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High light induced accumulation of two isoforms of the CF_1 α-subunit in mesophyll and bundle sheath chloroplasts of C_4 plants

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The effect of light irradiance on the amount of ATP synthase α-subunit in mesophyll (M) and bundle sheath (BS) chloroplasts of C_4 species such as maize (*Zea mays* L., type NADP-ME), millet (*Panicum miliaceum*, type NAD-ME) and guinea grass (*Panicum maximum*, type PEP-CK) was investigated in plants grown under high, moderate and low light intensities equal to 800, 350 and 50 μmol photons m^{-2} s^{-1}, respectively. The results demonstrate that α-subunit of ATP synthase in both M and BS chloroplasts is altered by light intensity, but differently in the investigated species. Moreover, we identified two isoforms of the CF_1 α-subunit, called α and $\acute{\alpha}$. The CF_1 α-subunit was the major isoform and was present in all light conditions, whereas $\acute{\alpha}$ was the minor isoform in low light. A strong increase in the level of the $\acute{\alpha}$-subunit in maize mesophyll and bundle sheath thylakoids was observed after 50 h of high light treatment. The α and $\acute{\alpha}$-subunits from investigated C_4 species displayed apparent molecular masses of 64 and 67 kDa, respectively, on SDS/PAGE. The presence of the $\acute{\alpha}$-subunit of ATPase was confirmed in isolated CF_1 complex, where it was recognized by antisera to the α-subunit. The N-terminal sequence of $\acute{\alpha}$-subunit is nearly identical to that of α. Our results indicate that both isoforms coexist in M and BS chloroplasts during plant growth at all irradiances. We suggest the existence in M and BS chloroplasts of C_4 plants of a mechanism(s) regulating the ATPase composition in response to light irradiance. Accumulation of the $\acute{\alpha}$ isoform may have a protective role under high light stress against over protonation of the thylakoid lumen and photooxidative damage of PSII.

Keywords: ATP synthase, bundle sheath, C_4 subtypes, CF_1 α-subunit isoforms, chloroplasts, light intensity, mesophyll

INTRODUCTION

Light is the energy source and a regulatory signal for photosynthesis. Depending on conditions under which plants are grown, major differences can be observed in the levels and activity of protein complexes in the thylakoid membranes (Lee & Whitmarsh, 1989; Anderson *et al.*, 1995). Under high light conditions there is an increase in the amount of electron transport complexes, ATP synthase and the components of the Calvin cycle, while there is a reduction in the level of light-harvesting complexes (Bailey *et al.*, 2001; Walters, 2005). These effects correlate with increases in the rate of photosynthesis (Evans & Vogelmann, 2003). On the other hand, exposure of plants to excess light can induce photoinactivation and photodamage some of photosynthetic proteins (Aro *et al.*, 1993). In order to overcome or protect photosynthetic apparatus against light stress, plants have evolved effective mechanisms of acclimation to minimize the harmful effects of excess light. The adjustments in the stoichiomery of the main proteins and in the light-harvesting complexes of the

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Abbreviations: BN, blue-native; BS, bundle sheath; CF_1, chloroplast coupling factor; HL, high light; M, mesophyll; ML, moderate light; LL, low light; NAD-ME, NAD-malic enzyme; NADP-ME, NADP-malic enzyme; PEP-CK, phosphoenolpyruvate carboxykinase; PSII, photosystem II; SDS/PAGE, sodium dodecyl sulfate/polyacrylamide gel electrophoresis; TCAA; trichloroacetic acid.

