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Polymorphism in galactolipid/phosphatidylglycerol model membranes initiated by chlorophylls: ^{31}P-NMR and electron-microscopy studies

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Electron microscopy and ^{31}P-NMR spectroscopic studies have revealed that chlorophylls (forms *A* and *B*) induce structural interconversions in the bilayer model membranes composed of monogalactosyldiacylglycerol (MGDG)/phosphatidylglycerol (PG) (at molar ratios of 2:1 and 3:1) and monogalactosyldiacylglycerol/digalactosyldiacylglycerol (DGDG)/phosphatidylglycerol (at molar ratios of 2:2:1 and 3:2:1), as well as in membranes composed of spinach chloroplast total lipids. The lipid/pigment molar ratio was 5:1 in all mixtures analyzed. The alterations in the bilayer structure of all the model membranes investigated are associated with the appearance of the intermembrane lipidic particles, complex hexagonal and hexagonal-(H_{II}-) phase structures. The inverted micelles have been demonstrated to arise from the fusion of adjacent bilayers initiated by the point or ring like contacts. The liposomes made of pure MGDG form bilayer structures after incubation at 0°C for not less than 48 h. It was shown that the polymorphic property of MGDG may be changed when the galactolipid is dispersed amongst other lipids.

Introduction

While the function of the pigments in the photosynthetic process is reasonably well defined, the structural role of the chlorophylls (Chl) in the thylakoid membranes is obscure, and is a subject of current interest. Extensive studies of the thylakoid membranes suggest that some of the chlorophyll molecules are associated with the lipid portion of the thylakoid membrane, but the main pool is incorporated into the membrane chlorophyll-protein complexes [1–5].

It is not clear at present whether the stacking of thylakoid membranes depends on the state of association of Chl with proteins and/or on the protein conformational state in the Chl-protein complexes. It is known, however, that membrane destacking coincides with disruption [6] or lacking [57], of the Chl-protein complexes in the membranes, but we know nothing about Chl participation in this process. There is evidence that the pigments can be incorporated into the model lipid membrane [8–12]. It has been postulated [11] and experimentally checked [8–9] that the lipid phosphorus headgroup interacts with the magnesium ion of Chl as well as the phitol chain of the

Abbreviations: MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; Chl, chlorophylls (forms *a* and *b*); TLC, thin-layer chromatography.

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pigment molecule structurally related to the hydrocarbon chain of lipids [10].

The lipid fraction of thylakoid membranes consists predominantly of uncharged galactolipids [13–15]. The first indication that chloroplast membrane galactolipids may undergo polymorphic transition was obtained from X-ray analysis and freeze-fracture electron microscopy [16–18]. It is well known that thylakoid membranes generally contain approx. 50% MGDG, 25–30% DGDG, 9% PG and a small amount of sulpholipid and phosphatidylcholine (PC) [14]. It has been demonstrated that pure MGDG suspensions adopt the hexagonal (H_{II}) phase in the temperature range of -15 to 80 °C, whereas DGDG suspensions favoured a lamellar structure under these conditions [18]. It has been shown also that MGDG//DGDG mixtures inhibited both the lamellar organization and the formation of a variety of intermediate polymorphic structures, depending on the composition of the mixture [19,20].

It was found that saturated MGDG formed flat bilayers, while the unsaturated analogue exhibited temperature-dependent polymorphic phase interconversions where the hexagonal (H_{II}) phase dominated [21]. Interesting results have been reported recently by Mansourian and Quinn [22]. It has been discovered by freeze-fracture that in the mixture of saturated and unsaturated MGDG, there is marked phase segregation with flattened bilayers (saturated MGDG) and hexagonal (H_{II}) structures (unsaturated MGDG), if the suspension is thermally quenched from 20 °C. When the same mixture is quenched from 55 °C, the suspension has only spherical bilayer liposomes.However, in all the above-mentioned studies, the influence of Chl on the structural properties of galactolipids had not actually been discussed. Meanwhile, the fluorescence studies of Trosper et al. [23] and Beddard et al. [24] have shown the actual interaction of Chl with galactolipids. Furthermore, according to ^{31}P-NMR spectroscopy results [25], the pigments, like Chl, markedly disturb the bilayer structure of PC.

