallel increase in the number of PSII centers capable of stable charge separation within fully dark-adapted leaves. The much smaller increase in ΔA_{FL1} during this period suggests that the ratio of functional PSII to functional PSI is very low within 14°C-grown plants.

Inactive PSII centers have previously been shown to constitute approximately 12% of ΔA_{FL1} in nonstressed, field-grown maize plants (Chylla and Whitmarsh, 1989). Within 14°C-grown plants, only 2.3% of ΔA_{FL1} could be attributed to inactive PSII centers. However, this number increased significantly (to 9.1%) within 4 h at 25°C.

It was recently demonstrated that increasing the temperature of 14°C-grown plants to 25°C resulted in a large increase in the rate of thylakoid protein synthesis and a change in the labeling profile of the polypeptides; at 14°C the D1 and LHCII polypeptides were the major proteins labeled, whereas on transfer to 25°C, a wider range of proteins became labeled (Nie et al., 1995). The large increases observed in the photochemically active PSII population within 14°C-grown leaves after 12 h at 25°C (Fig. 5) could be associated with synthesis of new PSII complexes or, alternatively, the repair of previously photodamaged PSII complexes by the insertion of newly synthesized D1 in the complexes. To examine these possibilities, net thylakoid protein synthesis in 14°C-grown leaves was studied during the recovery of ϕ_{PSII} at 25°C. The 14°C-grown leaves were infiltrated with [^{35}S]Met for 3 h following transfer to 25°C for 3, 9, and 24 h, and then thylakoids were isolated and the polypeptides separated by PAGE. Fluorography of the polypeptide profiles indicated that many proteins were synthesized within the 3- to 6-h period after transfer of the leaves from 14 to 25°C, and the pattern of synthesis remained similar between 9 and 12 h (Fig. 6). The major proteins accumulating in these periods had apparent molecular masses of approximately 58, 57, 43, 32, and 26 kD, which are almost certainly the α and β subunits of the coupling factor, CP43/47, D1, and LHCII apoprotein, respectively. Between 24 and 27 h at 25°C the protein accumulation profile changed, with by far the most label being incorporated into the band at approximately 32 kD during this interval (Fig. 6); labeling in the other bands was essentially similar to that seen in the 3- to 6- and 9- to 12-h samples. This suggests that the large increase in photochemically competent PSII complexes between 12 and 48 h at 25°C is associated with a large increase in accumulation of D1 but not of any other proteins. Consequently, these data would be more consistent with the repair of existing, photodamaged PSII complexes rather than the synthesis of new PSII complexes; if synthesis of new PSII complexes were occurring during this period, similar increases in labeling of both the 43- and 32-kD bands would be predicted. The labeling pattern of the polypeptide profile of

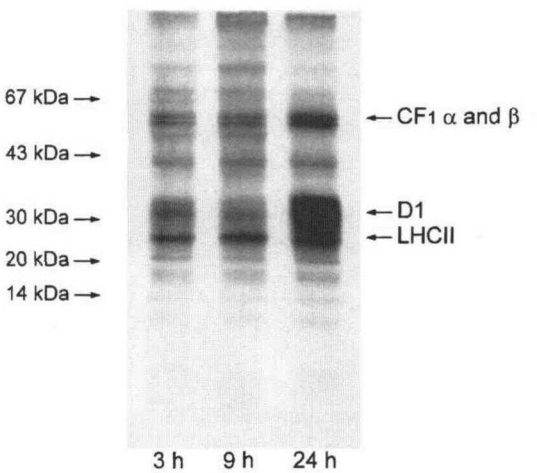

Figure 6. Fluorographs of maize mesophyll thylakoid polypeptide profiles showing net protein accumulation in 14°C-grown leaves during a 3-h incubation period with [^{35}S]Met after transfer to 25°C for 3, 9, and 24 h. Positions of molecular mass markers are given in kD at the left of the fluorograph. Locations of the α and β subunits of coupling factor 1 (CF_1), the D1 protein, and the major LHCII apoproteins are indicated.

the 14°C-grown leaves between 24 and 27 h after transfer to 25°C (Fig. 6) is similar to that found in control 25°C-grown leaves (Nie and Baker, 1991; Nie et al., 1995).

DISCUSSION

Increasing the ambient temperature of 14°C-grown maize leaves to 25°C resulted in an immediate increase in the rate of CO_2 assimilation (data not shown), which is almost certainly due to the release of temperature-related thermodynamic constraints on photosynthetic metabolism. This was followed by a slower increase in ϕ_{CO_2}, from 0.015 to 0.047 over 2 d (Fig. 1). Almost 70% of this gain occurred within the first 6 h after transfer to 25°C, despite the absence of any measurable increase in the number of photochemically active PSII centers (Fig. 5).

During the first 4 h at 25°C a rapid relaxation of nonphotochemical quenching of Chl fluorescence was observed, which was closely associated with increases in ϕ_{PSII} and ϕ_{CO_2}. This relaxation of nonphotochemical quenching was composed of two distinct phases. The first phase was completed within 14 min and can be attributed to reversal of the so-called "energy-dependent" quenching of Chl fluorescence (Krause and Weis, 1991). The second phase continued for at least 4 h, during which there was a substantial decrease in the level of zeaxanthin and a parallel increase in violaxanthin within these leaves (Fig. 4).

