Lipid Composition of Photosystem I and II in the Tobacco Mutant *Nicotiana tabacum* NC 95

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The lipids of photosystem II particles, of chloroplasts and leaves are compared in the variegated tobacco mutant NC 95. The mutant differs from other *N. tabacum* mutants by the phenomenon that it has variegated leaves with green and with yellow-green leaf patches. Chloroplasts from the green leaf areas exhibit photosystem II and photosystem I reactions and have a normal lamellar system with grana and intergrana regions. Chloroplasts from the yellow-green leaf areas, however, yield only photosystem I reactions and have only single stranded isolated thylakoids. Hence, this mutant offers the unique possibility to compare without the use of detergents within the same plant the lipid composition of photosystem II particles with that in intact chloroplasts, exhibiting either photosystem II and I reactions or those exhibiting exclusively photosystem I reactions.

The lipids of photosystem II particles are composed of 37% glycolipids, 4% phospholipids, 5% carotenoids and 54% chlorophyll. Lipids of chloroplasts with grana stacking are composed of 75% glycolipids, 7% phospholipids, 2% carotenoids and 16% chlorophyll. Chloroplasts with single isolated thylakoids have a lipid composition consisting of 83% glycolipids, 14% phospholipids and only 0.5% carotenoids and 2% chlorophyll. The chloroplast lipid mixture is characterized in comparison to the respective leaf lipid mixture by a 16–17% higher glycolipid portion and by a 13–70% lower phospholipid content.

The main difference in the lipid composition of photosystem I and II consists in the observation that chloroplasts active in only photosystem I contain more than double the amount of glycolipids and the 4-fold amount of phospholipids in comparison to photosystem II active preparations. The amount of monogalactolipid is even 3 times higher in chloroplasts active only in photosystem I when compared to those in photosystem II particles. In photosystem II particles phosphatidylethanolamine is completely lacking and phosphatidylglycerol and phosphatidylinositol occur only in traces.

The fatty acids of the sulfolipid are by 45% more saturated in the photosystem II particles and the digalactolipids of the photosystem II particles are by 28% more saturated than in chloroplasts exhibiting photosystem I and II activity.

Introduction

Earlier publications have demonstrated that photosynthetically active photosystem II preparations of higher plants are composed of the following polypeptides with the apparent molecular weights of 11, 17, 24, 33, 42 and 48 kDa [1–6]. Already 10 to 14 years ago we were able to demonstrate by means of preparation of specific antisera to different polypeptides of the thylakoid membrane, that the antisera to the polypeptides 11, 24, 26, 32 and 66 kDa inhibit electron transport reactions in the region of photosystem II [7–12]. All these antisera also agglutinated stroma-freed chloroplasts, the exception being the antiserum to the 24 kDa polypeptide which agglutinated these chloroplasts only after ultrasonication. This meant at that time, that the direct accessibility of the antigenic determinants was hindered by other proteins in the native thylakoid membrane. The 66 kDa polypeptide is now considered as being a dimer of D_1 and/or D_2 [13]. From the effect of the listed antisera in photosystem II we concluded at that time, that these polypeptides must be components of photosystem II and furthermore that these peptides must be situated in the thylakoid membrane surface which is directed towards the stroma in such a way that the antigenic determinants of these polypeptides

Abbreviations: DCPiP/ascorbate, 2,6-dichlorophenol-indophenol/ascorbate; DEAE-cellulose, diethylaminoethyl-cellulose; EDTA, ethylenediamine tetraacetic acid; Hepes, N-2-Hydroxyethyl-piperazine-N′-2-ethane-sulfonic acid; SDS, polyacrylamide, sodium dodecylsulfate polyacrylamide; Tris, tris(hydroxymethyl)-aminomethane; monogalactolipid, monogalactosyldiglyceride; digalactolipid, digalactosyldiglyceride; sulfolipid, sulfoquinovosyldiglyceride.

