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Modifications to Thylakoid Composition during Development of Maize Leaves at Low Growth Temperatures[1]

Gui Ying Nie and Neil R. Baker*

Department of Biology, University of Essex, Colchester, CO4 3SQ, Essex, United Kingdom

ABSTRACT

The effects of reductions in growth temperature on the development of thylakoids of maize (*Zea mays* var LG11) leaves are examined. Thylakoids isolated from mesophyll cells of leaves grown at 17° and 14°C, compared with 25°C, exhibited a decreased accumulation of many polypeptides, which was accompanied by a loss of activity of photosystems (PS) I and II. Probing the polypeptide profiles with a range of antibodies specific for thylakoid proteins demonstrated that a number of polypeptides encoded by the chloroplast genome failed to accumulate at low temperatures. Although thylakoid protein synthesis was reduced severely at 14°C compared with 25°C, major synthesis of both chloroplast and nuclear encoded polypeptides was detected. It is suggested that the lack of accumulation of some thylakoid proteins at low temperatures may be due to an inability to stabilize the proteins in the membranes. A number of thylakoid polypeptides were found to appear as the growth temperature was decreased. Analyses of pigments and polypeptides demonstrated that decreases in the photosystem reaction center core complexes occur relative to the light harvesting complex associated with PS II at reduced growth temperatures. Differential effects on the development of PSI and PSII were also observed, with PSII activity being preferentially reduced. Reductions in PSII content and activity occurred in parallel with decreases in the quantum yield and light-saturated rate of CO_2 assimilation. Fractionation of thylakoid pigment-protein complexes showed that the ratio of monomeric:oligomeric form of the light harvesting complex associated with PSII increased at low growth temperature, which is consistent with a chill-induced modification of thylakoid organization. Many, but not all, of the characteristic changes in thylakoid protein metabolism, which were observed when leaves were grown at low temperatures in controlled environments, were identified in leaves of a field maize crop during the early growing season when low temperatures were experienced by the crop. Chill-induced perturbations of thylakoid development can occur in the field in temperate regions and may have implications for the photosynthetic productivity of the crop.

Low temperatures experienced in many temperate regions by maize crops during the early growing season have long been considered to limit growth and productivity. Although chilling will have many effects on the physiology of maize plants, the large reductions in photosynthetic competence observed on exposure of leaves to low temperatures (19) are considered to be of particular importance in this context (2). Field studies in the United Kingdom have shown that the quantum yield of carbon assimilation of mature maize leaves can be 50% lower during the early growing season than is found to be the case later in the season (1). The depression of photosynthetic activity in chilling-sensitive plants at low temperatures has been attributed by some to be caused primarily by light-dependent damage to PSII (24). However, chilling maize leaves at low light results in a large decrease in the quantum yield of carbon assimilation, the basis of which is as yet unresolved, in the absence of any damage to PSII (25). Recently, severe impairment of the synthesis of some chloroplast proteins has been observed at chilling temperatures in rice (11) and rape (20), and on rewarming after chilling in tomato (5). Also, the accumulation of a 31-kD polypeptide in the thylakoids has been observed on chilling maize leaves; this polypeptide is considered to be an unprocessed precursor of the 29-kD apoprotein of the Chl-protein CP29 and its appearance is probably associated with an inhibition of its processing peptidase at low temperatures (12). Consequently, chill-induced depressions in the photosynthetic competence of maize leaves may be associated with perturbations of the synthesis and processing of proteins required for the development and repair of the photosynthetic apparatus.

In this study, the effects of suboptimal growth temperatures on the protein and pigment compositions of thylakoid membranes of maize leaves are examined in relation to their photosynthetic characteristics and ability to accumulate thylakoid proteins. We demonstrate that chilling perturbation of thylakoid protein metabolism has implications for the photochemical activities of the membranes and associated photosynthetic characteristics of the leaves. Also the characteristic perturbations to the accumulation and synthesis of specific thylakoid polypeptides observed when leaves are grown at chilling temperatures under controlled environmental conditions are identified in maize leaves grown in the field in the United Kingdom during May and June, when chilling conditions are experienced.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Maize plants (*Zea mays* L. cv LG11) were grown from seed in vermiculite watered with Hoagland nutrient solution and

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kept in controlled environment cabinets with 16 h light daily at a PPFD of 230 μmol m^{-2} s^{-1}. Three d/night growth temperature treatments were used: (a) 25/22°C, (b) 17/14°C, and (c) 14/12°C. Plants grown under these regimes will be referred to as 25°C-, 17°C-, and 14°C-grown, respectively. Second leaves that had reached full expansion in the 3 d before harvesting were used in all studies, with the exception that leaves that were approximately 50% expanded were used in the [^{35}S]methionine labeling studies to ensure that thylakoid protein synthesis was examined during chloroplast development.

For field studies, seeds were sown at weekly intervals from May 3, 1989 in 20-cm-diameter pots containing field soil and sunk into an experimental maize plot at Colchester (northeast Essex, UK). Analyses of the thylakoid polypeptides were made on the second leaves harvested within 3 d of reaching maximum size. The second leaves used in the [^{35}S]methionine incorporation studies of protein synthesis were approximately 50% fully expanded. The leaves were treated with [^{35}S]methionine at 9:00 AM and harvested 3 h later.

