Changes of the Polypeptide Composition in Thylakoid Membranes during Differentiation

Magdolna Droppa, Jiri Masojidek, and Gábor Horváth

Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Sciences, P.O. Box 521, Szeged, Hungary. H-6701

Z. Naturforsch. **45c,** 253–257 (1990); received October 13, 1989/January 2, 1990

Chloroplasts, Greening, Membrane Proteins, Photosynthesis, Thylakoids

Changes in membrane polypeptide composition during greening of etiolated maize were investigated to confirm the existence of the developmental polypeptides of 12–15 kDa described recently in virescent soybean mutant [M. Droppa, M. L. Ghirardi, G. Horváth, and A. Melis, Biochim. Biophys. Acta **932,** 138–145 (1988)]. These low molecular weight polypeptides were the most abundant proteins at the early stage of greening, but were largely absent from fully developed thylakoids. During greening the relative concentration of the 12–15 kDa polypeptides were inversely proportional to that of LHC II, suggesting a role of these polypeptides in the assembly of the LHC II and/or chloroplast development.

Introduction

The biogenesis of chloroplast thylakoids is an intriguing but still not completely understood process. The development of proplastids into photochemically active chloroplasts is arrested at an intermediate stage when plants are kept in the dark. Upon illumination, these etioplasts differentiate into chloroplasts. Many aspects of this transition have been intensively investigated (for review see [1–4]). During the past decade, for example, interest has been increasing in studying the protein assembly of differentiating thylakoids.

Mature chloroplast membranes contain approximately 45 different polypeptides [5]. A number of these polypeptides can even be detected in etioplasts, while others are newly synthesized upon illumination [5, 6]. The biosynthesis and accumulation of the light-harvesting complex of photosystem II (LHC II), which is the major protein component of the thylakoid, is also light-regulated. The light-harvesting complex proteins (LHCPs) are encoded in the nucleus and synthesized on cytoplasmic ribosomes as high molecular weight precursor forms [7]. LHCP is absent from etioplasts of angiosperms and accumulates during the greening process [6, 8–10]. The transcription of genes coding for LHCP is regulated by phytochrome [4], and its accumulation is coordinated with chlorophyll biosynthesis which is also light-dependent [8, 9, 11]. In addition, the structural development of the chloroplast is both triggered by, and dependent on, light [12]. One might expect, therefore, that aspects of the import and processing of LHCP as well as its assembly into the thylakoid membrane is related to plastid development. In spite of the fact that our knowledge about the synthesis, transport and processing of LHCP has been extended remarkably during the past years, the regulatory mechanism of the insertion of LHCP into the thylakoid is absolutely unknown at the moment.

In the Y_9Y_9 virescent mutant of soybean having a reduced LHC content, two novel polypeptides of 12–15 kDa were recently found [13]. These low molecular weight polypeptides were the most abundant protein in the thylakoid when the mutation was fully expressed but were largely absent from both the mature wild type and fully developed Y_9Y_9 chloroplasts. The relative concentration of polypeptides in the 12–15 kDa region was inversely proportional to that of LHC II, thus it was assumed that they have a functional role in the assembly of LHC II and chloroplast development. Since these observations were obtained with mutant soybean only, it is necessary to verify them with further experiments carried out on other plant species. Furthermore, if these proteins have a developmental role, they must be observed in normal chloroplasts during differentiation as well.

In the present work, therefore, the changes of the polypeptide composition of maize thylakoids

Reprint requests to Gábor Horváth, Biological Research Center, P.O. Box 521, Szeged, Hungary, H-6701.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/90/0300–0253 $ 01.30/0

were analyzed at various stages of greening. In agreement with our previous results [13], the low molecular weight polypeptides were the most abundant proteins of the thylakoid at the early stage of greening and their disappearance was inversely proportional with the enhancement of LHC II.

Materials and Methods

Seedlings of maize (*Zea mays* L. *cv.* MTC 255) were grown on moist filter paper at 25 °C in darkness. The 7 day-old seedlings were illuminated for different periods by continuous white light of 2500 lux.

Plastids from developing leaves were isolated in dim green light at 0 °C as described previously [14]. The isolated plastids were disrupted by osmotic shock in 20 mM Tricine-KOH buffer (pH 7.8) containing 40 mM sucrose, 10 mM NaCl and 5 mM $MgCl_2$.

