LIPID COMPOSITION OF CHLOROPHYL–PROTEIN COMPLEXES

Specific enrichment in *trans*-hexadecenoic acid of an oligomeric form of light-harvesting chlorophyll *a/b* protein

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1. Introduction

Thylakoid membranes of higher plants and green algae can be resolved by SDS–polyacrylamide gel electrophoresis into at least 2 different chlorophyll–protein complexes and ~30 polypeptides. The 2 chlorophyll–protein complexes correspond to the P700 chl *a*–protein complex (CPI) assumed to originate from the reaction centre of photosystem I and to the light-harvesting chl *a,b*–protein complex named LHCP or CP II [1] which is considered to represent the antenna chlorophyll of photosystem II. It is now well known that using milder conditions of solubilization, additional chl–proteins can be obtained. They correspond to a new chl *a*–protein complex generally termed CPa, which may represent the photosystem II reaction centre complex [2–4], and to several oligomeric forms of CP 1 and LHCP [3–13].

Although these different chl–protein complexes are characterized by some of their physiological, biochemical and physical properties (reviews [1,14–16]), little is known about their lipid composition other than pigments. The chl–protein complexes were firstly considered to contain only traces of lipids [17]. Two recent analyses [illegible]d the lipid composition of subchloroplast particles containing the LHCP complex isolated by density gradient centrifugation after thylakoid dissociation with Triton X-100 [18,19]. In [4,8], we described conditions for solubilization and electrophoresis which allowed us to obtain relatively large amounts of chl–protein complexes and especially a chl *a,b*–protein which we identified as probably a 'dimeric' form of LHCP.

We present here the lipid composition of these chl–protein complexes isolated from tobacco chloroplasts and discuss our finding that the phosphatidyldiacylglycerol-containing 3-*trans*-hexadecenoic acid is highly concentrated in the 'dimeric' form of LHCP in relation to the possible involvement of these diacyl lipids in the molecular organization of LHCP and in the stacking process.

Abbreviations: chl, chlorophyll; CPI, P700 chlorophyll *a*–protein; C 16:1-*trans*, 3-*trans*-hexadecenoic acid; C 16:0, palmitate; C 18:0, stearate; C 18:2, linoleate; C 18:3, α linolenate; DGDG, digalactosyldiacylglycerol; GLC, gas–liquid chromatography; LHCP, light harvesting chlorophyll *a/b* protein; MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PG, phosphatidyldiacylglycerol; SDS, sodium dodecylsulfate; SL, sulfoquinovosyldiacylglycerol; TLC, thin layer chromatography

2. Materials and methods

2.1. *Isolation of chl–protein complexes*

These were isolated from tobacco leaves, *Nicotiana tabacum* L. cv Xanthi. All technical operations were done as in [4,8]. The preparative electrophoresis was performed in 5% acrylamide gels using a 0.1 M Tris–borate buffer (pH 8.2) containing 0.1 % SDS.

Thylakoid membranes were solubilized in 50 mM Tris–borate buffer (pH 8.2), 1–1.3% SDS (final SDS/chl wt ratio of 5 or 2.5 mg chl/ml). Solubilized thylakoid membranes corresponding up to 200 μg chl were immediately loaded on cylindrical gels (1.8 × 10 cm) without any prior centrifugation and run at 3 mA/gel for 45 min (total migration 5 cm). Elution of complexes was done as in [8]. Chl and protein were determined according to [20] and [21], respectively.

2.2. *Lipid analysis*

Lipids were extracted from the isolated complexes by chloroform–methanol according to [22]. Lipid classes were analyzed by TLC as in [23]. Methyl-esters of fatty acids were prepared by direct *trans*-esterification [24] and analyzed by GLC on capillary colums. For the determination of lipid classes, spots were eluted from the thin-layer plate, *trans*-methylated with a nonadecanoic acid added as internal standard, and analyzed by GLC on capillary columns.

3. Results

3.1. *Electrophoretic separation of chl–protein complexes*

Five chl–protein bands, plus free chlorophyll bound to SDS micelles, were resolved when tobacco thylakoid membranes pre-solubilized by a limiting amount of SDS were subjected to electrophoresis (fig.1). All chlorophyll containing bands have been characterized by their absorption spectra [4–8]. For greater convenience, we used in fig.1 both our previous nomenclature and that now generally used [10].