Measurement of Leaf Gas Exchange Characteristics

The quantum yields of CO_2 assimilation were estimated from the linear portion of the light dosage response curves between PPFDs of 50 and 100 μmol m^{-2} s^{-1} in a light-integrating spherical leaf chamber as described previously (15). Estimates of the light-saturated rates of CO_2 assimilation were obtained at a PPFD of 1500 μmol m^{-2} s^{-1} as described previously (16).

Labeling of Proteins in Leaves

The method used to incorporate [^{35}S]methionine into leaf proteins was a modification of that used by Cooper and Ort (5). The upper surfaces of leaves attached to the plant were abraded once with carborundum paper and then aliquots of [^{35}S]methionine (400 μCi mL^{-1}, specific activity > 800 Ci $mmol^{-1}$) in 0.4% (v/v) Tween 20 were applied evenly using cotton wool. For protein synthesis inhibitor studies chloramphenicol (200 μg mL^{-1}) or cycloheximide (20 μg mL^{-1}) was added to the [^{35}S]methionine solution. After the application of label, leaves were left in their ambient growth conditions for 3 h and then rinsed in ice-cold, nonradioactive 1 mM methionine before isolation of thylakoids.

Isolation of Thylakoid Membranes

Thylakoids to be used in assays of electron transport were isolated from mesophyll cells essentially as described previously (13) using an isolation medium containing 500 mM sorbitol, 50 mM Hepes, 10 mM KCl, 5 mM $MgCl_2$, 2 mM EDTA, 10 mM sodium-isoascorbate, and 0.1% (w/v) BSA at pH 7.8. The membranes were washed in medium that was the same except that sorbitol, sodium-isoascorbate, and BSA were omitted and then resuspended in the wash medium with the addition of 330 mM sorbitol. For the analyses of polypeptides and pigment-proteins, the isolation and resuspension media were modified and contained 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.

Analyses of Thylakoid Polypeptides

Thylakoids were solubilized in 4% (w/v) SDS, 60 mM DTT, and 8% (w/v) sucrose using an SDS:Chl ratio of 32:1 (w/w). Thylakoids isolated from leaves grown at the three temperatures had similar protein:Chl ratios (approximately 6.6 μg/μg). PAGE of solubilized proteins was performed essentially as described by Chua (4) using the buffer system of Neville (22) and a resolving gradient gel of 10 to 18% acrylamide and 4 to 8% sucrose. All tracks on each gel were loaded with equal amounts of solubilized protein (80 μg or less). Gels were stained with 0.1% (w/v) Coomassie brilliant blue R in a methanol:acetic acid:water (4:1:5, v/v/v) solution and destained in a methanol:acetic acid:water (8:3:29, v/v/v) solution. Polypeptides labeled with [^{35}S]methionine were detected by fluorography at −70°C. Destained gels were impregnated with Autofluor in 5% (v/v) glycerol for 30 min before vacuum drying and exposure to Kodak XAR-5 x-ray film.

Immunoblotting of separated polypeptides was carried out essentially as described previously (21). Polypeptides were transferred onto nitrocellulose and probed with antibodies specific to thylakoid proteins. Antibody reactions were visualized by incubation with a peroxidase-conjugated rabbit anti-mouse immunoglobulin and development with saturated 4-chloro-1-naphthol solution. Antibodies to a range of thylakoid proteins were obtained from the following sources: CPI[2] apoprotein (65–70 kD) and LHC I polypeptides (20–25 kD) from R. J. Ellis (29); 26-kD polypeptide of LHC II from S. C. Darr (6); D_1 protein of PSII reaction center and the 33-kD extrinsic water splitting protein of PSII from J. Barber (23); subunit II of PSI (22 kD), subunit IV of the cytochrome b_6/f complex (17 kD), and Cyt f (33 kD) from R. Malkin (30); and the α- and β-subunits of the coupling factor (58 and 57 kD) from B. Jordan (7). The immunoblots presented for all antigens were produced from gels containing levels of the antigens below those required to saturate the antibodies applied. These nonsaturating antigen levels were determined by immunoblotting gels containing a dilution series of the solubilized thylakoid protein preparations.

Separation of Pigment-Protein Complexes

Pigment-proteins of mesophyll thylakoids were separated by mildly dissociating PAGE, employing a mixture of the detergents SDS and either n-octyl-β-D-glucopyranoside or deoxycholate. To solubilize and fractionate the pigment-proteins with SDS/octyl glucoside, the procedure of Dunahay and Staehelin (8) was used, except that the resolving gel contained 12% (w/v) acrylamide. A modification of the procedure of Waldron and Anderson (28) was used to solubilize the pigment-proteins with SDS/deoxycholate. Thylakoids were initially solubilized in 0.3 M Tris (pH 8.8) containing 13% (v/v) glycerol and 1% (w/v) SDS, then 2% (w/v) deoxycholate solution was added to give a deoxycholate:SDS:Chl ratio of 20:10:1.

[2] Abbreviations: CPI, CPII, Chl-protein I, II; LHC, light harvesting complex.

Pigment Analysis

The photosynthetic pigments were extracted from isolated thylakoids and separated using HPLC as previously described (27) except that a Beckman System Gold instrument, fitted with a Beckman RP-C18 15-cm column, and a flow rate of 1 mL min^{-1}, was used.

