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Purification and Monolayer Study of the Thylacoid Lipids of Moss *Marchantia polymorpha*

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ABSTRACT: The original lipid content of the thylakoid membranes of moss *Marchantia polymorpha* has been determined for the first time. In particular, the content of SQDG is almost 3 times higher than those for both of the other classes. The ratios for DGDG and MGDG are just a little bit lower than those for green algae, but almost 2 times less than those for plants. The distribution of unsaturated bonds has changed in C_{18}-residues of fatty acids. The total content of C_{18}-residues in thylacoid lipids have been almost the same but the content of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ are altered in the fractions of DGDG, PG and PE. The light stress produces only the quantitative, but no qualitative, changes of the thylakoid lipid composition. The properties of the thylakoid lipids and corresponding fatty acids in the monolayers at the liquid/gas interfaces have been studied. The changes in distribution of unsaturated bonds in C_{18} residues of fatty acids at light stress have been confirmed by Langmuir method.

Introduction

Mosses are very interesting for investigation of light stress because of their unique position between the high and primitive plants [1]. The thylakoid lipids from mosses, such as *Marchantia polymorpha*, have not been studied by now in contast to the thylakoid lipids from green algae and plants, as well as the pigment-protein complexes of photosystems I and II (PSI and PSII), whose structure and properties are studied intensively [2-6]. It is important that the qualitive and quantitative lipid content in the thylakoid membranes of the green algae and plants are different from those in other biomembranes. The mass content of the acyl-lipids in the thylakoid membranes is close to 35 %. About 75 % of these lipids are the unique glucolipids – monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol

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(DGDG), generally occurring in the ratio MGDG:DGDG close to 2:1 and slightly varying in particular plants [2]. In addition, they have unsually high content of the unsaturated fatty alkyl chains. These glucolipids are associating tightly with pigment-protein complexes that seems to be very important for the structure and function of the thylakoid membranes, especially, for the accomodation of the thylakoid membranes to extreme enviromental conditions (high temperature, light stress and so on). The third unique lipid is sulfoquinovosyldiacylglycerol (SQDG), not found in the other biomembranes, whose functional task in the membrane is still unknown. Some traditional lipids like phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) are present only in a relatively small amount [2].

The most interesting recent studies are concentrated on the investigation of the thylakoid lipids or PSI and PSII changes under high temperature [2, 3, 5] and light stress [5-7]. Such peculiarities in behavior of photosystems depend also on lipid content and lipid-protein interactions in thylakoid membranes. It is correlated with the recent view on the importance of lipids not only as matrix for the membrane proteins, but also as active functional component in the photochemical and biochemical processes in the membranes [8-10]. The first step in the study of such phenomenon is the determination of the original lipid content of the particular thylakoid membranes.

This work is devoted to the determination of the original lipid content of the thylakoid membranes of moss *Marchantia polymorpha* and purification of the particular lipids, investigation of their properties in the model membranes (such as monolayers at the liquid/gas interfaces) and their changes at various conditions, especially under light stress.

Experimental

Materials. The cell culture of moss *Marchantia polymorpha* was grown 3 weeks under standart conditions (16 hours light and 8 hours dark at constant temperature of 25°C) on the media Skoag. These samples were used as standards. The special photoirradiated samples were prepared by additional constant light stress with intensity of 1 mM/(m^2·s) during 30 hours. The thylakoid membranes were isolated by standard procedure [11]. First, moss washing with distilled water and buffer 1 (100 mM Tris/HCl pH 7.8, 400 mM sorbitol, 10 mM NaCl, 5 mM $MgCl_2$), cell destruction by pressure (300 atm), filtration through "Miacloth", centrifugation at 3500 /min. at 4°C during 10 min. Second, rest of the cells - destruction once more by pressure (300 atm) and centrifugation at 800 /min. at 4°C during