Obscurities in the data on interaction of Chl with lipid membranes [23–25], as well as on polymorphic behaviour of the thylakoid membrane in vivo [6], promote us to study the effect of Chl on the model membrane structure by means of ^{31}P-NMR and electron microscopic techniques.

We found that insertion of Chl into the thylakoid membrane lipid mixtures induced marked disturbances of lamellar membrane structure which finally lead to the formation of hexagonal (H_{II}) phase. The evidence obtained stresses that different intermembrane contact formation is indeed one of the initial steps in the process of bilayer hexagonal- (H_{II}-) phase transition. The experimental data obtained on any model system cannot be directly attributed to the properties of cellular membranes, but may be reasonably applied to the understanding of the functioning of membranes in vivo.

Materials and Methods

Isolation and purification procedures

Chromatographically pure PG was prepared by the phospholipase-D-catalyzed trans-esterification of chromatographically pure egg PC according to Dawson [26]. Phospholipase D (EC 3.1.4.4) was preparaed from cabbage according to Yang [27]. Egg PC was prepared according to Shvetz et al. [28]. MGDG and DGDG were extracted from wheat flour and purified by column chromatography [29]. The galactolipids were purified to homogeneity, as jugded by TLC, with the R_F values characteristic of these products [22]. TLC was performed on Silufol plates (Czechoslovakia) in a mixture of $CHCl_3/MeOH/H_2O$ (65 : 25 : 4). Galactolipid chemical structures were determined by ^{1}H-NMR spectroscopy on a Burker WM-250 spectrometer. For MGDG (δ, ppm): 5.40–4.80 and 4.32–3.50 (H^2, H^3, H^4, H^5, H^6 in galactose, CH_2O, CHO in glycerol); 4.56 (H^1, $J_{12} = 8$ Hz); 2.2–0.8 (CH_2, CH_3 in COOR). For DGDG (δ, ppm): 5.32–4.80 and 4.5–3.50 ($2H_2$, $2H_3$, $2H_4$, $2H_5$, $2H_6$ in galactose; CH_2O, CHO in glycerol), 4.6 ($^1H_{-\beta}$, $J_{1,2} = 7.0$ Hz) and 5.04 ($^1H_{-\alpha}$, $J_{1,2} = 3.3$ Hz), 2.4–2.2 and 1.80–0.7 (CH_2, CH_3 in COOR).

The fatty-acid composition of MGDG and DGDG was determined by gas-liquid chromatography (Chrom-41 Instruments, Czechoslovakia) of the methyl esters of acids obtained by alkaline deacetylation of galactolipids [30]. The fatty-acid composition of MGDG was: $C_{16:0}$, 15%;

$C_{18:1}$, 19%; $C_{18:2}$, 57%; $C_{18:3}$, 9%, and that of DGDG was: $C_{16:0}$, 8%; $C_{18:1}$, 9%; $C_{18:3}$, 83%. Total lipid fractions were isolated from spinach by a method described elsewhere [31]. Chl was isolated from nettle leaves as described by Iriyama et al. [32]. The chlorophyll *a*/chlorophyll *b* molar ratio was estimated according to Strain et al. [31] and was found to be 5.6 : 1. The concentration of purified pigment was determined from absorption spectra according to the conventional procedure [31,33].