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were accessible to antibodies. Antibodies to these photosystem II polypeptides are bound in relatively high amounts onto the thylakoid membrane. The bound amount makes up for 20% of total amount of antibodies which the outer surface of the thylakoid membrane is able to bind [14, 15].

There is only little information available on the relationship of glycolipids and phospholipids to photosystem II. From work by Gounaris *et al.* [16], Henry *et al.* [17] and Murphy and Woodrow [18] we may conclude, that the lipids are differently distributed between thylakoids of the grana and intergrana regions. Thus, it appears that the anionic lipids such as sulfolipid, phosphatidylglycerol and the dipolar ionic phosphatidylcholine are preponderently represented in grana thylakoids. According to Krupa [19] phosphatidylcholine can be considered to be a substantial component of the photosystem I as well as of the photosystem II reaction center. According to this distinct distribution of the lipids, a higher fluidity has been attributed to thylakoids of the intergrana region than to those of the grana region (Ford *et al.* [20]). This observation fits that of Hinz [21] namely that the membrane lipids of the grana regions of barley chloroplasts contain a higher amount of saturated fatty acids than the same lipids in membranes of the intergrana region. From the reactions of monospecific antisera to the galactolipids [22], which make up for 20% of the dry matter of the thylakoid membrane, and of antisera to the anionic lipids, sulfolipid [23] and phosphatidylglycerol [24] as well as from reactions of the membrane lipids phosphatidylcholine and phosphatidylinositol [24] with chloroplasts we were also able to demonstrate that these lipids were adsorbed onto photosystem II as well as onto photosystem I reaction centers. This was concluded from the fact that antisera to these lipids affected electron transport reactions on the donor side of photosystem II as well as of photosystem I. From the amount of antibodies bound to the mentioned lipids we estimated that these lipids occupy approximately 15% of the thylakoid membrane surface directed towards the stroma [25]. From this we concluded that these lipids fill up the space between protein molecules, facilitating thereby by means of their fluiditive properties, conformational changes of protein molecules.

In the present paper the lipid composition of chloroplasts and photosynthetically active photosystem II particles of the tobacco mutant NC 95 are compared. This mutant has been described earlier by Homann and Schmid [26], by Schmid [27] and Homann *et al.* [28] and differs from the mutants described by Okabe *et al.* [29] and Schmid *et al.* [30] by the fact that it is a variegated plant whose green leaf areas exhibit photosystem I and photosystem II reactions and have chloroplasts with a normal morphological structure of the lamellar system *i.e.* a normal ratio of thylakoids in the grana and intergrana regions. On the other side chloroplasts of the yellow-green leaf areas have only intergrana thylakoids and exhibit only photosystem I reactions [26, 27]. This offers the unique opportunity to compare directly within one plant the lipid composition of photosystem I with that in photosystem II.

Materials and Methods

Plant material

Plants of *Nicotiana tabacum* var. NC 95 were cultivated during the spring months in saucers in a greenhouse and transplanted into the field at the beginning of the summer, which means that growth occurred under normal light and humidity conditions. Leaves were harvested in August and September.

Chloroplast isolation and determination of enzyme activities

Freshly harvested leaves were ground in 50 mM Tris buffer, pH 7.8, containing 10 mM NaCl, 5 mM $MgCl_2$ and 0.4 M sucrose (buffer I) according to the procedure by Nakatani and Barber [31]. The tissue brei was filtered through 10 layers of cheese-cloth containing a cotton layer. The filtrate was centrifuged for 30 sec at $2200 \times g$. The looser upper layer of the sediment was removed by smooth shaking with Tris buffer and discarded. The lower denser sediment was resuspended in Tris buffer and again centrifuged for 20 sec at the same g value. The looser upper layer of the sediment was discarded again and the lower sediment containing pure chloroplasts was suspended in Tris buffer. The isolation procedure was carried out at 4 °C.

Cytochrome *c* oxidase activity was determined according to the procedure of Smith [32]. Catalase activity was determined according to the method of Bergmeyer [33]. Enzymatic reactions were carried out at 20 °C.

