Biochimica et Biophysica Acta 894 (1987) 49–58
Elsevier

BBA 42615

Organization of the photosynthetic membrane in maize mesophyll and bundle sheath chloroplasts studied by two-dimensional gel electrophoresis

Jiri Masojidek *, Magdolna Droppa and Gábor Horváth

Institute of Plant Physiology, Biological Research Center, Szeged (Hungary)

(Received 16 March 1987)

Key words: Bundle sheath; Chloroplast; Membrane polypeptide; Mesophyll; Photosystem II; Two-dimensional gel electrophoresis; (Maize)

Polypeptide composition and the distribution of Photosystem II polypeptides were studied in mesophyll and bundle sheath chloroplasts of maize by using a modified two-dimensional gel electrophoresis technique of O'Farrell (J. Biol. Chem. (1975) 250, 4007–4021). The modifications were LiDS solubilization instead of SDS, reverse isofocusing and sensitive silver staining procedure. This high-resolution technique allowed us to separate 160 polypeptides in the two types of thylakoid membrane. We found that both types of lamella contained nearly equal amounts of polypeptides, but 73 polypeptides were different in the two preparations. In mesophyll thylakoids, 75 polypeptides out of 140 were found to be characteristic, and 54 of them were exclusively present in mesophyll preparations. In bundle sheath, 24 polypeptides out of 106 were characteristic and 19 of these polypeptides were exclusively present in bundle sheath lamellae. In functional Photosystem II prepared from maize thylakoids, 68 individual polypeptides could be distinguished. Most of these polypeptides were present in both mesophyll and bundle sheath lamellae, but 19 of them were more pronounced in mesophyll than in bundle sheath lamellae. On the other hand, 11 polypeptides of Photosystem II were distinctly different in mesophyll and bundle sheath lamellae. These differences may be in close connection with the Photosystem II heterogeneity existing in the two types of thylakoid.

Introduction

Chloroplast dimorphism is a characteristic feature of malate type C_4 plants which have a leaf structure based on Kranz anatomy. In this type of plant, there are sheaths of cell containing agranal chloroplasts (also called bundle sheath chloroplasts) surrounding the vascular bundles of the leaf, and these in turn are surrounded by mesophyll tissue that contains granal chloroplasts (also called mesophyll chloroplasts). During the past decade many studies have been devoted to the biochemistry and physiology of C_4 photosynthesis. There is an extensive literature, which shows some disagreement over the PS II capacity of agranal chloroplasts. Earlier studies reported a low PS II content of bundle sheath chloroplasts [1,2]. Although the extent of PS II deficiency has been reconsidered [3–7], it has become apparent that bundle sheath chloroplasts lack the chlorophyll

* Permanent address: Laboratory of Autotrophic Microorganisms, Institute of Microbiology, CAS, 37981 Trebon, Czechoslovakia.

Abbreviations: Chl, chlorophyll; LHC, light-harvesting chlorophyll *a*/*b*-containing complex; PS I and II, Photosystem I and II; p*I*, isoelectric point; DPC, 2,5-diphenylcarbazide, DCIP, 2,6-dichlorophenolindophenol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Correspondence: G. Horváth, Institute of Plant Physiology, Biological Research Center, P.O. Box 521, Szeged, Hungary H-6701.

a/b-containing LHC associated with PS II [8–10] and, in addition, the photosynthetic unit size, measured as the ratio of chlorophyll/P-700, is significantly lower in bundle sheath compared to mesophyll chloroplasts [3]. Later studies, applying enzymatic digestion technique to separate the two types of cell, obtained high purity of mesophyll chloroplast and bundle sheath preparations and found very low PS II activity in bundle sheath thylakoids, as judged by delayed light-emission and fluorescence-induction measurements [11,12].

Parallel with the functional measurements, polyacrylamide gel electrophoresis has been also performed to analyse the polypeptide composition of granal and agranal chloroplasts. The early one-dimensional gel electrophoretic investigations found mainly quantitative differences only between the two types of chloroplast [9,13]. Recent experiments, however, demonstrated that the PS II reaction center polypeptides were absent in bundle sheath thylakoids [14–16], in agreement with the earlier results which found functional defect in PS II activity of bundle sheath chloroplasts [11,12]. By contrast, Shuster et al. [17] found at least 19 differences in the polypeptide composition of mesophyll and bundle sheath thylakoids; in their preparations, however, the 44–47-kDa reaction center polypeptides of PS II were present in bundle sheath thylakoids in correlation with the limited, light-dependent reduction of silicomolybdate with 1,5-diphenylcarbazide [17]. They have also demonstrated that the lack of the 32 kDa herbicide-binding polypeptide in bundle sheath chloroplasts is due to the regulatory inhibition of *psb*A gene at the transcriptional level [18].