Preparation of lipid suspensions

Non-sonicated lipid dispersions were prepared as follows: chloroform solutions containing 50–70 mg of lipid were evaporated, diluted with 1.2 ml of ^2H_2O (pH 5.6), and vortexed. The composition of the lipid suspensions was found to be: MGDG/PG (2 : 1 and 3 : 1); MGDG/DGDG/PG (3 : 2 : 1 and 2 : 2 : 1); DGDG/PG (2 : 1). Chl was incorporated into lipid mixtures in the following manner: Chl in ether (11–17 mg) was added to the lipid mixtures in chloroform (50–70 mg); the lipid/Chl mixtures were evaporated, diluted with 1.2 ml of ^2H_2O, and vortexed. The dispersion of total spinach thylakoid lipids was carried out in the same way. The molar ratio of lipid/Chl in all mixtures studied was 5 : 1 [34]. The lipid and lipid/Chl mixtures were used for ^{31}P-NMR and freeze-fracture examinations.

^{31}P-NMR spectroscopy

^{31}P-NMR of all hydrated lipid mixtures with or without Chl were recorded at the appropriate temperature on a Bruker WM-250 spectrometer (F.R.G. 101.4 MHz) with broad-band proton decoupling (15 W). The pulse waste 15 μs, the pulse delay was 0.8 s, and the number of scans, 5000. Chemical shifts were measured relative to 85% H_3PO_4.

Electron microscopy

For electron microscopy of thin sections, the same samples were fixed with 2.5% glutaraldehyde and 1% tannic acid in Hepes buffer (pH 7.8) and post-fixed with 0.25% osmium tetroxide for 15 min as described in detail elsewhere [35]. Fixed samples were dehydrated, embedded, sectioned and stained as described earlier [36].

For electron microscopy of freeze-fractured samples, all samples with or without glycerol, after incubation at an appropriate temperature, were frozen in liquid propane at −190 °C according to Borovyagin et al. [36]. Up to nine samples on copper sandwiches in liquid nitrogen were simultaneously inserted into a modified JEE-4 device (JEOL, Japan), fractured and replicated. After fracture, some samples were etched at −100 °C for 0.5–1 min. Replicas were cleaned [36] and examined in a JEM-100C electron microscope (JEOL, Japan) and micrographs were taken at a magnification of 5000–40000 ×. In all freeze-fracture micrographs, the platinum deposition direction is marked by double arrowheads.

Results

^{31}P-NMR spectroscopy

According to our ^{31}P-NMR data, the MGDG/PG (2 : 1) mixtures exhibited a stable lamellar-phase signal in the temperature range 5–50 °C (Fig. 1a). However, when the ratio of MGDG in the mixture was increased to 3 : 1, the bilayer structure of the membranes was markedly disturbed. In this mixture, more prominent structural changes occurred at 50 °C (Fig. 1e).

The bilayer phase of the MGDG/PG (2 : 1)/Chl mixture was still stable at 5 °C (Fig. 1b), whereas at 30–50 °C, some bilayer structure disturbances became detectable (Fig. 1c and d). However, the insertion of Chl into the MGDG/PG (3 : 1) mixture led to bilayer destabilization even at 5 °C (Fig. 1f), while at 30–50 °C, complete transition of lamellar to hexagonal (H_{II}) phase (Fig. 1g and h) was observed.

The addition of another thylakoid lamellar galactolipid, DGDG to the MGDG/PG (3 : 1) mixture stabilized its bilayer organization [37]. The model membranes composed of MGDG/DGDG/PG (3 : 2 : 1) exhibited a stabilized lamellar structure in the temperature range of 5 to 50 °C (Fig. 2a), whereas the MGDG/PG (3 : 1) binate system had a tendency to show polymorphic behaviour under these conditions (Fig. 1e). The mixing of the MGDG/DGDG/PC (3 : 2 : 1) system with Chl resulted in the disturbance of bilayers; the ^{31}H-NMR signal narrowed and became more symmetric (Fig. 2b). Therefore, DGDG