two photosystems are optimized in response to environmental conditions. There is a strong evidence that redox signals (Kim & Mayfield, 2002; Pfannschmidt, 2003), energy status (Melis *et al.*, 1985; Huner *et al.*, 1998) and sugar levels (Oswald *et al.*, 2001) play important roles in the regulation of light-induced changes in the composition, structure and function of chloroplasts. However, it is not clear whether in all C_4 species the same processes regulate the responses to light quantity. In C_4 plants, where chloroplasts in mesophyll (M) and bundle sheath (BS) cells differ structurally and functionally (Edwards *et al.*, 2001), light intensity may act in different manners on both types of chloroplasts. There is evidence that the amount of ATP may depend on light intensity and concentration of organic oxidants (Edwards & Huber, 1981; Sailaja & Rama Das, 2000). The different photosynthetic acclimation patterns to growth-limiting irradiance observed in the two metabolic types of C_4 plants (Sailaja & Rama Das, 2000) may indicate that in these plants acclimation to light intensity is realized by dynamic changes in chloroplast proteins and/or activity of main photosynthetic enzyme(s). How C_4 species are able to optimize the composition of the photosynthetic apparatus to light intensity in both mesophyll and bundle sheath chloroplasts to minimize the harmful effects of excess light is unknown. During our study on the effects of irradiance on photosystems' activity and content of protein complexes in chloroplasts of C_4 plants, we observed a significant increase of ATP synthase activity in response to high light in M and BS chloroplasts (not presented). Further results showed that two CF_1 α isoforms coexist in chloroplasts, the α' isoform being more abundant in high than in low light-grown plants. The main goal of this work was to gain information about the relative amounts of thylakoid ATP synthase α-subunits in mesophyll (M) and bundle sheath (BS) chloroplasts of various C_4 subtypes grown in different irradiances.

Our data suggest that ATP production in C_4 plants is regulated by two separate mechanisms operating: 1) under normal growth conditions, and 2) when plants are exposed to stressful environment. The metabolic separation of M and BS cells plays an important role in the acclimation processes because it enables the regulation of ATP/ADP ratio in both types of chloroplasts. We identified a light-dependent isoform of the CF_1 α-subunit, in all C_4 plants investigated. Because the level of the $\acute{\alpha}$-subunit significantly increased when light irradiance increased, accumulation of this isoform may have a protective role for C_4 plants under high light to prevent the thylakoid lumen from over-protonation and from photooxidative damage of PSII.

MATERIALS AND METHODS

Plant materials and growth conditions. The C_4 plants such as maize (*Zea mays* L., type NADP-ME), millet (*Panicum miliaceum*, type NAD-ME) and guinea grass (*Panicum maximum*, type PEP-CK) were grown on vermiculite in a growth chamber under a 14 h photoperiod and a day/night temperature of 24/19°C under low (LL), moderate (ML) and high (HL) light intensity (approx. 50, 350 and 800 μmol photons m^{-2} s^{-1}). Plants were fertilized with Knop's solution. Leaves were harvested from 3–4-week-old plants of maize and 4–5-week-old plants of millet and guinea grass.

Isolation of chloroplasts and thylakoids. Chloroplasts (and thylakoids) from mesophyll (M) and bundle sheath (BS) cells were isolated using the mechanical method described by Romanowska *et al.* (2006). Chlorophyll concentration was quantified after extraction with 80% acetone as described by Arnon (1949). Chloroplasts were used immediately or stored at –80°C. All isolation procedures were performed at 4°C using ice-chilled media. The protein content was determined by the method of Bradford (1976).

SDS/PAGE and protein immunodetection. Electrophoresis was conducted in 12% or 15% SDS/PAGE gels according to the method of Laemmli (1970). Gels were stained with a 0.25% solution of Coomassie Brilliant Blue R-250 and distained in 10% aldehyde-free acetic acid/45% methanol.

For immunodetection following electrophoresis, proteins were transferred onto a PVDF membrane (Millipore, Badford, MA, USA) as described by Towbin *et al.* (1979). The alkaline phosphatase color development reaction was used to visualize immunoreactive proteins. Membranes were probed with antibodies specific to the α-subunit of chloroplast coupling factor. INGENIUS densitometry (SynGene) was used for quantitative analysis of α and $\acute{\alpha}$ protein bands on the gels and membranes.

Two-dimensional BN/SDS/PAGE was performed as described by Kügler *et al.* (1997).

CF_1 purification. CF_1 was isolated as described by Leegood & Malkin (1986). Isolated chloroplast membranes were washed three times in cold 10 mM sodium pyrophosphate buffer (pH 7.4) and collected by centrifugation at 35 000 × *g* for 10 min at 4°C. Washed membranes were resuspended in 2 mM Tricine/NaOH buffer (pH 7.8) containing 0.3 M sucrose and stirred in the dark for 15 min. The membranes were collected by centrifugation at 35 000 × *g* for 15 min. The supernatant containing the CF_1 proteins was used for protein determination by the method of Bradford (1976). Proteins were precipitated with TCAA added to 20%, and the solution was incubated at 4°C for 10 min to allow precipitate formation (Sambrook & Russel, 2001). The pro-

teins were collected by centrifugation at 14 000 × *g* for 5 min. Pellet was washed with cold acetone and proteins were collected by centrifugation as above. The procedure was repeated two times. The pellet was heated at 95°C for 5 min to remove acetone and then was solubilized in electrophoresis sample buffer and used for electrophoresis as described above.