The inconsistencies of the above-mentioned results clearly indicate that there are still some uncertainties with respect to the polypeptide composition of mesophyll chloroplast and bundle sheath thylakoids as well as the presence or absence of PS II in agranal chloroplasts. The highly sensitive two-dimensional gel electrophoresis technique of O'Farrell [19] offers a good opportunity to analyze the polypeptide composition of mesophyll chloroplast and bundle sheath thylakoids and the distribution of PS II polypeptides within the two types of lamella.

This technique separates proteins by isoelectric points on an isoelectric focusing gel in the first dimension and by molecular weights on a SDS-polyacrylamide gel in the second dimension. Recently, two-dimensional gel electrophoresis has been applied to analyse the polypeptide composition of various chloroplast subfragments [20–24], but in most work, the gels were visualized by classical, Coomassie blue staining techniques, which resulted in a relatively poor resolution. The newly introduced silver staining technique [25] seems to be more advantageous [26,27] in visualizing even small differences existing amounts various preparations.

In the present work, we applied a modified two-dimensional gel electrophoresis technique of O'Farrell [19] to analyse the polypeptide composition of mesophyll chloroplast and bundle sheath lamellae as well as the distribution of PS II polypeptides within the two types of thylakoid. Using two-dimensional gel electrophoresis, we were able to separate 160 individual polypeptides of the thylakoid membranes and we found that about 73 polypeptides were different in mesophyll chloroplast and bundle sheath lamellae. Evidence is presented for the differences of the polypeptide composition of PS II located in bundle sheath and mesophyll chloroplast membranes. The results are discussed in terms of the heterogeneity of PS II. We concluded that the differences in the polypeptide composition of PS II in the two types of chloroplast membrane may play a role in determining the functional differences between PS II_α and PS II_β centers.

Materials and Methods

Plant material. Seedlings of maize (*Zea mays* L. convar. KSC 360) were grown in a greenhouse for 10–12 days. Leaves were harvested in the morning in order to obtain chloroplasts depleted of starch.

Isolation of protoplasts and intact chloroplasts from the mesophyll tissue [28]. The lower epidermis of the leaves was peeled off with a scalpel and floated on the surface of an enzyme solution containing 0.6 M D-sorbitol, 0.1 M KH_2PO_4, 0.25% Macerozyme R-10, 1.0% cellulase Onozuka R-10 and 0.5% potassium dextran sulphate, adjusted to pH 5.8, and incubated for 1.5 h at 37°C. The resulting protoplasts were washed three times in 0.6 M D-sorbitol and transferred into a suspending

medium containing 0.4 M D-sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 0.4% bovine serum albumin and 50 mM Hepes (pH 7.5) [29]. The protoplasts were ruptured by forcing them by a gentle pressure through a needle of 0.5 mm diameter. The cell debris and chloroplasts were separated by low-speed differential centrifugation ($300 \times g$ for 20 s, and $500 \times g$ for 2 min, respectively).

Isolation of bundle-sheath cells and intact bundle-sheath chloroplasts [28]. The isolation started from bundle-sheath strands prepared in a medium of 0.4 M D-sorbitol with a VirTis homogenizer, operating at 10000 rpm for 40 s. The strands were digested in an enzyme mixture containing 0.4 M D-sorbitol, 0.1 M KH_2PO_4, 0.5% Macerozyme R-10, 1.0% helicase and 1.0% cellulase for 2 h at pH 5.8 and 37°C. This enzyme treatment resulted in the release of the bundle sheath cells surrounding the vascular bundles, and a virtually complete loss of the cellulosic and pectinic compounds of the cell wall. The cells were washed three times in 0.4 M D-sorbitol, suspended and broken by the same procedure as described for mesophyll protoplasts, but with more pressure. Macerozyme and cellulase preparations were purchased from Kinki Yakult MFG, Nishinomiya, Japan; the helicase was obtained from Industrie Biologique Française, Gennevilliers, France; potassium dextran sulphate was from Meito Sangyo, Nagoya, Japan.