Electron Transport Assays

Electron transport activities of PSI and PSII in isolated thylakoids were determined polarographically in a Clark-type oxygen electrode at 25°C using a 3-mL reaction volume. Illumination was provided by a quartz-halogen source and the intensity in the reaction chamber was attenuated using glass neutral density filters. The light-dependent reduction of methyl viologen by PSI, using reduced tetramethyl-*p*-phenylenediamine as the electron donor, was monitored in 330 mM sorbitol, 20 mM Tricine, 7 mM $MgCl_2$, 5 mM sodium-azide, 3 mM sodium-isoascorbate, 0.2 mM tetramethyl-*p*-phenylenediamine, 0.1 mM methyl viologen, 15 μM DCMU, and 20 μg mL^{-1} Chl at pH 8.0. The reduction of *p*-phenylenediimine by PSII was determined in 100 mM sorbitol, 50 mM Hepes, 10 mM NaCl, 5 mM $MgCl_2$, 2 mM EDTA, 0.5 mM *p*-phenylenediamine, 2.5 mM $K_3Fe(CN)_6$, 2.5 mM NH_4Cl, and 20 μg mL^{-1} Chl at pH 7.8.

RESULTS

Lowering of growth temperature of maize plants from 25° to 17° and 14°C had large effects on the development of the second leaves (Table I). The length of the mature leaf decreased and the time taken to achieve this length increased dramatically. Photosynthetic performance measured at 25°C was markedly impaired in leaves of plants grown at the lower temperatures. Large decreases were observed in both the light-saturated rate and maximum quantum yield of CO_2 assimilation (Table I), indicative of decreases in the capacity for CO_2 assimilation per unit leaf area and in the efficiency of utilizing an absorbed photon for carbon assimilation.

Major differences were observed in the polypeptide profiles of thylakoids isolated from the mesophyll cells of second leaves grown at 14°, 17°, and 25°C (Fig. 1). With depressions of the growth temperature, decreases in many polypeptides were apparent, particularly those with apparent molecular masses of approximately 10, 16, 19, 24 to 28, 30 to 35, 40 to 45, and 50 to 70 kD. A number of other polypeptides, particularly at 14, 18, 21, and 31 kD, appeared to increase at 17° and 14°C. To determine how a number of known thylakoid proteins were affected by growth temperature, Western blots of the gels were performed using antibodies specific to polypeptide components of PSI, PSII, LHCII, Cyt b_6/f complex, and the extrinsic coupling factor 1 component of the coupling factor; *i.e.* CPI apoprotein (65–70 kD), PSI subunit II (22 kD), LHCI (20–26 kD), D_1 (32 kD), extrinsic water splitting protein (33 kD), LHCII (28 kD), cytochrome *f* (33 kD), Cyt b_6/f subunit IV (17 kD), and the α- and β-subunits of coupling factor 1 (58 and 57 kD). Growth temperature had a negligible effect on the content of the LHCI, LHCII, and extrinsic water splitting polypeptides in the thylakoids (Fig. 2). The amounts of the other polypeptides that were probed by Western blotting were found to decrease as a proportion of the total thylakoid protein with decreasing growth temperature. The polypeptides of PSI (CPI apoprotein and subunit II) and the coupling factor (α- and β-subunits) showed only small, if any, changes at 17°C, but large decreases at 14°C (Fig. 2), whereas the D_1 and Cyt b_6/f polypeptides were found to decrease at both 17° and 14°C (Fig. 2). It is of note that the polypeptides showing negligible change with growth temperature, *i.e.* LHCI, LHCII, and the 33-kD extrinsic water splitting protein, are encoded by the nuclear genome, whereas the CPI apoprotein, D_1, the Cyt b_6/f complex subunit IV, Cyt *f*, and the α- and β-subunits of the coupling factor are chloroplast gene products. These data suggest that reduction of growth temperature may inhibit selectively the accumulation of chloroplast, relative to nuclear, encoded polypeptides in the thylakoids.

To examine the effects of lowered growth temperature on

Table I. *Effects of Growth Temperature on Leaf Expansion, Chl Content, and Photosynthetic Parameters*

Measurements of CO_2 assimilation and electron transport were made at 25°C. PSI and PSII activities are the light-saturated rates of electron transport from reduced tetramethyl-*p*-phenylenediamine to methyl viologen and from water to *p*-phenylenediamine, respectively. Data are the means of at least five replicates, and often considerably more; SE of the means given were applicable.

Parameter	Growth Temperature		
	14°C	17°C	25°C
Maximum leaf length (cm)	9.9 ± 0.3	13.3 ± 0.3	16.2 ± 0.3
Time to reach maximum length (d from sowing)	28	19	6
Total Chl/area (μg cm^{-2})	12.9 ± 1.3	43.0 ± 2.5	46.2 ± 1.0
Chl *a*/area (μg cm^{-2})	9.3 ± 1.0	33.6 ± 2.1	35.5 ± 0.9
Light-saturated CO_2 assimilation/area (μmol m^{-2} s^{-1})	5.4 ± 1.8	16.3 ± 1.3	30.7 ± 0.9
Quantum yield of CO_2 assimilation (mol CO_2 mol^{-1} photon)	0.013 ± 0.003	0.044 ± 0.001	0.058 ± 0.001
PSI activity (nmol O_2 mg^{-1} Chl s^{-1})	19.0 ± 2.9	30.7 ± 4.1	33.1 ± 3.5
PSII activity (nmol O_2 mg^{-1} Chl s^{-1})	12.2 ± 2.3	16.8 ± 2.4	26.1 ± 1.4