Determination of protein sequences. Protein samples were separated by analytical SDS/PAGE and visualized with Coomassie Blue staining. Protein bands of interest (*ά*-subunit of ATPase) were excised from the gels and analyzed by mass spectrometry at the Laboratory of Mass Spectrometry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences (Warszawa, Poland). Gel slice was digested with semiTrypsin to short (preferably 5–25 aa long) fragments. Mass spectrometry data were analyzed with the use of Mascot search engines (www.matrixscience.com) (Perkins *et al.*, 1999).

All experiments were repeated at least 4–5 times.

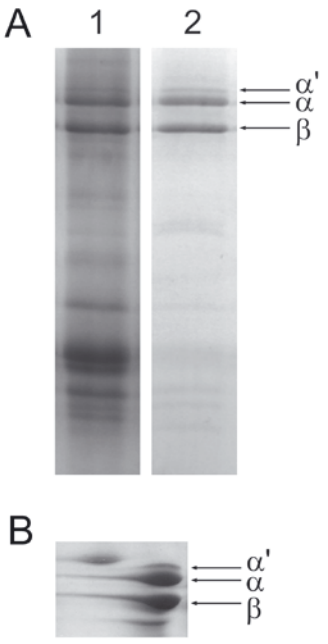

Figure 1 (A). Electrophoretic identification of α, ά and β CF_1 proteins in thylakoid membranes obtained from maize mesophyll chloroplasts (1) and in the isolated CF_1 (2).
Plants were grown under high irradiance (HL). Polypeptides were separated in 15% polyacrylamide gel and stained with Coomassie Blue. Sample containing 16 μg of chlorophyll was loaded in lane 1, 14 μg protein was loaded in lane 2.
(B) Fragment of 2D-BN/SDS-PAGE gel of solubilized mesophyll thylakoid membranes.
The proteins on the gel are identified as α, ά and β-subunits of CF_1 complex (as in lane 1, A). Electrophoresis was performed as described by Kügler *et al*. (1997). Thylakoids (equivalent to 50 μg chlorophyll) were solubilized with 1% *n*-dodecylmaltoside.

RESULTS

High light induced accumulation of 67 kDa protein

During our initial analysis of thylakoid proteins we found accumulation of a 67 kDa protein in leaves of maize plants grown in excess light conditions. By using antibodies this protein was identify as a component of CF_1. In order to identify this component CF_1 complex was isolated from mesophyll thylakoid membranes obtained from leaves of plants grown under high light conditions. Analysis of proteins on the gel showed a 64 kDa component to be the CF_1 α-subunit (Fig. 1). The upper band with molecular mass 67 kDa was additional α-subunit, which we named the ά isoform. This subunit was detected previously in monocot leaves (Burkey, 1992; Burkey & Mathis, 1998). To confirm the existence of the ά-subunit in the CF_1 complex, tylakoid proteins were separated by two-dimensiolal BN/SDS/PAGE. (Fig. 1B). The obtained results provided a direct evidence that both isoforms coexist in thylakoid membranes from maize mesophyll cells. We then asked if the ά-subunit could have a role in acclimation of C_4 plants to high light irradiance and whether its accumulation might be cell-specific.

Identification of ά-subunit in CF_1 complex isolated from mesophyll and bundle sheath chloroplasts of HL-grown maize. Effect of continuous high light treatment

The presence of the ά-subunit was also confirmed in the CF_1 complex isolated from M and BS chloroplasts of maize. The polypeptide composition of this complex was analyzed in a 12% acrylamide gel stained with Coomassie Blue (Fig. 2A) or by immunobloting analysis using CF_1 α antisera (Fig. 2B). The 67 kDa protein labeled ά was the highest molecular mass component observed in the extract and represented a small percentage of the total protein (Fig. 2A), but was better visible on immunoblots (Fig. 2B). The lower bands on the gel corresponded to the α and β CF_1 proteins with apparent molecular masses of 64 and 58 kDa, respectively. Thus, the ά-subunit is present in the CF_1 complex of both M and BS chloroplasts.