Chlorophyll determination. An aliquot of the chloroplast preparation was extracted in 80% acetone and the pigment extract was measured spectrophotometrically as described by Arnon [30].

Electron transport measurement. PS II electron transport was measured by using 0.25 mM *p*-benzoquinone in a Clark-type electrode under saturating white light [29].

Hill activity measurement. Hill activity was measured by the method of Vernon and Shaw [31] with an Aminco DW-2/UV-VIS spectrophotometer equipped with a side illumination. The reaction was carried out in a medium containing 0.2 M sucrose, 30 mM KH_2PO_4, 10 mM dichlorophenol indophenol (pH 6.5), chloroplast suspension with 10 μM chlorophyll content and, in some cases, 0.5 mM 2,5-diphenylcarbazide (DPC). Actinic red light (above 630 nm) was of saturating intensity.

Functional PS II particles were prepared as described in [32]. The intact thylakoids (2 mg Chl/ml) were suspended in a buffer containing 5 mM $MgCl_2$, 15 mM NaCl and 20 mM Hepes (pH 7.5) and incubated with Triton X-100 (25 mg/mg Chl) at 4°C for 30 min. Following the incubation, the suspension was centrifuged at $3000 \times g$ for 5 min and the supernatant at $40000 \times g$ for 30 min. The sediment resulted in the active PS II preparation capable of evolving 90–110 μmol O_2/mg Chl per h, as judged by measuring the electron transport from H_2O to *p*-benzoquinone.

Two-dimensional gel electrophoresis of thylakoids. The isolated chloroplasts were disrupted by osmotic shock in 25 mM Tris-HCl buffer (pH 7.5) containing 25 mM NaCl, 5 mM $MgCl_2$ and 0.06 M mercaptoethanol and centrifuged at $18000 \times g$ for 10 min. The sediment was washed once with Tris-HCl buffer and twice with water.

The two-dimensional gel electrophoresis was carried out using O'Farrell's technique [19], with several modifications [26,27]. The modifications were: Nonidet P-40 (first dimension) and LiDS (second dimension) solubilization instead of Triton X-100 and SDS, respectively, and reverse electrofocusing. In the first dimension, proteins were separated by electrofocusing on the rod gels (2 mm inner diameter, length 75 mm) with the following regime: at 50 V for 30 min; 100 V for 1 h; 200 V for 1 h; 400 V for 14 h and 800 V for 1 h without pre-focusing. 0.2% ethylenediamine was used instead of 0.02 M NaOH as the lower reservoir buffer. Aliquots of the sample containing about 80–120 μg of proteins were layered on the

TABLE I

PHOTOSYNTHETIC CHARACTERISTICS OF MESOPHYLL AND BUNDLE SHEATH CHLOROPLASTS ISOLATED FROM MAIZE LEAVES

Measurements of electron-transport activity were carried out in the presence of 10 mM methylamine.

	Chloroplasts	
	mesophyll	bundle sheath
Chl *a*/Chl *b* ratio	3.2	5.1
Electron-transport activity (μmol O_2/mg Chl per h)		
$H_2O \rightarrow$ *p*-benzoquinone	123.0	4.0
DPC → DCIP	160.0	25.0

rod gels. In the second dimension, 3-dimethylaminopropionitrile (DMAPD) was used instead of *N,N,N',N'*-tetramethylethylenediamine (TEMED) and 10–20% gradient gel instead of a homogeneous one. The parameters of the gel were: thickness, 1.5 mm; width, 130 mm; and length, 85 mm (separating gel), or 20 mm (stacking gel). After electrophoresis, the slabs were washed stepwise with 50% methanol, 25% ethanol/10% acetic acid, 50% methanol/0.1% glutaraldehyde and four times with water. The silver staining procedure was used for visualization of the protein spots as described by Wray et al. [25].