Preparation of photosystem II particles and measurement of oxygen evolution

According to the procedure of Berthold *et al.* [34] leaf material was blended in the above mentioned chloroplast isolation buffer in a star mix (Braun Mixer Typ MX 32). The tissue brei was filtered through 10 layers of cheese-cloth containing a cotton layer. The filtrate was centrifuged for 5 min at $2000 \times g$. The sediment was washed twice in Hepes I buffer (10 mM Hepes, 15 mM NaCl, 5 mM $MgCl_2$, pH 6.5) and resuspended in Hepes II (corresponds to Hepes I containing 0.4 M sucrose). Hepes I buffer, containing 25% Triton X-100, was applied to give a concentration ratio of 2 mg chlorophyll/20 mg Triton. By addition of Hepes II the suspension was adjusted to 2 mg chlorophyll/ml and incubated for 20 min at 4 °C. Subsequent centrifugation for 10 min at $15000 \times g$ sedimented impurities. The supernatant was again centrifuged for 30 min at $35000 \times g$. The sediment was suspended in Hepes II and was again supplemented with Hepes I-Triton buffer, to give a suspension containing 5 mg Triton per 1 mg chlorophyll. By addition of Hepes II buffer the concentration was adjusted to 2 mg chlorophyll/ml and immediately centrifuged for 30 min at $35000 \times g$. The sediment containing the photosystem II particles was washed with Hepes I and resuspended in the same buffer.

Oxygen evolving capacity of the photosystem II particles was measured in a Clark type oxygen electrode (Rank Brothers England). Photosystem I reactions were measured using anthraquinone-2-sulfonate as an electron acceptor and the electron donor couple DCPiP/ascorbate. For photosystem II reaction the electron acceptor phenyl-*p*-benzoquinone was used.

Isolation and characterization of lipids

Lyophilized chloroplasts or leaf material were extracted with methanol and diethylether according to earlier described methods [35]. Lipids were extracted with diethylether from the crude extract and purified over a Sephadex G-25 column according to the procedure by Wuthier [36]. The individual lipids were isolated from the mixture and characterized by combined column chromatography on DEAE-cellulose and Florisil (Table II) [37]. The isolated lipids were tested for purity by two dimensional thin layer chromatography. The solvent used for the 1st dimension was chloroform, methanol, 7 N ammonium hydroxide 65/30/4 (v/v) and for the second dimension chloroform, methanol, acetic acid, water 85/15/10/3.5 (v/v).

From the obtained lipids the fatty acids were obtained by transesterification with acetylchloride in a methanol-benzene mixture according to Lepage and Roy [38]. The methyl esters were identified by gas chromatography on 10% ethyleneglycole succinate-chromosorb columns, according to earlier described conditions (Radunz, [35, 37]). (Device by Hewlett Packard, Type 5750.)

The quantitative analysis of the galacto- and sulfolipids was carried out according to Roughan and Batt [39]. After the preceeding separation by thin layer chromatography the lipids were brought to reaction with a 2% or 5% aqueous phenolic solution in H_2SO_4 and photometrically analyzed at 480 respectively at 485 nm. Phospholipids were quantitatively determined according to Debuch *et al.* [40]. The carotenoid content was quantitatively determined by means of spectral analysis in ethanol after separation by thin layer according to the methods by Hager and Berthenrath [41]. Chlorophyll was determined according to Schmid [42] in methanol/water 90/10 (v/v).

In order to avoid decomposition of the lipids and the formation of degradation products, all used solvents were freshly distilled and were made peroxide-free. Lipids were flushed with nitrogen during the isolation procedure. All preparation procedures were carried out at 4 °C.