A strong increase in the level of the ά-subunit in mesophyll and bundle sheath thylakoids of maize was observed in plants exposed to HL and those illuminated continuously with high light (Fig. 3). After 50 h of HL-treatment the level of the ά isoform was as high as for the α-subunit in both types of chloroplasts.

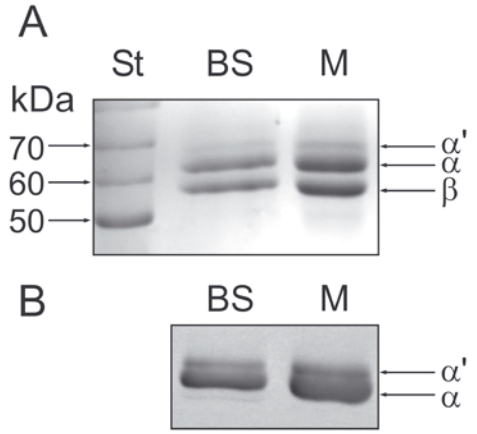

Figure 2 (A) Electrophoretic identification of α, ά and β CF_1 subunits in CF_1 isolated from mesophyll (M) or bundle sheath (BS) chloroplasts of maize plants grown under high irradiance (HL).
Polypeptides were separated in 15% polyacrylamide gel and proteins were stained with Coomassie Blue. 14 μg protein was loaded in each lane.
(B) Immunodetection of CF_1 α-subunit isoforms.
Polypeptides were separated in 12% polyacrylamide gel and blotted onto PVDF as described in Material and Methods. Each lane was loaded on an eqal protein basis (3 μg per lane) and probed with CF1 α antisera. Molecular mass markers (in kDa) are indicated at left.

Effects of light irradiance on the level of α and ά-subunit in M and BS chloroplasts of C_4 plants

We next examined whether changes in light intensity during growth have any influence on the amount and composition of isoforms of ATP synthase α-subunit in M and BS chloroplasts in plants of C_4 subtypes, represented by *Z. mays*, *P. maximum* and *P. miliaceum*. The level of α and ά-subunits was estimated in chloroplasts of plants grown under low, moderate and high light conditions (50, 350 and 800 μmol photons m^{-2} s^{-1}, respectively). An antiserum directed against maize CF_1 α-subunit was used for detection of the two isoforms. Both α and ά proteins

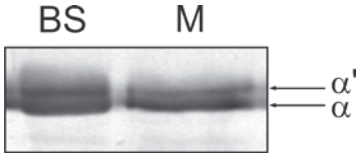

Figure 3. Immunodetection of CF_1 α-subunit isoforms in thylakoids isolated from mesophyll (M) and bundle sheath (BS) chloroplasts of maize.
Plants were grown under HL as described in Material and Methods, thylakoids were isolated from leaves after 50 h of continuous HL illumination. Polypeptides were separated in 12% polyacrylamide gel and blotted onto PVDF as described in Material and Methods. Equal amount of protein (3 μg) was loaded in each lane and probed with CF_1 α antisera.

of the CF_1 complex were present in M and BS thylakoids of C_4 plants investigated (Fig. 4). The level of the ά-subunit exhibited a similar irradiance response in all types of C_4 plants and increased when the light intensity increased during growth period. No differences were observed in the molecular mass of the isoforms isolated from the investigated species and they were approx. 67 and 64 kDa. The molecular masses estimated from SDS/PAGE are larger than those calculated from the sequence (Howe *et al.*, 1985). This discrepancy may be related to unknown characteristics of the primary structure that alter migration in the gel. Burkey and Mathis (1998) also observed α and ά-subunits in monocot plants, but with the molecular masses of 61 and 64 kDa. They found large differences in the molecular mass of CF_1 α obtained from spinach, pea, and soybean plants. As expected, in our experiment the accumulations of CF_1 α and ά-subunits were higher in M chloroplasts isolated from plants growing in higher light intensity than those in lower one (Fig. 4A). The ratio of α-subunit in ML-grown/LL-grown plants was about 1.2 for all investigated species. Whereas similar ratio for ά-subunit was 1.7 for both *Panicum* species and 2.4 for *Z. mays*. On the other hand these ratios in HL-grown/LL-grown maize plants were higher than in ML-grown/LL-grown plants and were found to be 2 and 4 for α and ά-subunit, respectively. In BS chloroplasts the α protein level was higher than in M for all species and was the same in ML-grown and LL-grown plants (Fig. 4B). However, the content of the ά-subunit in BS chloroplasts in *Z. mays* and *P. maximum* depended on light intensity whereas in *P. miliaceum* the ά-subunit level in ML-grown plants was comparable to that in LL-grown plants.