Results and Discussion

In all experiments dealing with mesophyll chloroplast and bundle sheath thylakoids, one of the most delicate problems is the purity of the preparations. The enzymatic separation of the two types of cell applied in our experiments had previously been found to result in insignificant cross-contamination, as proven by electron microscopy [28]. Table I shows some of the photosynthetic characteristics of our chloroplast preparations used in the two-dimensional electrophoretic analysis of membrane polypeptides. As seen, mesophyll chloroplast chloroplasts have lower Chl *a*/Chl *b* ratio than that of the bundle sheath chloroplasts. In contrast to granal chloroplasts, which exhibit normal photosynthetic activity, agranal chloroplasts practically did not show PS II activity, as measured from H_2O to *p*-benzoquinone. On the other hand, when DPC was used as an electron donor, about 25% of the PS II activity measured in mesophyll chloroplast chloroplasts could be restored in bundle sheath thylakoids. This result is in agreement with the observations which showed that some PSs II were present in bundle sheath, but in inactive forms [11,33]. Furthermore, these data indicate the high purity of our preparations used for mapping the polypeptide composition of the two types of thylakoids.

The polypeptide composition of mesophyll chloroplasts and bundle sheath chloroplasts of maize resolved by two-dimensional gel electrophoresis and visualized by silver staining is shown in Fig. 1. At least 140 well-defined spots in the mesophyll chloroplast and 106 spots can be observed in the bundle sheath preparation. In both slabs, the spots are concentrated in the region of 10–90 kDa and the pH 4.5–7.0 interval. The identification of the spots is difficult, since the apparent molecular weights of the polypeptides obtained by two-dimensional gel electrophoresis are slightly different from those observed by one-dimensional separation. The differences in molecular weights between the two types of gel electrophoresis are quite understandable because of the splitting of the virtual one-dimensional single bands into multiplied spots on two-dimensional gels. However, some of the proteins can be identified, even in this case. Remy et al. [34] have determined by two-dimensional electrophoresis and autoradiography that two 29-kDa polypeptides as well as 27- and 26-kDa polypeptides are present in LHC. Furthermore, the two 29-kDa polypeptides also appear in phosphorylated forms; thus, four distinct isoelectric forms can be observed at this point. In our experiments, the multiplied forms of LHC can also be observed in the 29–26-kDa region in Fig. 1.

Although a comparison of the individual spots is not easy at first sight, it can be seen, for example, that in granal chloroplasts the amount of LHC II is much higher in the 29–26-kDa region than in that of the bundle sheath preparation. Similar differences can be observed in the 34–32-kDa region as well. For an easier overview, Table II summarizes the numerical evaluation of Fig. 1(B) and 1(D), by listing all the spots which were visible in either the mesophyll chloroplast or in the bundle sheath preparation. The first column of the table contains the p*I* values of mesophyll chloroplast polypeptides. In the second column, the p*I* values of all bundle sheath polypeptides are listed. Spots which were more pronounced in one of the preparations than in the others were defined as characteristic polypeptides in one type of thylakoid. Spots visible only in one type of chloroplasts were defined as individual polypeptides of the appropriate thylakoids. It can be seen that both granal and agranal thylakoids contain nearly similar numbers of polypeptides and 73 of them were found to be different in the two types of membranes, also indicating the high purity of the preparations (marked in bold type).