Determination of proteins and characterization of polypeptides

For protein determination chloroplast proteins and photosystem II particles were precipitated with trichloroacetic acid and subsequently analyzed according to Lowry *et al.* [43]. For the determination of molecular weights of polypeptides we used SDS-polyacrylamide electrophoresis according to a slightly modified Laemmli procedure [44]. The acrylamide solution used consisted of 30% acrylamide and 0.8 bis-acrylamide in water. We worked with a 12.5% separation gel and a 3.8% collection gel. The assay buffer was 0.5 M Tris buffer, pH 6.8, containing in 4.5 ml, 2 g urea, 125 mg SDS and 250 µl mercaptoethanol. The separation buffer used for electrophoresis was a Tris-glycine mixture consisting of 50 mM Tris, 384 mM Glycine, 3.5 mM SDS and 4 M

urea. Electrophoresis was carried out at 20 mA. Standard proteins used were: bovine serum albumin, 68 kDa, catalase of bovine liver, 60 kDa, glutamate dehydrogenase of bovine liver, 53 kDa, egg albumin, 45 kDa, D-aminoacidoxidase from pig kidneys, 37 kDa, hexokinase from yeast, 25 kDa, and horse myoglobin with 17.2 kDa.

Results

The chloroplasts prepared from green or yellow-green leaf patches of the *N. tabacum* mutant NC 95 are to be considered as pure according to enzymic purity tests in which we tested for cytochrome *c* oxidase and catalase activity (Table I). Chloroplasts were isolated in the physiological intact condition, since rates of photosynthetic oxygen evolution measured as photosystem II or as photosystem II and I reaction correspond fully to activities described in the literature for higher plants (Table II [6]). The photosystem II particles described exhibit on a chlorophyll basis a three times higher activity of oxygen evolution than intact chloroplasts from which they are prepared. As to their polypeptide composition these particles correspond with molecular weights of 11, 14, 18, 25, 26, 28, 33, 34, 42 and 48 kDa to photosystem II preparations of *Spinacia oleracea* [1–5] and to preparations of the wild type *N. tabacum* var. John William's Broadleaf, as well as to preparations of the tobacco mutants *Su/su* and *Su/su* var. *Aurea* [6]. The obtained photosystem II particles are agglutinated by the antisera to the listed polypeptides. The protein/chlorophyll as well as the protein/lipid ratio of the used chloroplast preparations as well as of the photosystem II particles is characterized in Table II. The protein/lipid ratio is higher by a factor of two if com-

Table I. Catalase and cytochrome *c* oxidase activity in chloroplasts prepared from green and yellow-green leaf patches of the variegated mutant of *Nicotiana tabacum* NC 95.

	Catalase activity		Cytochrome *c* oxidase	
	Chloroplasts of green leaf areas	Chloroplasts of yellow-green leaf areas	Chloroplasts of green leaf areas	Chloroplasts of yellow-green leaf areas
Leaf filtrate	7350 ± 630	5900 ± 370	0.61 ± 0.04	1.21 ± 0.01
Supernatant I	7590 ± 320	5620 ± 260	0.77 ± 0.03	1.18 ± 0.02
Sediment I	3970 ± 320	1980 ± 250	0.36 ± 0.03	0.50 ± 0.02
Supernatant II	5790 ± 1050	5080 ± 120	0.85 ± 0.07	1.02 ± 0.06
Sediment II	810 ± 40	1160 ± 40	0.17 ± 0.02	0.30 ± 0.07

Catalase and cytochrome *c* oxidase activities are expressed in μmol substrate × mg protein^{-1} × h^{-1}.
Leaf filtrate: Obtained after homogenization of the leaves and subsequent filtration.
Supernatant I/II: Supernatant after 1st and 2nd centrifugation.
Sediment I/II: Sediment after 1st and 2nd centrifugation.

Table II. Characterization of chloroplasts and photosystem II particles from the variegated mutant *Nicotiana tabacum* NC 95 with respect to their protein, lipid and pigment content as well as to their photosynthetic activity.