Identification by sequence analysis of the 67-kDa protein as an isoform of the CF_1 α-subunit

Mass spectrometry data analysis identified the 67-kDa protein as α-subunit of CF_1 (Fig. 5). The 291 aa identified cover 57% of the amino acids sequence of maize CF_1 α-subunit. These results provided direct evidence that the 67-kDa protein was an isoform of the α-subunit of CF_1.

Accumulation of the CF_1 α isoforms in M and BS chloroplasts of maize during light acclimation

A light acclimation experiment was conducted to answer the question if both isoforms coexist during chloroplast acclimation and whether changes are characteristic for granal and agranal chloroplasts. Maize was selected for this experiment because the effect of light irradiance on the polypeptide content and ATP syntase activity was clearly visible in M and BS chloroplasts (Drozak

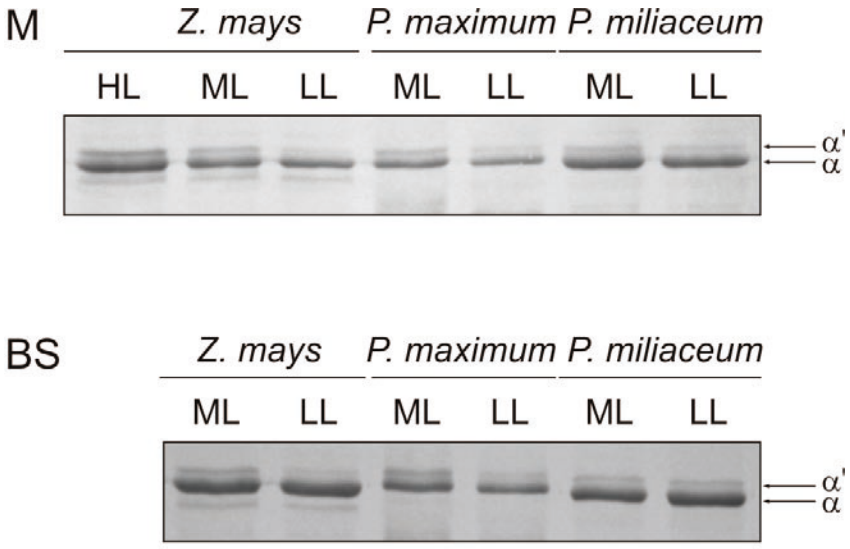

Figure 4. Effects of growth irradiance on the level of CF_1 α-subunit isoforms in chloroplasts of C4 subtypes.
Thylakoid membrane polypeptides from mesophyll (M) and bundle sheath (BS) chloroplasts of *Z. mays*, *P. maximum* and *P. miliaceum* were separated in 12% polyacrylamide gel and blotted onto PVDF as described in Material and Methods. Lanes used for immunoblots contained thylakoid equivalent to 2 μg of chlorophyll. LL, ML and HL indicate thylakoids from plants grown under low, moderate and high irradiance, respectively.

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  1 MATLRVDEIN KILRERIEQY NRKVGIENIG RVVQVGDGIA RIIGLGEIMS GELVEFAEGT
 61 RGIALNLESK NVGIVLMGDG LMIQEGSFVK ATGRIAQIPV SEAYLGRVIN ALAKPIDGRG
121 EIVASESRLI ESPAPGIISR RSVYEPLQTG LIAIDSMIPI GRGQRELIIG DRQTGKTAVA
181 TDTILNQKGQ DVICVYVAIG QRASSVAQVV TTFHEEGAME YTIVVAEMAD SPATLQYLAP
241 YTGAALAEYF MYRERHTLII YDDLSKQAQA YRQMSLLLRR PPGREAYLGD VFYLHSRLLE
301 RAAKLNSLLG EGSMTALPIV ETQSGDVSAY IPTNVISITD GQIFLSADLF NAGIRPAINV
361 GISVSRVGSA AQIKAMKQVA GKSKLELAQF AELQAFAQFA SALDKTSQNQ LARGRRLREL
421 LKQSQSNPLP VEEQVATIYT GTRGYLDSLE IEQVKKFLDE LRKHLKDTKP QFQEIISSSK
481 TFTEQAETLL KEAIQEQLER FSLQEQT
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Figure. 5. Identification of peptides from the 67-kDa protein (ά-subunit of CF_1) as internal sequences of the α-subunit of CF_1.
The predicted amino-acid sequence of the maize CF_1 α-subunit is presented (Strittmatter & Kassel, 1984). In bold, amino acids definitely identified and corresponding to the sequences of maize CF_1 α-subunit.