the rate of net synthesis of thylakoid proteins, developing leaves approximately halfway through expansion were infiltrated with [^{35}S]methionine and after 3 h at the ambient growth temperature, thylakoids were isolated. Incorporation of label into thylakoid proteins was approximately 3 times greater per unit of thylakoid protein in the 25°C-grown compared with the 14°C-grown leaves (see legend of Fig. 3). Fluorography of the polypeptide profiles demonstrated that the most heavily labeled proteins at both growth temperatures were found between 25 and 35 kD (Fig. 3), and are presumably the apoproteins of LHCII and the D_1 polypeptide of the PSII reaction center. No synthesis of proteins below 20 kD was detected in the 14°C-grown leaves (Fig. 3), suggesting that during the 3-h pulse label the polypeptides between 10 and 22 kD, which accumulate at the lower growth temperature (Fig. 1), are not synthesized *de novo* in any significant amount during the 3-h pulse label.

The apparent inhibition by low temperature of the synthesis of chloroplast-encoded proteins was further examined by infiltrating 14°C- and 25°C-grown leaves with chloramphenicol and cycloheximide, inhibitors of chloroplast and cytoplasmic protein synthesis, respectively, before a [^{35}S]methionine pulse. These protein inhibitor treatments of 25°C-grown leaves demonstrate clearly that the majority of the polypeptide synthesis is associated with chloroplast gene products (Fig. 3);

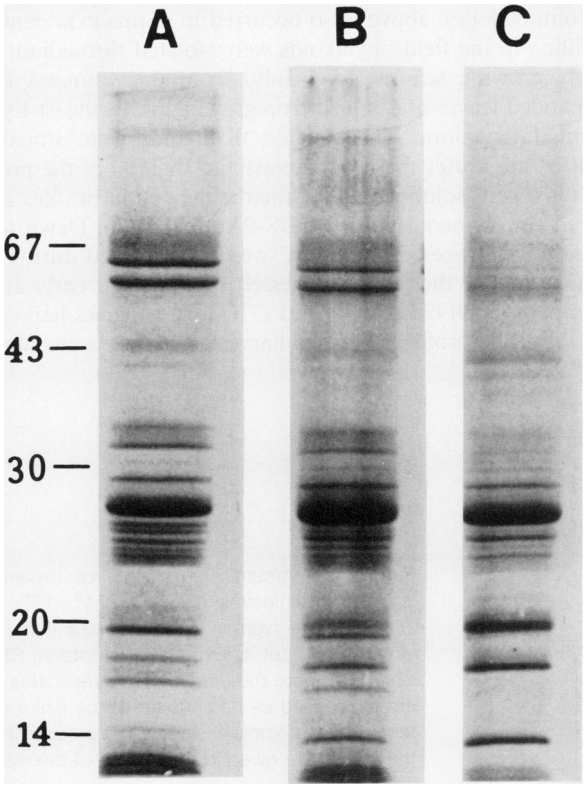

Figure 1. Coomassie blue-stained polypeptide profiles of thylakoid membranes isolated from fully expanded maize leaves grown at (A) 25°, (B) 17°, and (C) 14°C. Molecular masses of marker proteins are given on the left of the gel in kilodaltons. Lanes were loaded with equal amounts of protein.

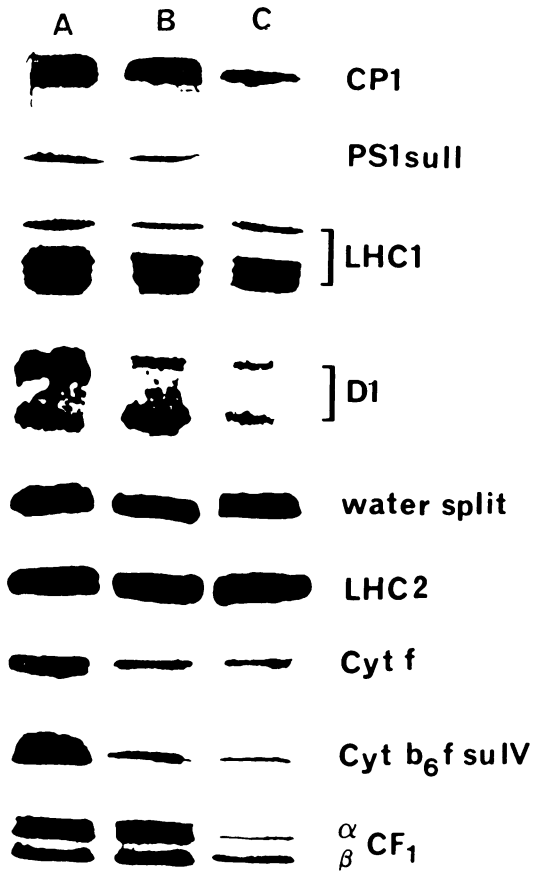

Figure 2. Immunoblots of thylakoid proteins from leaves grown at (A) 25°, (B) 17°, and (C) 14°C. Polypeptide profiles similar to those shown in Figure 1 were probed with antibodies specific to CPI apoprotein (65–70 kD), PSI subunit II (22 kD), apoproteins of LHCI (20–26 kD), D_1 protein (32 kD), extrinsic water splitting protein (33 kD), apoprotein of LHCII (26–28 kD), Cyt *f* (33 kD), Cyt b_6/f subunit IV (17 kD), and the α and β subunits of the coupling factor (58 and 57 kD).