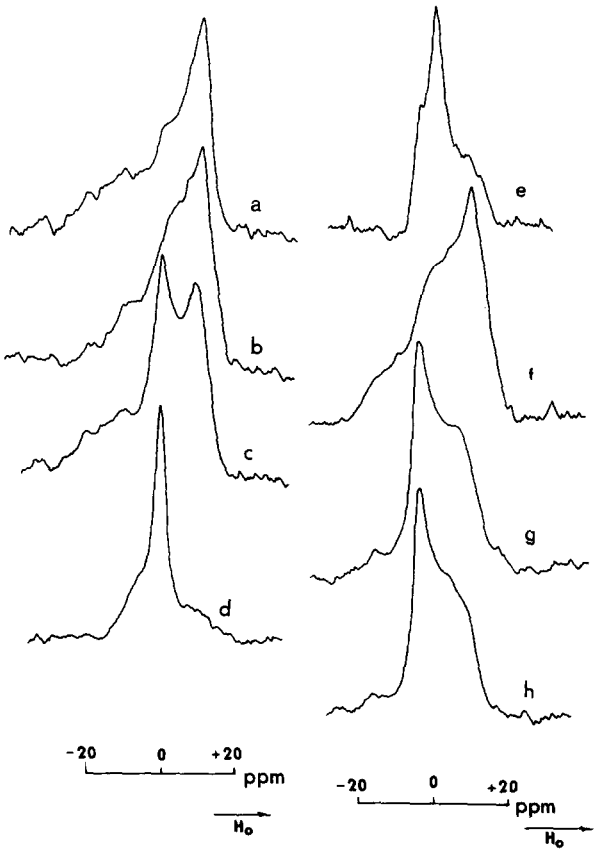


Fig. 1. 101.4 MHz, ^{31}P-NMR spectra of aqueous suspensions of: MGDG/PG with a molar ratio of 2:1 at 50 °C (a); MGDG/PG (2:1) with chlorophylls (lipids/Chl, 5:1) at 5 °C (b); at 30 °C (c); and at 50 °C (d); MGDG/PG with a molar ratio 3:1 at 50 °C (e); MGDG/PG (3:1) with chlorophylls at 5 °C (f); at 30 °C (g); and at 50 °C (h).

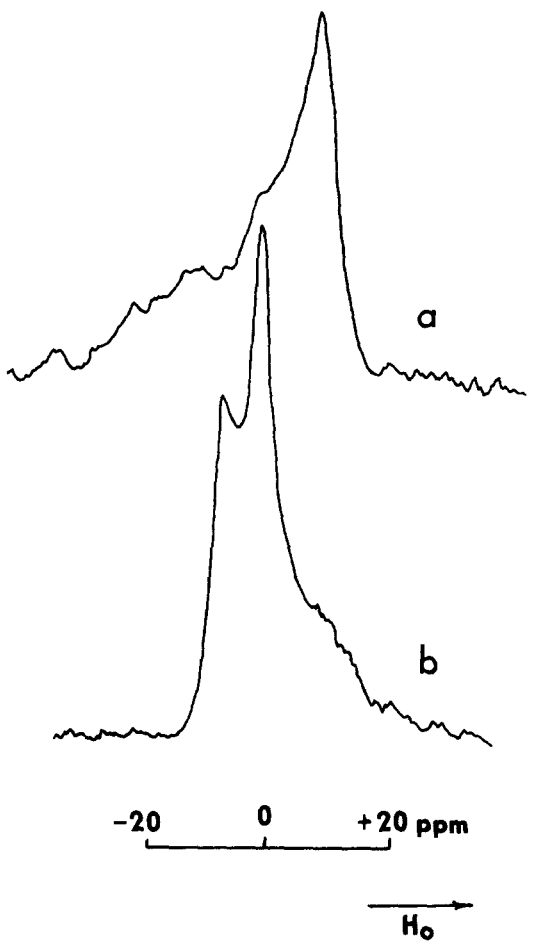


Fig. 2. 101.4 MHz, ^{31}P-NMR spectra of aqueous dispersions of model membranes composes of: MGDG/DGDG/PG with a molar ratio of 3:2:1 at 50 °C (a); MGDG/DGDG/PG (3:2:1) with chlorophylls (lipid/Chl, 5:1) at 30 °C (b).

stabilized the bilayer structures in the MGDG/DGDG/PG system, whereas Chl shifted this system to an unstable state.