& Romanowska, 2006). Maize plants were grown in LL (50 μmol m^{-2} s^{-1}) and then were transferred to ML (350 μmol m^{-2} s^{-1}) (LL → ML) or to HL (800 μmol m^{-2} s^{-1}) (LL → HL) for 72 h. Thylakoids were isolated from M and BS chloroplasts of LL-grown plants, and after acclimation to ML or HL. Polypeptides were subjected to immunoblotting analysis using CF_1 α antisera (Fig. 6). Interestingly, the level of the CF_1 ά-subunit was very low in both M and BS chloroplasts in LL-grown plants but transfer to higher irradiance caused its enhanced accumulation. The level of the 67 kDa protein in CF_1 depended on light intensity and its relative amount strongly reflected the new light environment. The accumulation of ά isoform was higher in plants transferred from LL to ML or to HL than in plants continuously grown at ML or HL conditions.

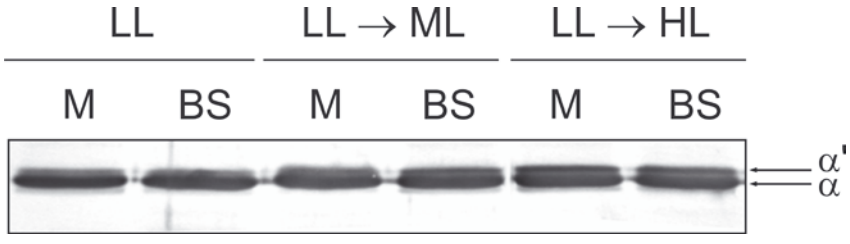

Figure 6. Effect of light acclimation on the levels of α and ά CF_1 proteins in mesophyll (M) and bundle sheath (BS) chloroplasts of maize.
Thylakoids from LL-grown plants and from plants transferred from LL to ML (LL → ML) or to HL (LL → HL) were isolated. Polypeptides were separated in 12% polyacrylamide gel and blotted onto PVDF as described in Material and Methods. Sample equivalent to 2.5 μg of chlorophyll was loaded in each lane and probed with CF_1 α antisera.

DISCUSSION

In chloroplasts, synthesis of ATP is coupled with the utilization of the proton gradient formed by photosynthetic electron transport (Hisabori *et al.*, 2002). The ATP synthase activity is modulated by reversible reduction of a disulfide bridge in the γ-subunit and light intensity is an important factor responsible for this modulation (Strotmann *et al.*, 1986; Groth & Strotmann, 2000). Light irradiance also controls the level of chloroplast proteins (Chow & Anderson, 1987) and influence on photosynthetic electron transport capacity (De la Torre & Burkey, 1990). Photosynthetic organisms show various acclimation responses to changing light intensity in the environment.

We examined the level of ATP synthase α-subunit isoforms in mesophyll and bundle sheath chloroplasts of C_4 plants grown in low (LL), moderate (ML) and high (HL) light intensities and during acclimation maize plants transfered from LL to ML or to HL conditions.

We found two isoforms of CF_1 α-subunit with apparent molecular masses of 64 and 67 kDa in the all investigated C_4 plant species and at all light intensities (Figs. 1, 2 and 4). The 64 kDa protein was routinely identified as CF_1 α-subunit whereas the 67 kDa protein was an ά isoform (Fig. 5). The CF_1 α-subunit was weakly stimulated by light intensity in mesophyll (M) and bundle sheath (BS) chloroplasts of *Z. mays* and *P. maximum* and in M of *P. miliaceum*. In contrast, accumulation of the ά isoform was strongly irradiance-dependent and was correlated with the electron transport activity in the chloroplasts (not presented). The level of the ά isoform in maize chloroplasts increased proportionally to light intensity. Increased ATPase activity and accumulation the of α and β isoforms in high light was also observed in monocot plants (Burkey, 1992; Burkey & Mathis, 1998) and in *Brassica rapa* (Jiao *et al.*, 2004), respectively. The presence of an additional β subunit was described in pollen mitochondria from *Nicotiana silvestris* (De Paepe *et al.*, 1993). Very little is presently known about the signal transduction pathways underlying photosynthetic acclimation. It is known that expression of many genes is light-regulated, including that for CF_1 α-subunit (Walters & Horton, 1994). Rodermel and Bogorad (1987) suggested that the plastid genome responds actively to adaptive signals generated by changing environment and that the flanking regions of *atpA* contain species-specific regulatory sequences (in maize photoregulated promoter sequences).