The thylakoids were washed twice in hypotonic buffer and resuspended in 20 mM Tricine and 10% sucrose, and denatured at 100 °C for 3 min in the presence of 2% SDS and 2% of mercaptoethanol. The analysis of membrane polypeptides was carried out by SDS polyacrylamide gel electrophoresis in a 10–17% (w/v) linear gradient of polyacrylamide containing 0.1% SDS using the buffer system of Chua [15, 16]. The proteins in the polyacrylamide gels were stained with Coomassie brilliant blue and scanned linearly at 580 nm for the quantitation of the density of Coomassie stain. Care was exercised to ensure the linearity between A_{580} and the amount of protein loaded to the gel.

The chlorophyll content was determined in 80% acetone extract according to the method of Arnon [17], and the protein content of the washed thylakoids was estimated on the basis of the technique by Markwell *et al.* [18].

Results and Discussion

The time course of greening strongly depends on the environmental conditions such as the intensity of illumination, light quality, water supply and humidity, etc. [19]. These factors markedly influence the onset and velocity of pigment and protein synthesis, the membrane content of chloroplasts and others. Thus a comparison of developmental data obtained in various laboratories is difficult, especially if the time course of greening is not characterized. Usually, the chlorophyll accumulation is used as such a "marker" since its synthesis is completely light-regulated. Fig. 1 A shows that, in our experiments, the Chl accumulation has a relatively short lag period, and is complete within 72 h with the major changes in the Chl *a*/*b* ratios between 0 and 24 h of illumination. For the protein content of thylakoids, a similar time course could be observed (Fig. 1 B). The changes in the protein/chlorophyll ratio indicate that at the early stages of greening, the chlorophyll synthesis was more intense than that of the proteins, but after 24 h of illumination, both Chl and proteins accumulated at the same velocity.

The polypeptide composition of thylakoid membranes isolated at various times of greening is shown in Fig. 2. In etiolated thylakoids, practically three major groups of polypeptides could be distinguished in the 56–63 kDa, 36–40 kDa and 12–17 kDa region with several additional weak bands but no detectable amount of LHCP was present in the 29–25 kDa region. This result is

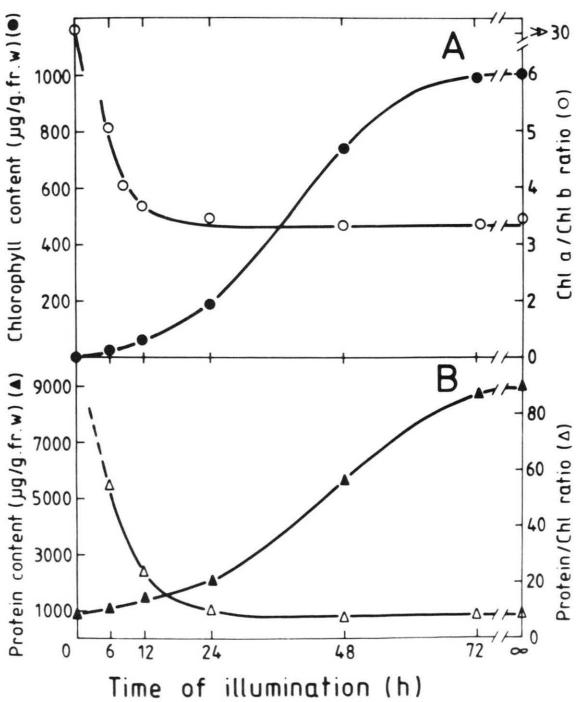

Fig. 1. The time course of the chlorophyll (A) and protein (B) accumulation during greening of maize leaves.

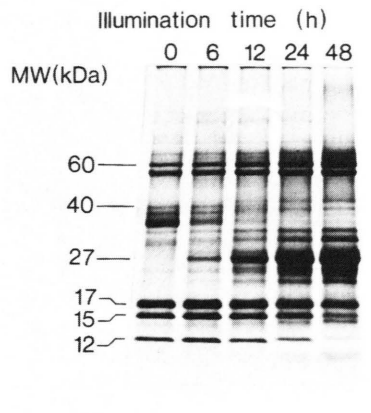

Fig. 2. Changes of the polypeptide pattern of isolated chloroplast membranes by progress of greening. (Thylakoids were washed twice by 20 mM Tricine/KOH buffer pH 7.8 containing 40 mM sucrose, 10 mM NaCl and 5 mM $MgCl_2$.)

consistent with that of Lindsten *et al.* [20] who found that the major polypeptides of the prolamellar body and prothylakoids were the α and β subunits of CF, in the 60 kDa region and the NADPH-protochlorophyllide oxidoreductase of 37 kDa with some additional small polypeptides. As we expected, however, on the basis of our earlier results obtained with Y_9Y_9 soybean mutant [13], an abundance of proteins was found in the 12–17 kDa low molecular weight region. During greening, the amounts of the 12 and 15 kDa polypeptides decreased and at the end of greening, the LHCP became the most abundant protein of the chloroplast thylakoid (Fig. 2).