In the mesophyll chloroplast preparation, 75

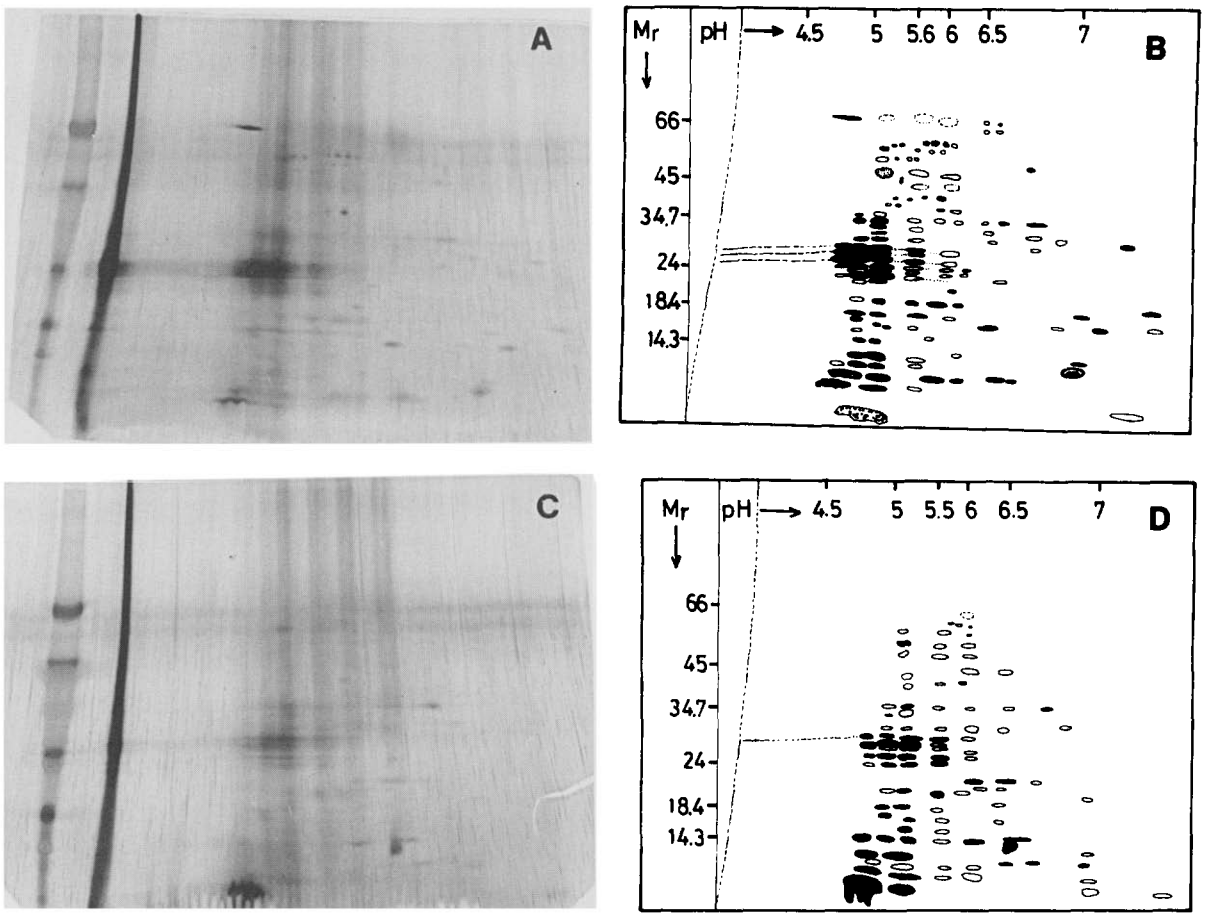

Fig. 1. Membrane polypeptides of mesophyll (A, B) and bundle sheath thylakoids (C, D) separated by two-dimensional gel electrophoresis. (A, C), Two-dimensional electrophoretograms; (B, D), schematic interpretations of (A) and (C). Unshaded areas enclosed by a continuous or broken line appeared as light or very light spots, whereas the shaded areas represent the more intensively stained spots. The spots were visualized on the original electrophoretogram by silver staining technique [25]. M_r values are mentioned in thousands.

spots were more intesively stained than in bundle sheath thlakoids and 54 of them could be exclusively seen in mesophyll chloroplast only (bold type). In bundle sheath thylakoids, 24 spots were found to be more intesively stained than in mesophyll chloroplast and 19 of them could not be found in the granal preparation. The result shows that the polypeptide composition of the two types of lamella is markedly different. Earlier work, which studied the protein composition of mesophyll chloroplast and bundle sheath lamellae, found much fewer polypeptides and mainly quantitative differences only [9,10,13]. Using one-dimensional gel electrophoresis and Coomassie blue staining, only 15–20 bands were found in both granal and agranal thylakoids. The relatively low number of bands can be explained by two factors. On the one hand, polypeptides with the same or very similar molecular weights, but different p*I* value cannot be separated by one-dimensional electrophoresis, and these polypeptides appear as one intensive band on the gels [35]. On the other hand, the Coomassie staining is not sensitive enough to detect a number of minor compounds on the gel [25]. The technique applied in our work is a combination of two-dimensional electrophore-

TABLE II

POLYPEPTIDE COMPOSITION OF MESOPHYLL AND BUNDLE SHEATH THYLAKOIDS OF MAIZE

The following typefaces are used:
italics: not characteristic for any type of chloroplast;
Roman type: characteristic for one type of chloroplast;
bold face: visible only in one type of chloroplast.
Strong spots on the gel are underlined.