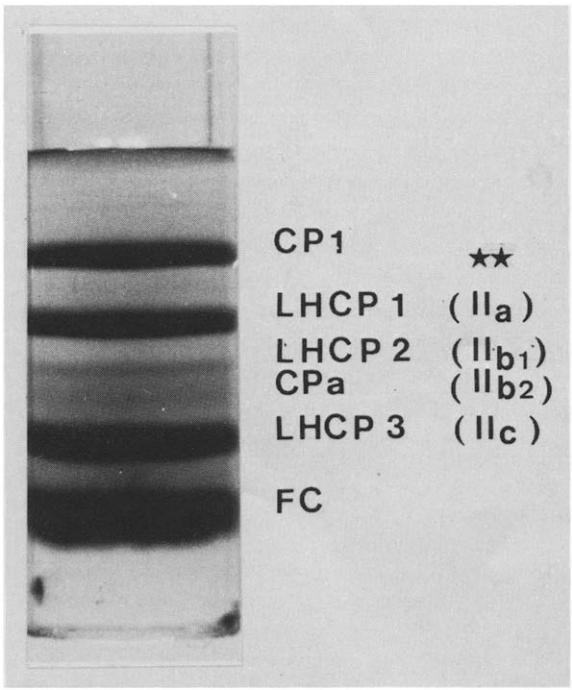

Fig.1. Sodium dodecylsulfate preparative polyacrylamide gel pattern of tobacco thylakoid membranes. Localization of chlorophyll containing bands in an unstained gel. The designation marked by two asterisks was used in [4,8].

Table 1
Total polar lipids and 3-*trans*-hexadecenoic acid content (μg/mg chl) of chl–protein complexes

Complexes	LHCP[1]	LHCP[3]	CP 1
Total polar lipids	175	275	86
C 16:1-*trans*	17	5,3	1

3.2. *Lipid analysis of LHCP[1], LHCP[3] and CPI*

Due to their minor amounts (<5% of the total chlorophyll on the gels) LHCP[2] and CPa were not eluted from the gels. Lipid analysis shows that CPI is relatively poor in polar lipids containing only 86 μg/mg chl as compared to LHCP[1] (175 μg) and LHCP[3] (275 μg).

The content of C 16:1-*trans* in LHCP[1] is 3-times higher than in LHCP[3] and is present only in trace amounts in CPI (table 1).

Analysis of polar lipid classes also shows marked differences between each complex (table 2).

An important observation is the high concentration of PG in the LHCP[1] where this lipid represents 39% of polar lipids and its amount is 68 μg/mg chl. This PG concentration is ~4-times lower in LHCP[3] where its percentage does not exceed the percentage found in intact lamellae (11% of polar lipids).

As shown in fig.2, the high concentration of PG in LHCP[1] (dimeric form) is also visible on the pattern of lipids separated by TLC. In the 3 complexes, high percentages of galactolipids are found. If DGDG represents

Table 2
Lipid composition of chlorophyll protein complexes expressed in % of polar lipids (A) and in μg/mg chl (B)

Complexes	LHCP[1]		LHCP[3]		CP 1	
	A	B	A	B	A	B
PC + SL	5	9	9	24	23	20
PG	39	68	11	30	5	4
DGDG	22	38	23	63	27	23
MGDG	34	59	57	156	45	39

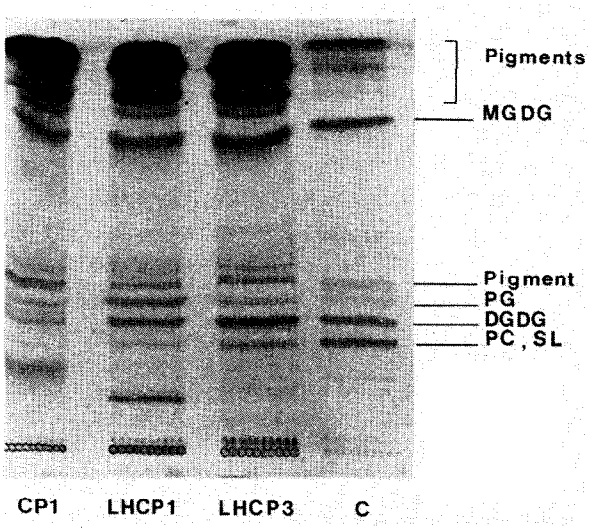

Fig.2. Thin-layer chromatography on silica gel G of polar lipids of chl–protein complexes. Elution solvent: chloroform/acetone/methanol/acetic acid/water (50:20:10:10:5; by vol). Spots were visualized by iodine vapor. C = control with a lipid extract of known composition.

about the same percentage in each complex, MGDG is far more concentrated in LHCP[3] where it represents about twice of the percentage found in LHCP[1] (table 2).

Table 3 shows the fatty acid composition of the main lipid classes found in chl–protein complexes. Since all the C 16:1-*trans* was contained in PG, differences in C 16:1 reflect the differences observed in PG concentration as well. On the other hand, we noticed that the galactolipids, especially MGDG, were rich in linolenic acid (C 18:3).