the only nuclear encoded thylakoid polypeptides showing large net synthesis at this stage of leaf development were the 26- to 28-kD apoproteins of LHCII and a 33- to 34-kD protein. The chloramphenicol and cycloheximide treatments of 14°C-grown leaves show that synthesis of chloroplast proteins does occur at the low growth temperature. Consequently, the selective inhibition of the accumulation of chloroplast, relative to nuclear, encoded proteins in the thylakoids, cannot be attributed simply to an inhibition of chloroplast protein synthesis at low growth temperatures.

Leaves grown at 25° and 17°C contained similar total Chl and Chl *a* contents per unit area, however in 14°C-grown leaves this was decreased by over 70% (Table I). The changes in the relative proportions of Chl *b* and the major carotenoids in the thylakoids as a function of growth temperature are shown in Figure 4. Only minor differences were found between 25° and 17°C-grown leaves. Leaves grown at 14°C exhibited a near doubling of lutein along with a substantial increase in Chl *b* relative to the Chl *a* content. Smaller increases were found in neoxanthin and violaxanthin and β-

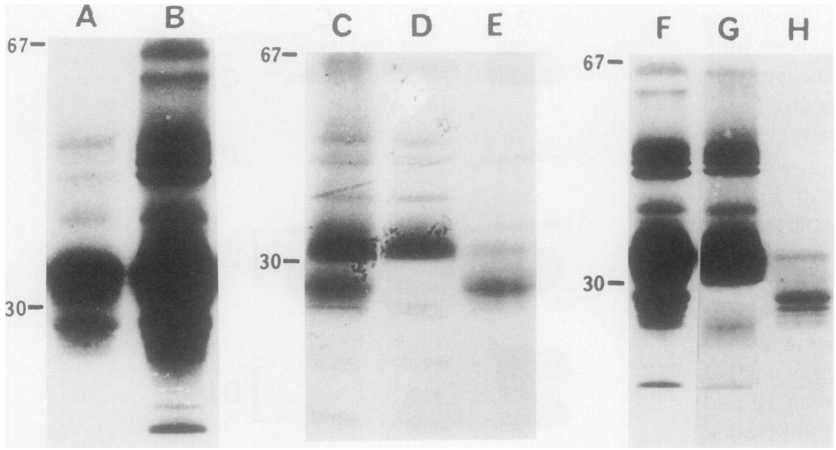

Figure 3. Fluorographs of thylakoid polypeptide profiles showing net protein synthesis in developing leaves approximately half way through expansion. Leaves were labeled with [^{35}S]methionine for 3 h. A, 14°C-grown leaf; B, 25°C-grown leaf; C, D, and E, 14°C-grown leaves, infiltrated with cycloheximide (D) and chloramphenicol (E); F, G, and H, 25°C-grown leaf, infiltrated with cycloheximide (G) and chloramphenicol (H). Positions of molecular mass markers are given in kilodaltons. Lanes were loaded with equal amounts of protein. The amounts of label loaded onto lanes A and B were 1.03×10^7 and 3.16×10^7 dps, respectively.

carotene decreased. Since lutein, neoxanthin, and violaxanthin are associated predominantly with light-harvesting antenna complexes and β-carotene with the reaction center cores of PSI and PSII (3), these data suggest that the proportion of reaction center core complexes to LHCII is decreased in 14°C-grown compared with 17° and 25°C-grown leaves. Chl-protein complexes of thylakoids isolated from 25° and 14°C-grown leaves were examined using mildly denaturing gel electrophoresis employing a mixture of the detergents SDS and octyl glucoside or deoxycholate (Fig. 5). Due to large differences in the amounts of free pigment on these gels, it is difficult to interpret the differences observed in the pigment-protein profiles of 25° and 14°C-grown leaves. However, it would appear that decreases in the CPI complexes associated with PSI cores and the oligomeric form of LHCII together with an increase in the monomeric form of LHCII are associated with samples from 14°C-grown compared with 25°C-grown leaves. It should be stressed that such differences may be indicative of differential stabilities of the complexes of the two leaf types, which results in differences in solubilization and fractionation of the complexes, rather than being attributable to differing contents of the complexes in the thylakoids (9, 10).

Electron transport activities of PSI and PSII in thylakoids isolated from 14°, 17°, and 25°C-grown leaves demonstrate that growth temperature influences the light-saturated rates of electron transport per unit Chl (Table I). Although reduction of the growth temperature from 25 to 17°C had a negligible effect on PSI activity, a large decrease was observed in PSII activity. Both PSI and PSII activities were severely reduced when leaves were grown at 14°C.