An interesting ancillary issue is the curious, but highly reproducible, transient decrease in both F_v/F_m and ϕ_{PSII} that occurs between 4 and 6 h after transfer to 25°C. After a short interval, this transient gives way to further slow increases in both parameters, which continue up to 48 h. During this transient, the zeaxanthin content continues to decline and the number of functional PSII centers increases slightly (Fig. 5). It therefore seems extremely unlikely that either the changes in carotenoid content or in the capacity for PSII photochemistry could have been responsible for this transient decrease in both F_v/F_m and ϕ_{PSII}. The zeaxanthin content of leaves continued to decline between 8 and 24 h after transfer to 25°C, whereas the number of functional PSII centers increased markedly from 12 h after transfer. Consequently, there was a period of several hours during which both relaxation of nonradiative decay processes (decrease in nonphotochemical quenching) and an increase in the capacity for PSII photochemistry contributed to the increase in ϕ_{PSII}.

The observed changes in atrazine-binding capacity of isolated mesophyll thylakoids indicate that the PSII population increases markedly between 12 and 48 h after the 14°C-grown leaves are transferred to 25°C (Fig. 5). This increase in the PSII population is likely to be an important factor in the increase in ϕ_{CO_2} between 12 and 48 h at 25°C. The [^{35}S]Met-labeling studies show that the increase in the PSII population after 12 h is accompanied by an increased rate of synthesis of D1 that is much greater than for other PSII core complex proteins, such as CP43 and CP47 (Fig. 5). These data are consistent with the repair of photodamaged D1 in inactivated PSII complexes rather than the synthesis of entirely new PSII complexes. The repair of photodamaged PSII complexes requires the proteolytic degradation of the damaged D1, together with the synthesis and insertion of D1 into the damaged complexes. Degradation of D1 is known to be highly temperature sensitive (Aro et al., 1990) and, in maize chloroplasts, has been shown to be reduced when thylakoids have developed at 14°C compared to 25°C (Bredenkamp and Baker, 1994). Therefore, the proportion of PSII complexes containing damaged D1 would be expected to be greater in maize leaves grown at 14°C as compared to 25°C. It should be noted that an immediate increase in the rate of net synthesis of PSII CPs and many other thylakoid proteins on transfer of 14°C-grown leaves to 25°C has been reported previously (Nie et al., 1995). Thus, it appears that some biogenesis of PSII complexes does occur, which could account for the small increases in the atrazine-binding capacity of thylakoids observed during the first 12 h at 25°C (Fig. 4).

After transfer to 25°C, the increase in ΔA_{FL1} (which is directly proportional to the number of PSII plus PSI centers capable of stable charge separation) is much smaller than the increase in atrazine-binding (which reflects the number of PSII centers capable of binding plastoquinone). Assuming the increase in atrazine binding represents an increase in the number of PSII centers capable of stable charge separation, a comparison of the atrazine-binding and ΔA_{518} data (Fig. 5) indicates that the level of PSII centers capable of stable charge separation within 14°C-grown plants is extremely low, relative to PSI, or that an increase in PSII centers is paralleled by a decrease in PSI centers following transfer to 25°C.

The small increase in the contribution of inactive PSII centers to ΔA_{FL1} (Fig. 5), after the transfer of 14°C-grown plants to 25°C, occurs before any measurable increase in ΔA_{FL1} per se. Consequently, inactive PSII centers must be formed at a faster rate than active PSII centers during this period. Although there is relatively little incorporation of [^{35}S]Met into D1 and the 43-/47-kD proteins during the 3- to 6-h period after transfer to 25°C, it should be noted that relatively little protein synthesis would be required to account for the increase in inactive PSII centers observed, since these centers represent a small proportion of the total PSII pool. An alternative explanation, however, might be that these centers are synthesized from precursors within the existing polypeptide pool.

To conclude, the data presented here demonstrate that the major factor determining the low photosynthetic efficiency of leaves from 14°C-grown maize plants is zeaxanthin-related quenching of excitation energy within PSII antennae. Since this quenching of absorbed excitation energy through nonradiative decay competes directly with energy trapping by P_{680}, the potential for damage to PSII reaction centers is consequently decreased. Despite the protection afforded by high levels of zeaxanthin-related quenching, the data presented in Figure 5 clearly show that the concentration of functional PSII reaction centers within maize leaves developed at 14°C is much lower than within the same leaves 48 h after transfer to 25°C. Potentially, this low concentration of functional PSII centers also plays a significant role in depressing photosynthetic activity

within these leaves once the ambient temperature has been increased to 25°C.

It is now widely accepted that the replacement of light-damaged D1 requires degradation of the damaged protein before replacement with functional D1 can occur (Ohad et al., 1994) and that this process is inhibited by low temperature (Aro et al., 1990). Consequently, it seems reasonable to suggest that, although the high levels of zeaxanthin present within the leaves of 14°C-grown maize plants will offer some protection against light-induced damage to D1, the inhibition of D1 degradation by low temperature prevents the accumulation of functional PSII reaction centers.

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