	Ratio of		Photosynthetic activities	
	$\frac{\text{Protein}}{\text{Chlorophyll}}$	$\frac{\text{Protein}}{\text{Lipid}}$	PS I	PS II
Chloroplast preparations of green leaf areas	6.1 ± 1.0 (10)	1.0 ± 0.2 (10)	320 ± 53	117 ± 16
Chloroplast preparations of yellow-green leaf areas	36.4 ± 5.8 (10)	0.8 ± 0.1 (10)	668 ± 178	< 12
Photosystem II particles	3.8 ± 0.5 (13)	2.1 ± 0.4 (13)	52 ± 19	368 ± 71

Photosynthetic activities are given as μmol O_2 evolved × mg chlorophyll^{-1} × h^{-1}. Numbers in brackets give number of determinations.

pared with that of chloroplasts, which means that photosystem II particles contain only half the amount of lipids than chloroplasts.

The lipid composition of photosystem II particles shows in comparison to chloroplasts of green leaf areas, exhibiting photosystem II and photosystem I reactions and to those of yellow-green leaf areas exhibiting photosystem I reactions, not only quantitative but also qualitative differences (Table III). The photosystem II particles are characterized by a content of only half the amount of glycolipids as well as of the membrane forming phospholipids when compared to chloroplasts. On the other hand chlorophylls and carotenoids are in photosystem II particles present in a two to three times higher amount than in intact chloroplasts.

It is noteworthy that in chloroplasts exhibiting only photosystem I activity, the monogalactolipid occurs in a 3-fold higher amount than in photosystem II particles. Moreover, among the phospholipids in photosystem II particles, phosphatidylethanolamine is completely lacking and the amount of phosphatidylcholine is only half the content in photosystem I exhibiting chloroplasts. Phosphatidylglycerol and phosphatidylinositol occur in photosystem II particles only in traces.

These differences in the lipid composition between photosystem II and photosystem I find their confirmation when lipids of chloroplasts with photosystem I activity are compared to those with photosystem I and II activity. Also here can be seen that in chloroplasts active in photosystem I the sum of glycolipids is higher by 10% and the sum of phospholipids is higher by 100% when compared to chloroplasts exhibiting photosystem I and II activity. However, for the two anionic lipids and the digalactolipid differences in the distribution exist. Thus, chloroplasts with photosystem I and II activity contain more sulfolipids and more phosphatidylglycerol, than chloroplasts with only photosystem I activity. The digalactolipid is present in the chloroplasts active in photosystem I and II in almost double the amount than in chloroplasts active in photosystem I.

If the lipids of chloroplasts from green and yellow-green leaf tissue are compared to the corresponding leaf lipids, it is seen that chloroplasts contained a higher portion of glycolipids and a considerable lesser portion of phospholipids, with the exception of the digalactolipid. This glycolipid is in contrast to monogalactolipid and sulfolipid contained in a higher amount in the leaf lipids. These results fit into earlier observations on the lipid composition of chloroplasts and leaves in chlorophyll-deficient mutants of *Antirrhinum majus* and the mutant of *Nicotiana tabacum* var. *Xanthi* D_{523} [45].

Concerning the fatty acid composition the galactolipids of the tobacco mutant are, just as in chloroplasts of other higher plants, such as *Antirrhinum*

Table III. Per cent of lipids referred to the total lipid content of green and yellow-green leaf areas, chloroplasts and photosystem II particles of the mutant *Nicotiana tabacum* NC 95.