An interesting question is why these low molecular weight proteins are visible only in particular, but not in all experiments. Further experiments, therefore, were conducted to answer the question by washing the thylakoids with various buffer systems. When tricine or HEPES were used for washing, all low molecular weight polypeptide were present in the gel (see Fig. 2). This result is consistent with those in which low molecular weight polypeptides were observed in developing chloroplasts [5, 21–26]. In all of these experiments, tricine or HEPES buffer system was used for thylakoid washing. When strong ionic buffers such as phosphate, pyrophosphate, TRIS, borate or EDTA were applied in all cases, the polypeptides of the 12–17 kDa region were present in matured thylakoids only (Fig. 3). From etioplasts or partially developed thylakoids, however, the low molecular weight polypeptides were mostly removed by washing, indicating that these peptides are only loosely bound to the prolamellar body or prothylakoids. It seems reasonable to assume that in those greening experiments which used strong ionic buffers for thylakoids isolation, the low molecular weight compound were removed by washing.

Fig. 4 presents a quantitation of the relative amounts of the major protein groups as a function of the greening time. It can be seen that the relative content of various polypeptides show very different time dependence during the progress of greening. The amount of polypeptides in the 12–15 kDa region, however, is inversely proportional to that of LHCP present in the membrane. This result is consistent with the earlier observation obtained with Y_9Y_9 virescent soybean mutant where the 12–15 kDa and 27–29 kDa polypeptides were also inversely proportional at various stage of chloroplast development [13]. It was demonstrated by immunoprecipitation that these low molecular weight polypeptides were not proteolytic products of LHC II and were not identical with the subunits of ATP synthase or the small subunit of ribulose bisphosphate carboxylase. The latter was also confirmed by our recent experiment, since maize mesophyll chloroplasts, operating with C_4-dicar-

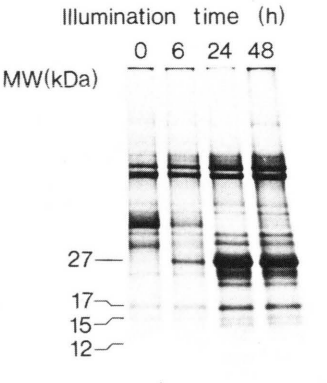

Fig. 3. The effect of pyrophosphate washing on the polypeptide pattern of greening thylakoids. (The washing medium as in Fig. 2 except the 20 mM Tricine was replaced by 20 mM pyrophosphate.)

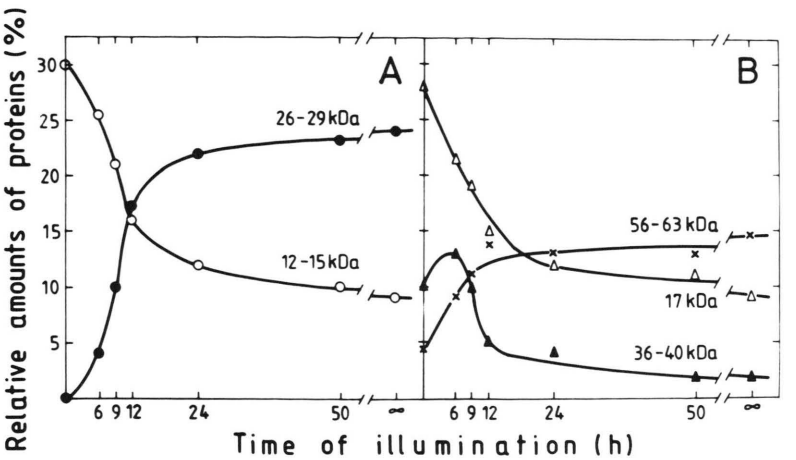

Fig. 4. Changes of the relative amount of membrane proteins during greening. The relative amounts of proteins were calculated from the integrated area of the respective bands of densitometric tracings scanned at 580 nm.

boxylic acid pathway, still exhibited a large number of low molecular weight polypeptides at 12–15 kDa region. Previously, it was proposed that these novel polypeptides have a functional role in the assembly of LHC II into the chloroplast membrane [13].