To assess whether the low growth temperature-induced changes in thylakoid composition observed in controlled environments (see above) also occurred in plants experiencing chilling in the field, thylakoids were isolated throughout the early growing season (May–July) from the youngest fully expanded leaves of a maize crop growing in northeast Essex, United Kingdom. The isolated thylakoids were stored at −40°C until after the final harvest and then all of the preparations were solubilized and their polypeptide profiles analyzed simultaneously using SDS-PAGE (Fig. 6). Dawn temperatures between 2° and 14°C were experienced during development of the leaves harvested in May and early June, compared with between 8° and 17°C for the leaves harvested in July. The profiles of leaves harvested in July were similar

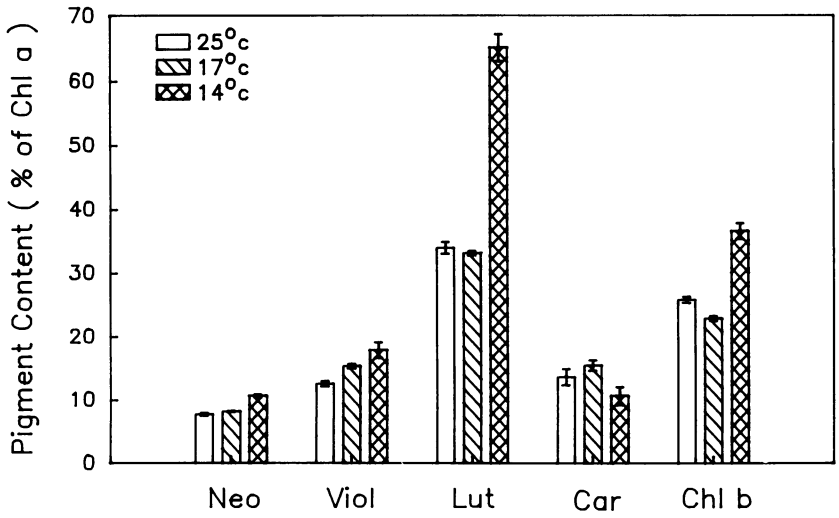

Figure 4. Pigment composition of thylakoids isolated from leaves grown at 14°, 17°, and 25°C. Key to pigments: Neo, neoxanthin; Viol, violaxanthin; Lut, lutein; Car, β-carotene; Chl b, Chl *b*. Data are the means of five replicates and are expressed as percentage of the Chl *a* content of the membranes. The Chl *a* contents of the leaves are given in Table I. SE of the means are shown.

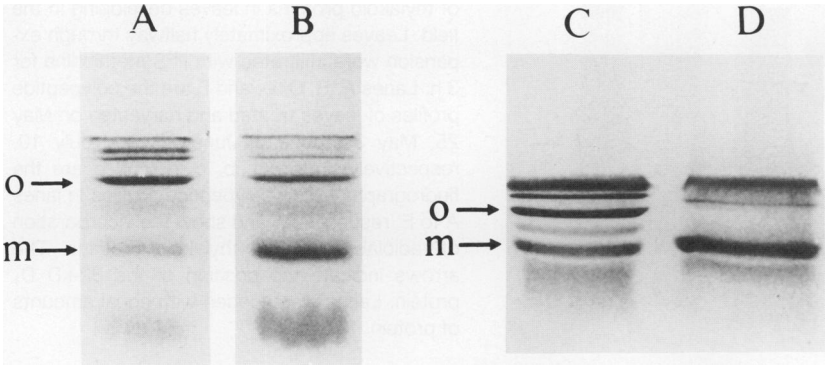

Figure 5. Pigment-protein complexes of thylakoids isolated from leaves grown at 25°C (lanes A and C) and 14°C (lanes B and D). Unstained gels of pigment-protein complexes extracted and solubilized with SDS and octyl glucoside (lanes A and B) or with SDS and deoxycholate (lanes C and D) are shown. The oligomeric (o, CPII*) and monomeric (m, CPII) forms of LHCII are identified. For the 25°C-grown samples, the two complexes above the LHCII oligomer are LHCI + CPI (the core complex of PSI) and CPI, the band between the LHCII oligomer and monomer contains CP47 and CP43 (complexes associated with the core of PSII), and the band below the LHCII monomer is free pigment. Lanes were loaded with equal amounts of Chl.

to those of leaves grown at 25°C in a controlled environment chamber (Fig. 6). This was not the case for leaves harvested in May and early June, when a loss of polypeptides with molecular masses of approximately 16 and 42 kD was observed. However, no major deficiencies in many of the polypeptides, which were found to decline in 14°C compared with 25°C-grown leaves (*e.g.* at 25–28, 30–35, and 50–67 kD; Fig. 1), were apparent. An accumulation of polypeptides at approximately 17 and 21 kD occurred in leaves harvested in May and early June and was similar to the situation observed in 14°C-grown leaves (Fig. 1).

The ability of leaves developing in the field to synthesize thylakoid proteins was examined by infiltrating leaves, which were approximately 50% of mature size, with [^{35}S]methionine at 9:00 AM for 3 h and then isolating the thylakoids and analyzing their polypeptide profiles for radiolabel incorporation. Large differences in the fluorographs of the thylakoid polypeptide profiles of leaves developing during the period May to July were observed (Fig. 7). Leaves developing during late June and early July exhibited thylakoid labeling patterns (Fig. 7) similar to those of leaves developing at 25°C in a controlled environment cabinet (Fig. 3), with the exception of the net synthesis of a low molecular mass (less than 20 kD) polypeptide. Net protein synthesis was considerably reduced in leaves developing earlier in the season (Fig. 7), during May and early June, with the labeling patterns being more similar to those observed in 14°C-, than 25°C-, grown leaves (Fig. 3).