The thylakoid total lipid fraction contained sulpholipid and PG as compared to the MGDG/DGDG/PG suspension [38]. In order to determine the relative contribution of these lipids (sulpholipids and PG) to the structural properties of the MGDG/DGDG/PG membranes, comparative ^{31}P-NMR experiments were carried out on these two lipid suspensions.

The ^{31}P-NMR spectrum of the total membrane lipid fraction has an effective chemical shift anisotropy of about 50 ppm, with a complex signal of isotropic and an H_{II} phase located down-field as compared to the maximum of the anisotropic signal (Fig. 3a). Compared to the MGDG/DGDG/PG bilayers (Fig. 2a), the total polar lipids showed some bilayer disturbances under the same conditions. These polymorphic changes could be attributed to the presence in the total lipid fraction of sulpholipid rather than PG, since, as has been previously demonstrated [39], the addition of sulpholipid to the MGDG/DGDG mixture leads to the appearance of a large amount of non-bilayer structures in the suspension. As is clearly seen from the ^{31}P-NMR spectrum (Fig. 3b), the insertion of Chl into the total lipid extract markedly increases its polymorphic transition property.

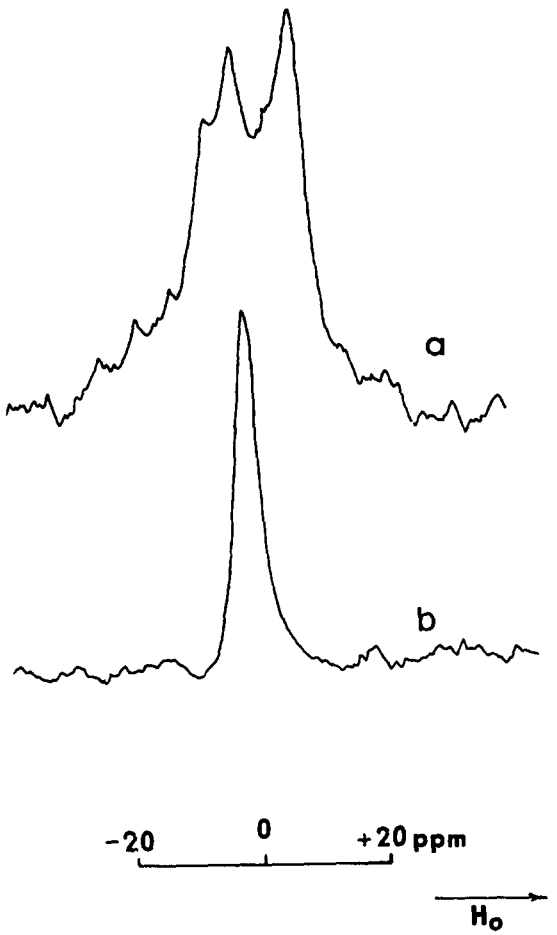


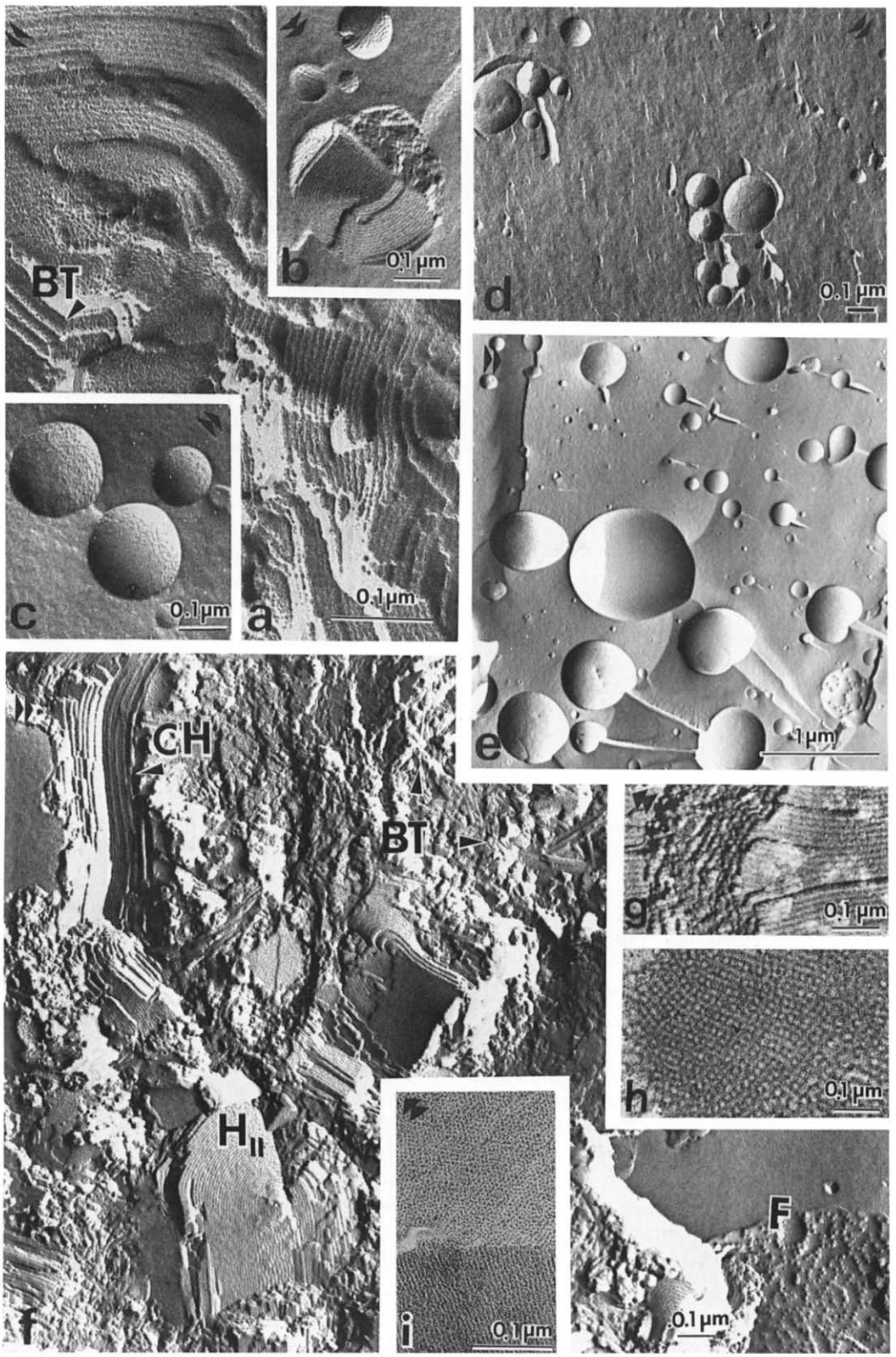
Fig. 3. 101.4 MHz, ^{31}P-NMR spectra of aqueous suspensions of total thylakoid lipids at 30 °C (a) and the same lipid fraction with chlorophylls (lipid/Chl, 5 : 1) at 30 °C (b).