10 min. Third, the precipitate resuspended in the buffer 2 (10 mM Tris/HCl pH 7.8, 10 mM NaCl, 10 mM $MgCl_2$) - centrifugation at 9000 $min.^{-1}$ at 4°C during 10 min. Forth, the precipitate resuspended second time in the buffer 2 - centrifugation at 9000 $min.^{-1}$ at 4°C during 10 min and solution in glucerol for total chlorophyll detection (in this case - 728.6 mg/ml). The lipid extraction from thylakoid membranes was done by mixture of methanol-chloroform 7:3 with the following filtration and washing by pure chlorofom. The preparative purification of lipids was fulfilled by column chromatography on DEAE-cellulose in the system I (chloroform-methanol 6:4) and system II (chloroform-methanol 6:4 with 1.5 ml ammonia) or by TLC on Kiselgel 60 DC-Fertigplatten in the system chloroform-methanol-acetic acid-water 85:15:10:3 [12]. By these methods various fractions of lipids from thylakoid membranes of moss, grown at normal and high light conditions, were isolated and measured (Table1). In order to study precisely the changes in the lipids by light stress, the methanolys of the lipid fractions was fulfilled and pure fatty acids were modified with phenacylbromide, isolated and detected by HPLC [13] (Table 2).

Table 1

	Control (standart conditions) (Th_c)	Light stress (Th_{30})
Rf	0.21/0.30/0.33/0.40/0.43/0.55/0.83	0.22/0.27/0.31/0.41/0.45/0.56/0.85
ratio, %	3.4/38.6/5.7/9.1/9.1/5.7/28.4	4.1/41.2/5.4/8.7/8.7/5.5/26.6
mass parts	0.4/4.3/0.6/1.0/1.0/0.6/3.1	0.5/4.8/0.6/1.0/1.0/0.6/3.1

Methods. Monolayers of the isolated lipids and fatty acids have been prepared and studied on a commercial film balance ("Lauda", FRG). The samples of lipids (10 μl from 10 mM chloroform solutions) have been spread onto 100 mM Tris-HCl buffer subphases pH 6.0, 20°C between moving and measuring barriers. The prepared monolayers have been compressed by moving barrier with constant speed of about 1 cm^2/s and the isotherms of surface pressure (π) vs. area (A) of the monolayer have been recorded [14].

Results and Discussion

The purification of the particular lipids and determination of the original lipid content of the thylakoid membranes of moss *Marchantia polymorpha* have been fulfilled for the first time. The lipid content of the thylakoid membranes (in mass parts and %) have been determined by preparative TLC according to the relative mobility (R_f) of the lipids in the system chloroform-methanol-acetic acid-water 85:15:10:3 [12] (Table 1).

The fraction with R_f 0.21 can be considered, in general, as phosphatidylinositol (PI), the fraction with R_f 0.30 – as sulfoquinovosyldiacylglycerol (SQDG), the fraction with R_f 0.33 – as phosphatidylcholine (PC), the fraction with R_f 0.40 – as digalactosyldiacylglycerol (DGDG), the fraction with R_f 0.43 – as phosphatidylglycerol (PG), the fraction with R_f 0.55 – as phosphatidylethanolamine (PE) and the fraction with R_f 0.83 – as monogalactosyldiacylglycerol (MGDG). To our opinion, the fractions with R_f 0.30 and R_f 0.33 can contain the mixture of PC and SQDG which is difficult to separate. The fractions with R_f 0.40 can contain some amount of PG in addition to DGDG.

It is important that all the lipids, found in the moss, are typical for the thylakoid membranes of green algae and plants. The ratios for PI, PC, PE and PG are in the range of the average values for these thylakoid lipids both from green algae and plants (such as spinach, tobacoo, pea). In contrast, content of SQDG is almost 3 times higher than those for both of the other classes. The ratios for DGDG and MGDG are just a little bit lower than those for green algae, but almost 2 times less than those for plants. Thus, the content of the traditional lipids found in moss is almost the same as for the thylakoid membranes of green algae and plants. But according to the content of the specials lipids, like DGDG and MGDG, this moss can be considered in the evolutional scale more close to the green algae than to plants. The extreme high content of the unique sulfolipid (SQDG) is difficult to explain. The general consideration may be that this lipid is important for retention of the thylakoid membranes from light stress, because at high light the content of SQDG (Th_{30}) is increasing drastically (on about 2.6 %). Probably, the same is reasonable for the MDGD, which content at high light (Th_{30}) is decreasing on 1.8 % as compared to control (Th_C). The small increase of PI content (one of the traditional lipids with the lowest R_f 0.21-0.22) at high light on 0.7 % is, probably, due to the partional decomposition of the other lipids and appearence of their lyso-forms, which generally have lower mobility (R_f) as compared to the diacyl-forms. The ratios for other found lipids (DGDG, PC, PE and PG) at high light are smaller on less than 0.4 % as compared to control that is in the range of the experimental error and can not be considered as essential for the retention of the thylakoid membranes from light stress.