Molecular mass (kDa)	Mesophyll (p*I*)	Bundle sheat (p*I*)
65	**4.7–4.85, 5.1, 5.6**, *5, 95*, **6.42, 6.5**	*5.95*
63	**6.42, 6.5**	–
58	**5.25, 5.35**, 5.7, 5.9, 5.95, **6.05**	*5.6, 5.77, 5.96*
55	5.15–5.2, *5.57–5.1, 5.9*	*5.15, 5.51, 5.95*
52	*5.15*, **5.25, 5.4**, *5.51*	5.15, *5.51*, **5.95**
49	**6.77**	–
47	5.15, *5.51, 6.0*	*5.15, 5.51, 5.95*
45	**5.51**, *6.0*	*5.95*, **6.35**
42	–	**5.15**
40	**5.25**, *5.4*, **5.6**, 5.9	*5.5*, 5.95
39	**5.22, 5.3**	**5.15**
36	**6.0, 6.15**	–
35	**4.9, 5.15**	–
34	4.9, *5.15, 5.51, 5.9*, **6.15**, *6.41*, **6.5**, *6.77*	*4.95, 5.15, 5.5, 5.95, 6.41, 6.7*
32	5.15, **5.51, 6.41**	*5.15*
30.5	**5.15**, *6.77*	**4.9**, *6.77*
30	4.9, 5.15, *5.51, 6.5*, **6.87, 6.99**	*4.9, 5.15, 5.51*, **5.95**, *6.32*
29	*4.85, 4.9, 5.15, 5.51, 5.95*, **6.77**	*4.85, 4.95, 5.15, 5.51, 5.95*
27	4.85, *4.9, 5.15*, 5.51	*4.85, 4.95, 5.15, 5.51*
26	4.85, 4.9, 5.15, 5.51, *5.95*	*4.85, 4.95, 5.15, 5.51, 5.95*
24	4.85, 4.9, 5.15, 5.51, 5.95, **6.2**	*4.85, 4.95, 5.15*, 5.51, *5.95*
23.5	**4.9, 5.15, 5.51, 5.95, 6.2**	–
23	*4.85, 4.9*, 5.15, *5.51*, **6.5**	*4.85, 4.9, 5.15*, 5.51
21.5	**4.9, 5.15**	–
21	*5.95*	*5.95*, **6.3, 6.5**
20.5	–	**6.0, 6.3**
19.5	*4.9, 5.15, 5.51, 5.8*, **6.15, 7.0**	*4.95, 5.15, 5.51, 5.8*
18	*4.9, 5.15*, 5.51, **5.96**, 6.95	*4.95, 5.15, 5.5, 6.85*
17	*4.9*	*4.95*
16.5	**5.15**	–
16	*5.22, 5.57*, **6.41, 6.84, 6.95, 7.0**	*5.22, 5.51*, **6.28**
15.5	**4.95**	–
15	**5.75**	–
14.5	**4.95**, *5.15*	*5.15*, **5.51**
14	*6.1*	5.95, **6.3, 6.41**
13.5	*4.95, 5.15, 5.51*, **6.92**	**4.85**, *4.95, 5.15, 5.51*
12.5	*4.85, 4.95, 5.15*, **5.51**, *6.9*	4.85, *4.95–5.15, 6.85*
11.5	4.75–5.0, *5.15*, **5.75**, 6.1, 6.5, 6.6	*4.87, 5.15*, **5.51**, *5.95, 6.3, 6.5*, **6.8**
10.5	*4.7–5.0, 5.15, 5.51*	*4.7–4.95, 5.05–5.25, 5.51*, **5.95, 6.8**
10	*4.85–5.22, 6.97–7.0*	*4.7–4.95, 5.05–5.25, 6.8, 7.0*

sis and the sensitive silver straining [25], thus it gives much higher resolution than the earlier works.