DISCUSSION

Besides reducing the amounts of thylakoid pigments and proteins per unit leaf area, depression of the growth temperature of maize from 25° to 14°C clearly has major effects on the polypeptide and pigment composition of the thylakoid membranes of mesophyll cells. Analyses of pigments, pigment-proteins, and polypeptides demonstrate that decreases in the reaction center core complexes occur relative to LHCII. Also, differential effects on the development of PSI and PSII were observed; PSII appears to be more sensitive to reduction in growth temperature than PSI (Table I). Restrictions on the development of PSII could contribute to the decreases observed in the quantum yield and light-saturated rate of CO_2 assimilation at the lowered growth temperatures (see Table I). Large decreases in these carbon assimilation parameters are found in 17°C, compared with 25°C-, grown leaves, yet little change in PSI activity occurs, consequently it is unlikely that PSI can be implicated as a limiting factor in the chill-induced depression of CO_2 assimilation in maize leaves.

The effects of reduction in growth temperature on the relative accumulation of polypeptides in the thylakoid membranes are complex and fall into two categories. The accumulation of thylakoid proteins, which are the products of chloroplast genes, *e.g.* CPI, subunit II of PSI, D_1 protein, cytochrome *f*, cytochrome b_6/f subunit IV, and the α- and β-subunits of the coupling factor, is repressed as growth tem-

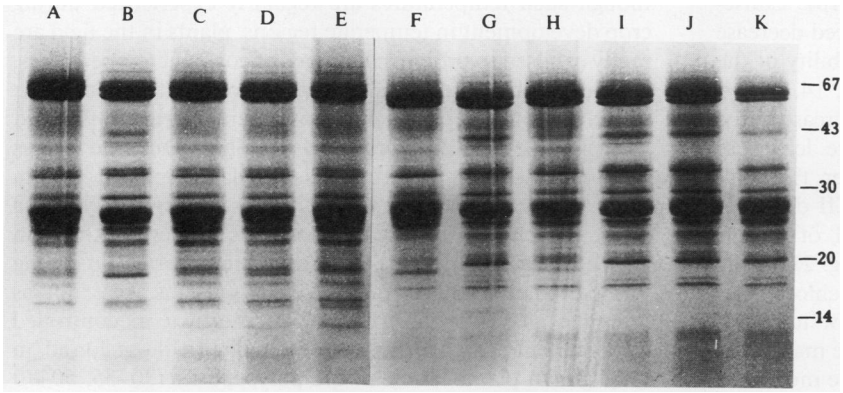

Figure 6. Coomassie blue-stained polypeptide profiles of thylakoids isolated from the youngest, fully expanded leaves of a maize crop growing in northeast Essex, United Kingdom, during 1989. Lanes A to J are the profiles for leaves harvested on the following dates: A, May 23; B, May 31; C, June 1; D, June 2; E, June 6; F, June 13; G, June 19; H, June 26; I, July 10; J, July 31. Lane K shows the profile of thylakoids isolated from fully expanded leaves grown at 25°C in a controlled environment cabinet. The molecular masses of marker proteins are given in kD. Lanes were loaded with equal amounts of protein.

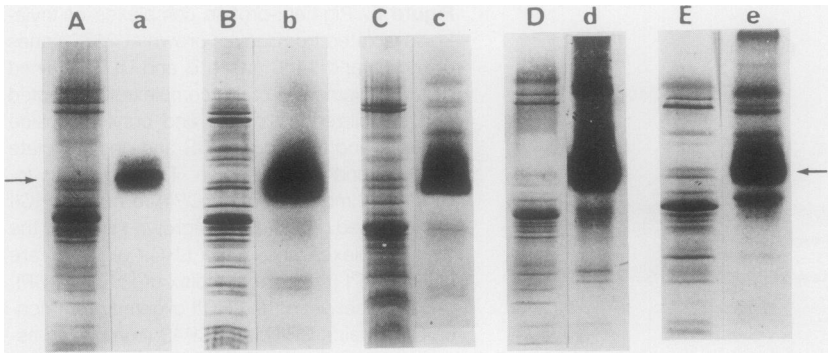

Figure 7. Fluorographs showing net synthesis of thylakoid proteins in leaves developing in the field. Leaves approximately halfway through expansion were infiltrated with [^{35}S]methionine for 3 h. Lanes A, B, C, D, and E are the polypeptide profiles of leaves treated and harvested on May 25, May 31, June 6, June 26, and July 10, respectively. Lanes a, b, c, d, and e are the fluorographs of the polypeptide profiles in lanes A to E, respectively, and show the incorporation of radiolabel into the thylakoid proteins. The arrows indicate the position of the 32-kD D_1 protein. Lanes were loaded with equal amounts of protein.