Work in other laboratories has indicated the presence of early light-induced proteins with unknown function migrating in the 13.5–17 kDa region [23, 25, 26]. Since these peptides appeared immediately after, or prior to, the illumination, it was suggested that these polypeptides play a regulatory role during development [23]. The relation of the 12–15 kDa polypeptides found in soybean [8] and maize and the 13.5–17 kDa polypeptides described in barley and peas [23, 25, 26] is not known. It is interesting to note, however, that the accumulation of LHC II in barley and peas was found to be delayed to that in soybean and maize indicating that the regulation of LHC II accumulation might be controlled by species differences [27]. One can speculate that the regulation of the appearance of low molecular weight polypeptides in the 12–17 kDa region preceding LHCP synthesis, may depend on plant species used. In other words, we assume that in those plants, for example maize and soybean, in which the LHC synthesis starts immediately after illumination, the low molecular weight polypeptides are synthesized even in the dark. By contrast, in barley and peas which have delayed LHC synthesis, the 12–15 kDa polypeptides are mainly accumulated after the onset of illumination. Since this is a speculation, it has to be confirmed by further experiments with different plant species having various timing in LHC II accumulation.

As a summary, we can say that the polypeptides of 12–15 kDa found earlier in soybean mutant [13] are present in developing maize thylakoids as well. Since their relative concentration was inversely proportional to that of LHC II, we propose that these novel polypeptides have functional role in the assembly of LHC II and/or chloroplast development.

[1] N. R. Baker and J. Barber (eds.), Chloroplast Biogenesis, Topics in Photosynthesis, **Vol. 5,** pp. 1–379, Elsevier/North Holland, Amsterdam 1984.
[2] J.-P. Dubacq and A. Trémolières, Physiol. Vég. **21,** 293 (1983).
[3] J. E. Mullet, Annu. Rev. Plant Physiol. **39,** 475 (1988).
[4] E. M. Tobin and J. Silverthorne, Annu. Rev. Plant Physiol. **36,** 569 (1985).
[5] G. Hoyer-Hansen and D. J. Simpson, Carlsberg Res. Commun. **42,** 379 (1977).
[6] K.-J. Dietz and L. Bogorad, Plant Physiol. **85,** 808 (1987).
[7] G. W. Schmidt, S. G. Bartlett, A. R. Grossman, A. R. Cashmore, and N.-H. Chua, J. Cell Biol. **91,** 343 (1982).
[8] E. Harel, Progr. Phytochem. **5,** 127 (1978).
[9] R. G. Hiller, T. B. G. Pilger, and S. Genge, Chloroplast Development (G. Akoyunoglou, ed.), pp. 215–220, Elsevier/North Holland Biomedical Press, Amsterdam 1978.
[10] A. Tanaka and H. Tsuji, Plant Cell Physiol. **26,** 893 (1985).
[11] J. Bennett, Eur. J. Biochem. **118,** 61 (1981).
[12] P. R. Chitnis, E. Harel, B. D. Kohorn, E. M. Tobin, and J. P. Thornber, J. Cell Biol. **102,** 982 (1986).
[13] M. Droppa, M. L. Ghirardi, G. Horváth, and A. Melis, Biochim. Biophys. Acta **932,** 138 (1988).
[14] J. Masojidek, M. Droppa, and G. Horváth, Eur. J. Biochem. **169,** 283 (1987).
[15] N.-H. Chua, Methods Enzymol. **69,** 434 (1980).
[16] P. Delepelaire and N.-H. Chua, Proc. Natl. Acad. Sci. U.S.A. **76,** 111 (1979).
[17] D. I. Arnon, Plant Physiol. **24,** 1 (1949).
[18] M. A. Markwell, S. M. Haas, N. E. Tolbert, and L. L. Beiber, Methods Enzymol. **72,** 296 (1981).
[19] G. Horváth, L. A. Mustárdy and Á. Faludi-Dániel, Chloroplast Development (G. Akoyunoglou, ed.), pp. 863–869, Elsevier/North Holland Biomedical Press, Amsterdam 1978.
[20] A. Lindsten, M. Ryberg, and C. Sundquist, Physiol. Plant. **72,** 167 (1988).
[21] T. M. Bricker and L. K. Frankel, Arch. Biochem. Biophys. **256,** 295 (1987).
[22] N. Farineau, T. Guillot-Salomon, C. Cantrel, A. Oujja, and C. Tuquet, Plant Cell Physiol. **29,** 925 (1988).
[23] B. Grimm and K. Kloppstech, Eur. J. Biochem. **167,** 493 (1987).
[24] G. Hoyer-Hansen, R. Bassi, L. S. Honberg, and D. J. Simpson, Planta **173,** 12 (1988).
[25] K. Kloppstech, Planta **165,** 502 (1985).
[26] G. Meyer and K. Kloppstech, Eur. J. Biochem. **138,** 201 (1983).
[27] J. N. Mathis and K. O. Burkey, Plant Physiol. **85,** 971 (1987).