In order to clearify the changes in the lipid structure at light stress, the hydrolysis of the lipid fractions in methanol has been fulfilled and the obtained fatty acids have been carefully analyzed (Table 2).

Table 2

Sample	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Control						
1+2	++		+			30 %
3			++		14 %	14 %
4	++	+	++		23 %	
5			++		19 %	
Light stress						
1+2	+		++			23 %
3			++	21 %		
4			++			48 %
5	+	+	++		8 %	

From these data it is evident that the main changes by light stress are concentrated in the fractions 3 and 4 due to the changes in degree of unsaturation in fatty acids, in particular, in the fraction 4 - the creation of one more double bound, but in the fraction 3 - reduction of one or two double bounds to the final oleic acid. The content and quality of the saturated fatty acids are almost the same before and after light stress that is in a good agreement with the known data [7] and means that light stress do not influence on the saturated fatty acids. That is why, the main attention further was devoted to the unsaturated fatty acids (Table 3).

As it is seen from the Table 3, after light stress the re-distribution of the double bounds in the fatty acids occured. The amount of the linolic acid in the fractions after light stress decrease drastically (on 48 %), where as the amount of linolenic acid is increasing on 27 %

and oleic acid appeared in the amount of 21 %. Thus, the changes in the unsaturated fatty chains in the thylakoid lipids at light stress are more pronounced as the changes in the lipid head groups.

Table 3

Fatty acids	Control (Th $_c$)	Light stress (Th $_{30}$)
$C_{18:2}$	56 %	8 %
$C_{18:1}$		21 %
$C_{18:3}$	44 %	71 %
Σ	100 %	100 %

C16:0 – palmitic acid
C16:1 – palmitelaidic acid
C18:0 – stearic acid
C18:1 – oleic acid
C18:2 – linolic acid
C18:3 – linolenic acid

All purified lipids can form stable monolayers at the liquid/gas interfaces with the collapse pressures are in the range 30-45 mN-m (Fig. 1). The surface-active properties of the thylakoid lipids of moss *Marchantia polymorpha* in monolayers at the liquid/gas interfaces and their changes at various conditions, especially under light stress, were investigated. According to the profile of the isotherm all monolayers of these lipids are in the liquid-expanded states.