4. Discussion

The above results clearly indicate that chl–protein complexes prepared in a highly purified state by SDS gel electrophoresis contain lipids. Isolated CPI was relatively poor in lipids. In LHCP[3] polar lipids represented ~25% of chl content. Consequently, we have dealt not simply with chl–protein complexes but more precisely with lipo–protein pigment complexes.

In these complexes, the major lipids of photosynthetic membranes such as MGDG, DGDG and PG were found. The most interesting result is that the diacyl-lipid content in the oligomeric form of LHCP (LHCP[1]) was different from that in the monomeric form (LHCP[3]). The most important observation was the presence in LHCP[1] of high concentration of PG containing the 3-*trans*-hexadecenoic acid. Such a high concentration of PG has never been found up to now in any other subchloroplast fragment. The high concentration of PG in LHCP[1] strongly argues for a true physiological significance of this oligomeric form of the LHCP and strengthens our assumption that this form may reflect the state of LHCP in vivo. This assumption was based on the comparison of the fourth derivative analysis of the absorption spectra at −196°C [8] from LHCP[1], LHCP[3] and intact lamellae.

Photosynthetic lamellae are very rich in galactolipids but the most characteristic lipid in the lamellae

Table 3
Fatty acid composition of the main lipid classes of the chl–protein complexes (in % of total fatty acids in each class)

Fatty acids		C 16:0	C 16:1-*trans*	C 18:0	C 18:1	C 18:2	C 18:3
PG	LHCP[1]	19.5	35	3.5	10.5	11.5	20
	LHCP[3]	20	30	6	20	9	15
	CP 1	26	46	trace	trace	trace	26
DGDG	LHCP[1]	27.5	–	5	10	5	52.5
	LHCP[3]	17	–	3	3	trace	77
	CP 1	21	–	8	15	8	48
MGDG	LHCP[1]	7	–	2	5	6	80
	LHCP[3]	7	–	2	5	6	80
	CP 1	8	–	3	8	5.5	75.5

is the PG containing C 16:1-*trans*. The 3-*trans*-hexadecenoic esterified the *sn*-glycerol specifically at the C 2 position [25]. This monounsaturated fatty acid with a *trans*-configuration has not been found in any biological membrane, except in the photosynthetic lamellae [26] containing LHCP; it is absent from blue–green algae [27]. Studies on leaf greening have shown that the PG content with C 16:1 increases only after illumination [28] and a good correlation exists between C 16:1-*trans* synthesis and the stacking process in chloroplasts [28,29].

The removal of PG and C 16:1-*trans* by phospholipase A_2 in pea class II chloroplasts, strongly modified the kinetics of fluorescence induction, suggesting that PG must play a role in the LHCP or in the relationship between LHCP and the photosystems [31]. The results presented agree with this hypothesis and make a more precise localization of PG and C 16:1-*trans* possible, suggesting that this lipid must be implicated in the molecular organization of the oligomeric form of LHCP.

The question arises whether or not the monomeric form $LHCP^3$ results from a complete dissociation of the oligomeric form of $LHCP^1$. In [8], re-electrophoresis of $LHCP^1$ yielded, after further dissociation, $LHCP^3$ and the subunit polypeptide composition of these 2 complexes, revealed by disc electrophoresis, yielded the same major polypeptide of app. M_r 24 000. A reinvestigation of this question at higher resolution (slab gel with a 10–18% acrylamide gradient) allowed us to observe that with this major polypeptide a minor component of app. M_r 23 000 is associated. However the 2 LHCPs have apparently the same polypeptide composition detectable with our techniques. Thus, the differences observed between the lipid composition of $LHCP^1$ and $LHCP^3$ suggest that these 2 forms must probably correspond to 2 different molecular organizations in situ in the intact lamellae.

In [32] the participation of galactolipids as integral components of these complexes was questioned because an artifactual trapping of lipids could have occurred during the thylakoid solubilization. The different lipid compositions between the chl–protein complexes and especially between the 2 LHCPs reported, favour the hypothesis that these lipids are integrated parts of the chl–protein complexes.

In [33], a differential distribution of acyl lipids between CP 1 and LHCP was reported; the latter was shown to be enriched in galactolipids and phosphatidylglycerol containing a high amount of 3-*trans*-hexadecenoic acid. Although the LHCP was prepared by a method other than electrophoresis and consequently may be a mixture of the oligomeric and monomeric forms, there is agreement with our result concerning the preferential localization of the 3-*trans*-hexadecenoic acid.

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