For studying the polypeptide composition of the PS II complex, functional PS II particles were isolated from maize chloroplasts according to the

method of Berthold et al. [32]. The preparation was capable for evolving 100 μmol O_2/mg Chl per h, as measured from water to *p*-benzoquinone. The two-dimensional electrophoretogram of such a preparation is shown in Fig. 2. We could visualize 68 individual polypeptides on the gel. This result is in good agreement with the very recent publication of Baier et al. [35], who could also detect 60 polypeptides in PS II preparations by two-dimensional gel electrophoresis. Of course, we do not assume that all of these polypeptides are involved in PS II reactions. It has been shown previously, for example, that a significant amount of catalase is present in PS II preparations but it has no apparent role in PS II [35]. The large number of polypeptides found in our experiments differs from those of other laboratories who found 9–13 polypeptides on one-dimensional gel stained with Coomassie blue [36,37]. It may be argued that perhaps some proteolysis occurred during isolation which multiplied the spots on our gels. Our arguments against this assumption are the following: (1) On one-dimensional gel stained with Coomassie blue, in PS II preparations, we found 11 bands only, similarly to Refs. 36 and 37 (data not shown). (2) On the two-dimensional gels, these bands were also observed but they were resolved into several spots each. Numerous minor spots were also detected spanning the whole molecular weight range of the gel. Usually, when proteolysis occurs, the resulting minor spots show up mainly in the low-molecular-weight range. On the basis of these arguments, however, it cannot be completely excluded that proteolysis occurs, but its evaluation requires further detailed investigations.

In our experiments, we compared the polypeptide composition of PS II to that of mesophyll chloroplast and bundle sheath preparations. Table III shows that most of PS II polypeptides were found in both mesophyll chloroplast and bundle sheath lamellae. 58 out of 68 PS II proteins could be found in granal and 48 of the PS II proteins were present in agranal thylakoids. Ten polypeptides of PS II could not be found in either preparation. 19 polypeptides were characteristic mesophyll chloroplast polypeptides and only one of them was characteristic of bundle sheath thylakoids. Ten of the PS II proteins were found exclusively in mesophyll chloroplast and one one of them were unique for bundle sheath lamellae (marked in bold type). The results indictes that both mesophyll chloroplast and bundle sheath membranes contain PS II but the polypeptide composition of the PS II located in different types of chloroplast is not exactly the same. This result is in good agreement with the conclusions of Ghirardi and Melis [33], who compared the PS II content of mesophyll chloroplast and bundle

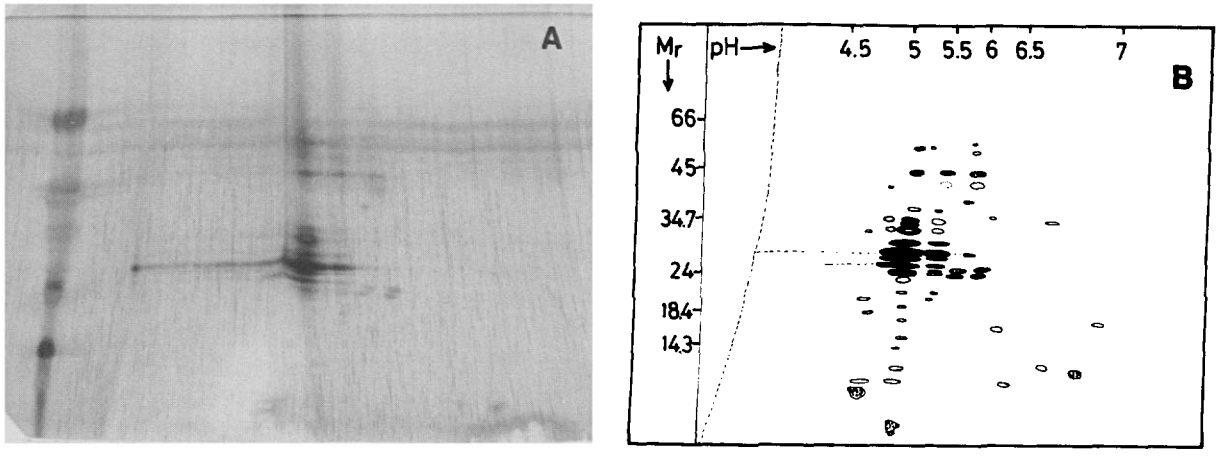

Fig. 2. Polypeptide composition of functional PS II particles isolated according to ref. 32. The oxygen-evolving capacity of the preparation was 100 μg O_2/mg Chl per h, as judged by measuring the electron transport from H_2O to *p*-benzoquinone. (A), Two-dimensional electrophoretogram; (B), schematic map. See legend to Fig. 1 for explanation. M_r values are mentioned in thousands.