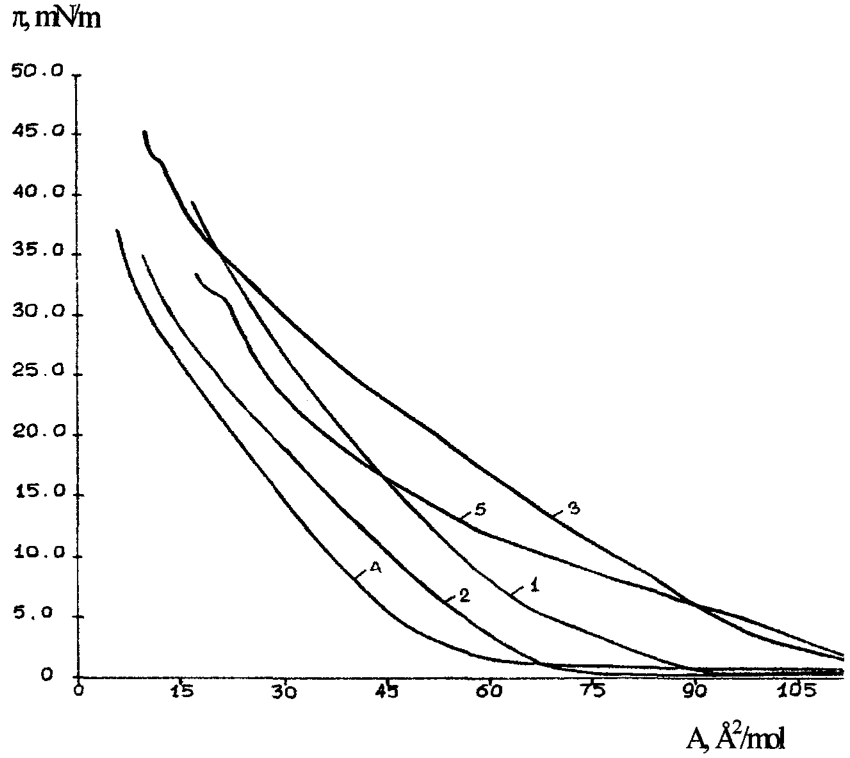

Fig. 1. Surface pressure (π) - molecular area (A) isotherms for monolayers of lipid fractions purified from thylakoid membranes, grown at normal conditions: 1 – fraction 1 (PI), 2 – fraction 2 (SQDG and PC), 3 – fraction 3 (DGDG), 4 – fraction 4 (PG and PE), 5 – fraction 5 (MGDG) at the surface of 100 mM Tris-HCl, pH 6.0, 20°C.

It was found that the monolayer parameters of the thylakoid lipids are changing drastically with the temperature. In general, the areas per lipid molecules are increasing with increasing temperature in the range 5 - 30°C.

The only exceptions were found for the fraction 1 at 20°C and fraction 3 at 30°C, when the areas were smaller as compared to those at lower temperatures. These effects can be explained due to the possible phase transitions at these temperatures for the particular lipid fractions, as well as the inhomogeneity of the fractions, which can contain some times more than one lipid.

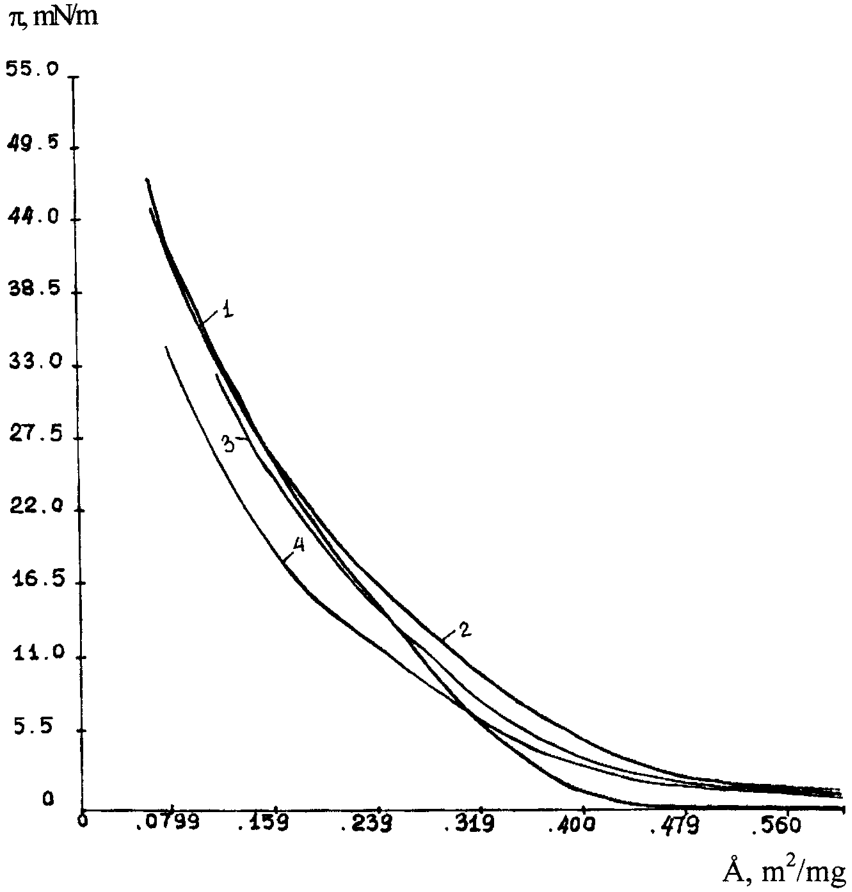

Fig. 2. Surface pressure (π) - molecular area (A) isotherms for monolayers of fatty acids purified from particular lipid fractions of thylakoid membranes, grown at normal conditions: 1 – fractions 1 and 2, 2 – fraction 3, 3 – fraction 4, 4 – fraction 5 at the surface of 100 mM Tris-HCl, pH 6.0, 20°C.