perature is lowered, whereas the apoproteins of LHCI and LHCII and the 33-kD extrinsic water splitting enzymes, which are nuclear encoded, do not decrease significantly. The chilling-induced decrease in accumulation of the chloroplast-encoded polypeptides in the thylakoid membranes would not appear to be attributable to the inhibition of the synthesis of chloroplast-, compared with nuclear-, encoded polypeptides. As expected, labeling studies indicated that the rate of net synthesis of the total thylakoid proteins was inhibited at 14°C. However, the inhibition of net synthesis of chloroplast-encoded polypeptides did not appear to be markedly different from that of nuclear-encoded proteins (Fig. 3). A more detailed quantitative study is required to confirm this observation, especially since it has been suggested previously that chilling may affect the coordination of the large and small subunits of ribulose-1,5-biphosphate carboxylase (11), which are the products of chloroplast and nuclear genes, respectively. The decrease in the accumulation of chloroplast-encoded polypeptides in the thylakoids at low temperatures may be due to a reduced ability to stabilize the newly synthesized polypeptides in the membranes, which could involve changes in polypeptide processing or lipid-protein interactions.

The possibility of chill-induced changes in the thylakoid lipid composition altering the stability of protein complexes in the membranes is attractive. Leaves of winter rye grown at 5°C show a large decrease in the *trans*-Δ^3-hexadecanoic acid (*trans*-16:1) level associated with phosphatidyldiacylglycerol (14). LHCII isolated from these leaves had markedly decreased levels of this fatty acid (18) and was found to produce an increased ratio of the monomeric:oligomeric forms of LHCII when solubilized and fractionated on nondenaturing gels compared with leaves grown at 20°C (9, 18). Consequently, it has been suggested that the cold-induced decrease in the *trans*-16:1 is associated with a reduced stability of the oligomeric form of LHCII in the thylakoids (10, 14, 18). A similar situation may account for the observed increase in the ratio of monomeric:oligomeric LHCII in maize leaves as growth temperature is decreased (Fig. 5). However, the chill-induced decrease in stability of oligomeric LHCII does not appear to be associated with a decreased level of LHCII polypeptides in either winter rye (9) or maize (Fig. 2). In the case of the decrease in the accumulation of the chloroplast-encoded polypeptides in maize thylakoids at low temperatures, a decrease in their stability in the membrane may occur due to changes in lipid complement. However, the molecular basis of this instability could be quite different from that determining the loss of stability of oligomeric LHCII.

At low growth temperatures, a number of polypeptides of molecular mass 10 to 22 and 31 kD accumulate in the membranes. The 31-kD polypeptide has previously been identified tentatively as an unprocessed precursor of the apoprotein of the pigment-protein CP29, which accumulates at low temperatures in maize, probably due to inactivation of the processing peptidase (12). The lower molecular mass polypeptides, as yet unidentified, may be synthesized in response to low temperatures, although their net synthesis would have to be associated with early stages of leaf development since in leaves approximately halfway through expansion these proteins do not accumulate to detectable levels during a 3-h pulse of [^{35}S]methionine (Fig. 3). There are many reports of the accumulation of plant proteins in response to temperature change (5, 17, 26), and it has been suggested that stress-induced proteins may function to optimize metabolic activities that have been perturbed by the stress (5). Alternatively, the low molecular mass polypeptides that accumulate in maize at low temperatures may be breakdown products of higher molecular mass proteins, which have been exposed to peptidases as a result of changes in protein folding or membrane organization.

In the context of chill-induced damage to maize crops, it is important to assess whether the perturbations in thylakoid protein metabolism observed when maize leaves are grown at suboptimal temperatures in controlled-environment cabinets also occur in the field during the early growing season. In controlled environments temperatures of 17°C and below were found to perturb thylakoid protein metabolism. Although such temperatures are regularly experienced during crop development in temperate regions, plants in the field are rarely exposed to prolonged periods of constant temperatures, as would be the case in controlled environment cabinets. The varied and often rapid fluctuations in temperature in the field may produce quite different effects on plants. Our field studies have indicated that perturbations to thylakoid protein metabolism did occur in maize leaves during May and early June when the crop is exposed to low temperatures. Although temperatures well below 14°C were often experienced during this period, the effects on thylakoid proteins were not as dramatic as those observed in plants grown in controlled environments at 14°C and more similar to those found in 17°C-grown plants. Some of the polypeptides (30–35, 50–70

kD), which failed to accumulate in significant amounts in 14°C compared with 25°C-grown leaves, were found in leaves developing in the field during May and early June. However, 16- and 42-kD polypeptides did not accumulate during May and early June at the levels found in leaves developing later in the season and in 25°C-grown leaves. Also, leaves developing in the early growing season accumulated a number of thylakoid proteins (14–20 kD), similar to those found in 14°C-grown leaves, and exhibited considerably reduced rates of net thylakoid protein synthesis than were found in late June and July. Consequently, it would appear that in our maize field crop, chill-induced perturbations of thylakoid protein metabolism did occur during the early growing season and could have important implications for the photosynthetic competence of the crop during this period.

In conclusion, it is clear that suboptimal growth temperatures can produce perturbations of thylakoid protein metabolism in maize leaves, which reduce the capacity for electron transport and may have important consequences for carbon assimilation. Chill-induced perturbations of thylakoid protein metabolism can also occur in the field during the early growing season in temperate regions. However, the relationships between such thylakoid perturbations and chill-induced depressions in the quantum yield and maximum capacity of CO_2 assimilation during leaf growth have yet to be established.

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