TABLE III

DISTRIBUTION OF PS II POLYPEPTIDES IN MESOPHYLL AND BUNDLE SHEATH CHLOROPLASTS OF MAIZE

The following typefaces are used:
Italics: not characteristic for any type of chloroplasts;
Roman type: characteristic for one type of chloroplast;
bold face: visible only in one type of chloroplast.
Strong spots on the gel are underlined.

Molecular mass (kDa)	Mesophyll (p*I*)	Bundle sheath (p*I*)	PS II only
58	*5.95*	*5.95*	–
55	5.15–5.2, *5.95*	*5.15–5.2, 5.95*	–
47	5.15, *5.51*, 5.95	*5.15, 5.51, 5.95*	–
45	**5.51**, *5.95*	*5.95*	–
42	–	–	4.9
40	*5.8*	*5.8*	–
39	**5.15**	–	5.4
34	4.9, *5.15, 5.4*, **6.15**, *6.75*	*4.9, 5.15, 5.4, 6.75*	–
33	5.15	*5.15*	–
32	**5.15, 5.4**	**4.9**	–
31	–	–	4.7
30	4.9–5.2, *5.4*	*4.9–5.2, 5.4*	–
29	*4.9–5.3, 5.35–5.6, 5.9*	*4.9–5.3, 5.35–5.6, 5.9*	–
27	*4.9–5.3, 5.35–5.6*	*4.9–5.3, 5.35–5.6*	–
26	*4.9–5.3, 5.35–5.6*	*4.9–5.3, 5.35–5.6*	–
24.5	*4.9–5.2, 5.4, 6.0*	*4.9–5.2, 5.4, 6.0*	5.75
22.3	**4.9–5.2, 5.4, 6.0**	–	5.75
21.5	**5.15**	–	–
21	–	–	5.15, 5.4
20.5	*5.15*	*5.15*	4.7, 5.35
19.5	*4.85*, 6.92	*4.85*, 6.85	–
16.5	**5.15**	–	–
16.4	*6.3*	*6.3*	–
16.1	*5.15*	*5.15*	–
14.5	*5.1*	*5.1*	–
13	–	–	6.7
12.5	*5.1*, 6.9	*5.1, 6.9*	–
11.5	*4.85, 5.1, 6.35*	*4.85, 5.1, 6.35*	–
10.5	*4.8*	*4.8*	–
10	*5.1*	*5.1*	–

sheath chloroplasts by the kinetic analysis of the PS II photoactivity in the two types of chloroplasts. They demonstrated the complete absence of PS II_α in bundle sheath chloroplasts and could identify the small complement of Photosystem II as PS II_β [33].

PS II is known to occur in two structural-functional configurations in granal chloroplasts, denoted as PS II_α and PSII_β [38–44]. During the last years, the following differences have been established for the two types of PS II complex; in granal chloroplasts of C_3 plants, PS II_α is located in grana region and PS II_β can be found in the stroma exposed thylakoids [38,43,44]. The two types of PS II center differ in the effective light-harvesting chlorophyll antenna size [42] and in the apparent midpoint potential of their primary electron acceptor, Q [45,46]. Mg^{2+} affects the organization of PS II_α in the membrane, but not that of PS II_β [41]. PS II_β is not connected to the intermediate plastoquinone pool of the photosynthetic electron transport [44]. In malate type C_4 plants

the mesophyll chloroplast chloroplasts contain both PS II_α and PS II_β, similarly to the granal chloroplasts of C_3 plants. In bundle sheath chloroplasts, however, PS II_α is completely absent and only a small amount of PS II_β is present [33]. It has been demonstrated previously that at the early steps of development, bundle sheath chloroplasts also contain grana but bundle sheath chloroplasts become grana-less in the fully differentiated leaves [47,48]. Ghirardi and Melis [33] concluded that the elimination of the grana partition region from the bundle sheath during ontogeny resulted in the selective elimination of PS II_α but not the elimination of PS II_β. Although all data mentioned above indicated that considerable structural differences might be expected between PS II_α and PS II_β centers, direct analysis of their protein composition has not been performed until now. We believe that, in our experiments, the observed differences in the PS II polypeptide composition of mesophyll chloroplast and bundle sheath thylakoids reflect the structural differences of PS II_α and PS II_β located in granal and agranal thylakoids, respectively.

Acknowledgement

We wish to thank Dr. A.H. Nagy and her group, Department of Genetics, Eötvös L. University, Budapest, for introducing J.M. to the application of two-dimensional gel electrophoresis.

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