The presence of the ά protein in certain species, and its absence in others may suggest that this component requires special factors responsible for the changes in its concentration. The presence of the ά isoform in C_4 plants might, for instance, explain their resistance to high temperature and strong light (Edwards *et al.*, 2001).

There is no evidence that the difference in molecular mass of about 3 kDa between the α-subunit of CF_1 and its ά isoform could result from the use of an alternative translation start site (Howe *et al.*, 1985). The lack of peptides homologous to the amino-acid sequence generated by conceptual translation of the nucleotide sequence preceding the usual start codon of the *atpA* gene seems to exclude the presence of the alternative translation start site. Also editing events which have been detected for several transcripts of the maize plastome, including *atpA* transcript, can not explain such significant differences, because they usually result in single amino acid substitute (Maier *et al.*, 1995). All of those lead to the conclusion that the most likely mechanism of the ά isoform formation is by posttranslational modification. Protein glycosylation within CF_1 (Maione & Jagendorf, 1984) seems to be an attractive possibility because it could also explain the discrepancy between the SDS/PAGE estimate of the α-subunit molecular mass and that calculated from sequence data. Andreau *et al.* (1978) reported that carbohydrates bound to CF_1 amounted to 4.5% (wt/wt) of the protein, which can explain the differences in the molecular mass of both isoforms. However, the mechanism of isoform formation and its physiological role need further comprehensive studies. We believe that in high light conditions where the electron transport efficiency is enhanced the amount of the electron transport components and the potential for photodamage also increase (Drożak & Romanowska, 2006), and under such conditions glycosylation of the α-subunit would play a role in determining the activity of the whole ATP synthase. The identification of the isoform(s) of CF_1CF_o subunits and knowledge how environmental factors affect ATP synthase structure, allows in the future on investigation the regulation of the ATP synthase activity. When plants grow in stable environmental conditions they develop mechanism(s) responsible for optimal efficiency of photosynthesis and the redox signal derived from photosynthetic electron transport plays an important regulatory role by modulation of expression of genes encoding photosynthetic components. It is also possible that in constant conditions redox changes are too small to be measured but plants respond to these changes. When the plants are suddenly subjected to stress conditions (e.g. LL → HL) another acclimation mechanism may be activated, including metabolic signals and the alteration of ATP/ADP ratio. When we illuminated maize plants with continuous high irradiance, enhanced accumulation of CF_1 ά-subunit was observed in both mesophyll and bundle sheath chloroplasts (Fig. 3). Transferring of plants from LL

to ML or to HL caused increase the level of the ά isoform more than would be expected (Fig. 6). We suppose that enhanced accumulation of ά isoform in HL-grown maize plants contributes to the photoprotection of ATP synthase. According to our hypothesis, excess light may induce glycosylation of CF_1. It may be essential in the regulation of proton gradient and dissipation of excess energy. The extent of acclimation varies between species in accordance with metabolic differences among chloroplasts and energy demand (Edwards *et al.*, 2001). In BS chloroplasts of *P. maximum* where the demand for ATP is higher than in *P. miliaceum* (Romanowska & Drożak, 2006) both the α and ά isoform accumulation are HL-stimulated.

The observation that in maize HL-dependent increased level of the ά isoform is intriguing. This may indicate that not the same signal operates in low and high light intensity. The extent of acclimation does not simply depend on the photon flux density, but rather depends both on protein content and intensity of electron transport (Drożak & Romanowska, 2006).

We can conclude that acclimation to light intensity observed for α CF_1 isoforms in both granal and agranal chloroplasts may suggest that this kind of responses to light are universal. The presence of regardless CF_1 α isoforms, of their physiological significance, may be a general feature of chloroplast ATP synthase complexes in many other plant species.

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