The comparative investigation has been performed with the lipid fractions, obtained at normal conditions and at light stress. The main changes were found for the monolayers of the fatty acids obtained from the thylakoid membrane, grown at normal conditions (Fig. 2) and at high light (Fig. 3). The areas per molecule in monolayers of the fractions 3 and 4 (isotherms 2 and 3, Fig. 3) after light stress are almost 2 times lower (0.11 and 0.08 nm^2/mg, respectively) as compared to those (0.22 and 0.20 nm^2/mg, respectively) at normal

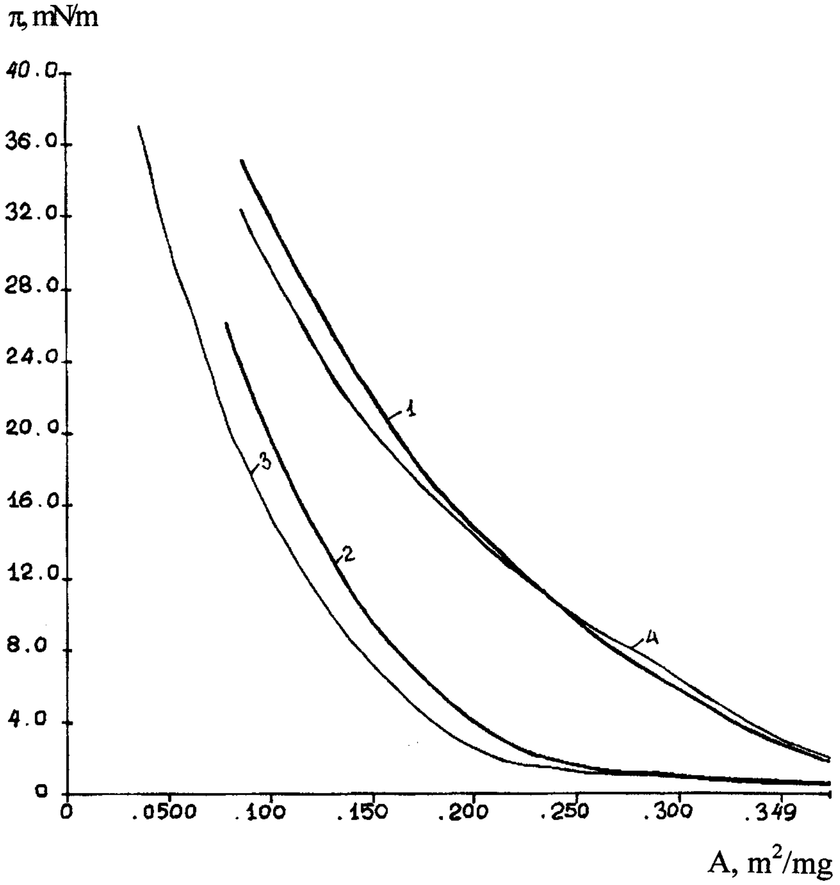

Fig. 3. Surface pressure (π) - molecular area (A) isotherms for monolayers of fatty acids purified from particular lipid fractions of thylakoid membranes, grown at light stress: 1 – fractions 1 and 2, 2 – fraction 3, 3 – fraction 4, 4 – fraction 5 at the surface of 100 mM Tris-HCl, pH 6.0, 20°C.

conditions (isotherms 2 and 3, Fig. 2). These changes are in a good correlation with the changes of the amount of double bounds in the fatty acids of the respective fractions (Table 3). The isotherms of all fatty acid fractions at normal conditions (Fig. 2) and light stress (Fig. 3) have graduale slope (liquid-expanded state of the monolayer) that is an additional evidence of the high content of double bounds in the fatty acids. Thus, monolayer study supports the data, obtained by TLC and HPLC, on the initial structure and change by high light.

Conclusions

Thus the original lipid content of the thylakoid membranes of moss *Marchantia polymorpha* has been determined for the first time. All lipids have been isolated and purified. It has been found that light stress produces only the quantitative, but not qualitative changes of the thylakoid lipid composition. In particular, the content of SQDC is almost 3 times higher than those for both of the other classes. The ratios for DGDG and MGDG are just a little bit lower than those for green algae, but almost 2 times less than those for plants. The distribution of unsaturated bonds has changed in C_{18} -residues of fatty acids. The total content of C_{18}-residues in thylacoid lipids have been almost the same but the content of $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ are altered in fraction of digalactosyldiacylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The properties of the thylakoid lipids and corresponding fatty acids in the monolayers at the liquid/gas interfaces have been studied. The changes in distribution of unsaturated bonds in C_{18} -residuces of fatty acids at light stress have been confirmed by monolayer measurements.

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