	Per cent composition of lipids				
	Green leaf areas	Yellow-green leaf areas	Chloroplasts of green leaf areas	Chloroplasts of yellow-green leaf areas	Photosystem II particles
Monogalactosyldiglyceride	32.9 ± 5.0	25.2 ± 4.7	42.6 ± 7.5	62.7 ± 8.9	21.6 ± 6.2
Digalactosyldiglyceride	23.3 ± 1.7	15.3 ± 2.0	21.6 ± 3.0	11.7 ± 2.5	9.0 ± 2.4
Sulfoquinovosyldiglyceride	8.2 ± 1.1	6.8 ± 1.7	10.7 ± 2.3	8.4 ± 0.7	6.0 ± 2.7
Phosphatidylcholine	8.9 ± 1.8	22.7 ± 0.8	3.0 ± 0.5	8.7 ± 0.2	3.7 ± 0.7
Phosphatidylglycerol	3.4 ± 0.4	4.7 ± 0.8	2.0 ± 0.6	1.2 ± 0.2	0.14 ± 0.06
Phosphatidylinositol	2.3 ± 0.1	7.0 ± 1.2	1.4 ± 0.2	3.2 ± 0.2	< 0.1
Phosphatidylethanolamine	4.4 ± 0.2	15.7 ± 1.2	0.5 ± 0.2	1.2 ± 0.1	
β-Carotene	0.40 ± 0.05	0.11 ± 0.01	0.54 ± 0.03	0.10 ± 0.01	0.90 ± 0.16
Lutein	0.91 ± 0.08	0.30 ± 0.02	0.90 ± 0.08	0.30 ± 0.01	2.90 ± 0.19
Neoxanthin	0.24 ± 0.04	0.13 ± 0.02	0.30 ± 0.05	0.10 ± 0.01	0.83 ± 0.13
Violaxanthin	0.05 ± 0.01	0.06 ± 0.02	0.36 ± 0.06	0.14 ± 0.01	0.53 ± 0.07
Chlorophyll *a*	11.5 ± 0.5	1.5 ± 0.2	10.7 ± 0.8	1.54 ± 0.02	37.6 ± 4.3
Chlorophyll *b*	3.5 ± 0.1	0.5 ± 0.2	5.4 ± 0.5	0.72 ± 0.02	16.7 ± 1.7

Total lipids were normalized to 100.

Table IV. Per cent fatty acids of polar lipids of chloroplast preparations of green leaf areas and photosystem II preparations from the mutant *Nicotiana tabacum* NC 95.

	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
Monogalactosyldiglyceride of:						
chloroplasts		2.0 ± 0.5	18.6 ± 0.3*		1.6 ± 0.1	77.6 ± 0.9
photosystem II particles		1.9 ± 0.1	18.3 ± 0.1*		1.5 ± 0.3	78.3 ± 0.8
Digalactosyldiglyceride of:						
chloroplasts		9.8 ± 0.2	3.0 ± 0.2		1.4 ± 0.1	85.8 ± 0.1
photosystem II particles		9.5 ± 0.5	7.0 ± 1.0		1.5 ± 0.5	82.0 ± 1.7
Sulfoquinovosyldiglyceride of:						
chloroplasts		40.9 ± 0.9	5.1 ± 0.4	2.0 ± 0.2	2.6 ± 0.6	49.4 ± 1.0
photosystem II particles		35.9 ± 0.5	30.9 ± 0.5			33.2 ± 1.0
Phosphatidylcholine of:						
chloroplasts	0.8 ± 0.3	19.3 ± 3.0	27.5 ± 7.0	3.6 ± 0.1	9.0 ± 0.7	39.8 ± 7.0
photosystem II particles	1.8 ± 0.1	26.0 ± 1.9	19.1 ± 3.4		13.4 ± 1.1	39.5 ± 7.7

The total fatty acid content of a given lipid correspond to 100%. * Characterized as hexadecatrienoic acid.

majus, *Urtica dioica* [37, 46] and *Spinacia oleracea* [47], characterized by a higher content in linolenic acid. However, the monogalactolipid of the tobacco mutant contains in addition just as the monogalactolipid of *Spinacia oleracea* up to 20% hexadecatrienoic acid. Only half of the fatty acids of the sulfolipid and phosphatidylcholine are unsaturated fatty acids. If the digalactolipid and sulfolipid of photosystem II particles are compared with intact chloroplasts it becomes evident that the sulfolipid of photosystem II particles has a 45% higher content of saturated fatty acids and the digalactolipid a 28% higher content of saturated fatty acids. Whereas in both lipids of intact chloroplasts the main component is found to be palmitic acid, the sulfolipid and digalactolipid of photosystem II particles contains both palmitic and stearic acid in approximately comparable amounts. This means that the degree of saturation of the fatty acids is considerably higher in grana thylakoids than in thylakoids of the intergrana regions. From this it is deduced that thylakoids of the intergrana regions have a higher fluidity.

Discussion

Considerable progress in knowledge of the structure of photosystem II has been achieved [48]. The current general view tends to a reaction center model in which only the two 30 kDa polypeptides D_1/D_2 together with cytochrome b_{559} would form the minimal protein entity on which the charge separation should take place [48]. However, it should be always borne in mind that in the intact membrane these polypeptide associations are according to the respective molecular structure of the membrane always embedded in or in close contact with lipids. For the functioning of structures such as the reaction center of photosystem II the vicinity of lipids plays an essential role. In the present paper we make the approach to determine which thylakoid lipids can be preferentially associated with photosystem II and which with photosystem I.

Our earlier investigations on the maximal binding of antibodies to glycolipids [14, 49] phospholipids, pigments [50, 51] and proteins [52, 53] onto different chloroplast preparations in which different surface areas of the thylakoid membrane were accessible to antibodies, have led to the clear result that lipids and proteins are asymmetrically distributed in the thylakoid membrane. Although the individual lipids are all accessible in the outer surface to antibodies, it can be demonstrated that the main portion of lipids is located inside the membrane or at the inner thylakoid membrane surface.

Correspondingly, the individual lipids also showed an asymmetrical distribution in the membrane [54, 55]. Thus, we were able to demonstrate that monogalactolipid is situated in a domaine in the immediate vicinity of the coupling factor of photophosphorylation (CF_1 complex). This was concluded from the fact that removal of coupling factor by EDTA-wash-

ing permitted binding of 3 times the amount of antibodies to monogalactolipid. This clearly shows that lipids have not only a transversal asymmetry but that certain membrane lipids also exhibit a distribution with lateral asymmetry. For plastocyanin, cytochrome *f*, ferredoxin-$NADP^+$-reductase we were able to demonstrate by means of the "antibody adsorption method" that these proteins are located in functional units in the thylakoid membrane [52].

The asymmetric distribution of lipids in the thylakoid membrane was further confirmed by enzymic lipid degradation through glycolipases [56, 57], phospholipases [58–60] as well as by means of lipid extraction methods using solvents of different polarities [61, 62]. This means that if different particle structures *i.e.* functional areas as photosystem I, photosystem II or light harvesting complexes are isolated from the thylakoid membrane, it is to be expected that these entities differ with respect to their lipid composition when compared to intact chloroplasts. The results of the quantitative lipid and fatty acid analysis of the tobacco mutant NC 95 confirm this conclusion. Photosystem II particles with high photosynthetic activity and chloroplasts active in photosystem I, having only single stranded isolated thylakoids differ with respect to their lipid composition. The differences in lipid composition of the two preparations should correspond to the natural condition of the two photosystems, since "photosystem I chloroplasts" were isolated without the use of any detergent and the photosystem II particles were isolated by addition of only the unpolar detergent Triton X-100 and should therefore also correspond to their native composition.

Photosystem I chloroplasts in comparison to photosystem II particles are characterized by a high content of monogalactolipid and phosphatidylcholine. The photosystem II particles in turn differ from photosystem I chloroplasts in their high pigment content and in the interesting fact that the phospholipids phosphatidylinositol and phosphatidylglycerol occur only in traces. The differences in the lipid composition between chloroplasts with normal grana and intergrana structure and those having only intergrana thylakoids also correspond to the native condition.

The higher fluidity of thylakoids of the intergrana region in comparison to a lower fluidity of grana thylakoids measured by Ford *et al.* [20] is confirmed by our lipid analysis with chloroplasts of *N. tabacum* NC 95. In photosystem II particles the protein/lipid ratio is twice as high than in chloroplasts having only single isolated thylakoids. This lower fluidity of photosystem II particles is further confirmed by a higher degree of saturation of the fatty acids of the sulfolipid and the digalactolipid. The degree of saturation of fatty acids concerning two main components of the lipids, namely the monogalactolipid and phosphatidylcholine, is identical in photosystem II particles and chloroplasts.

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