The spectrum suggests that the total lipid extract plus Chl has no bilayer structure at all. Spectroscopic data presented above strongly suggest that Chl markedly destabilizes the bilayer structure in the membranes studied. The level of polymorphic transition under the same experimental conditions depends on the lipid composition of the suspensions. The changes of line-shape in the ^{31}P-NMR spectra of MGDG/PG mixtures (2 : 1, Fig. 1a–d; 3 : 1, Fig. 1e–h; or MGDG/DGDG/PG, 3 : 2 : 1, Fig. 2a and b) showed that PG (which usually forms stable bilayers in the hydrated state) also takes part in the polymorphic transition. The fact that the addition of Chl to the aqueous dispersions of DGDG, PG or DGDG/PG mixtures did not trigger any changes in the ^{31}P-NMR spectra directly confirms the suggestion that the polymorphic interconversions of MGDG-containing lipid mixtures are due to the presence of this galactolipid [19–21,37]. This, however, does not mean that only MGDG contributes to the detectable polymorphic transition of lipid systems. As mentioned above, the NMR spectra confirm the participation of PG in polymorphic transition. Moreover, it has recently been shown by ^{2}H-NMR and ^{31}P-NMR spectroscopy that in different lipid mixtures [40,41], as well as in the MGDG/PG mixture [40], each of which being in liquid crystalline state, all lipid components take part in the detectable polymorphism of the system. This is a very important point (see below).

NMR spectroscopy does not give direct and precise information with regard to the morphological details of the polymorphic interconversions triggered by the insertion of Chl. An extensive electron microscopic study was therefore performed on the same lipid dispersions.

Electron microscopy of MGDG suspensions thermally quenched from 0 to 50 °C usually reveal the hexagonal complex [35,42] and hexagonal (H_{II}) phase (Fig. 4a and b), which is in agreement with earlier data [18,38]. However, if the MGDG suspensions were preincubated at 0 °C for not less than 2 days, all replicas demonstrated bilayer organization (Fig. 4c). When quenched in the temperature range of 0–40 °C, the aqueous dispersions of pure DGDG or PG, as well as DGDG/PG (2 : 1), MGDG/PG (3 : 1 and 2 : 1) and MGDG/DGDG/PG (3 : 2 : 1 and 2 : 2 : 1) mixtures showed smooth fracture faces of closed liposomes (Figs. 4d, 5a and 6a). In the case of depigmented total lipid fraction, the same situation could be observed, although about 3–5% of the liposome fracture faces (Fig. 4e) contained a few conical or crater-like lipidic intramembrane particles.

In all the above-mentioned lipid mixtures except for DGDG, PG and DGDG/PG suspensions, Chl induced significant changes in the lamellar organization. This agrees with NMR results. For example, the replicas showed that Chl induced marked structural changes in the lamellar organization of the total lipid samples (Fig. 4f–i). The fracture faces of the depigmented total lipid


BT
b
0.1 μm
d
0.1 μm
c
0.1μm
a
0.1μm
e
1 μm
CH
BT
g
0.1 μm
h
0.1 μm
H_{II}
i
0.1μm
F
0.1 μm
f

fraction (Fig. 4e) were easily distinguishable from those containing Chl (Fig. 4f–i). The fracture face of the sample showed that the lipids were generally in aggregated state with areas occupied by different polymorphic structures: (i) small bilayer area (F) studied by lipidic intramembrane particles; (ii) large areas composed of randomly occurring bilayer tubular structures (12–18 nm in diameter) and regular, tightly packed 'complex hexasomal' spacing of about 15 nm) tubular structures; Thin sections (Fig. 4h) and the dimensions and type of packing of these structures on freeze-fracture replicas (Fig. 4g) allowed us to consider them as bilayer tubular or in some cases as 'complex hexagonal' structures; (iii) areas occupied by tightly packed structures with spacing of about 7–7.5 nm (H_{II}). The dimensions and appearance of the latter are characteristic of the hexagonal phase (Fig. 4i) [20,21,35]. When these samples were thermally quenched from 40 or 50 °C, the replicas predominantly displayed the H_{II} phase, which is in good agreement with our NMR data. The presence of Chl in MGDG/DGDG/PG (molar ratios, 2 : 2 : 1 or 3 : 2 : 1) mixtures (thermally quenched from 20 °C) stimulated (in non-tightly packed multiwalled liposomes, Fig. 5a) the formation of randomly distributed numerous point and linear intermembrane contacts (Fig. 5b). In tightly packed multilayered liposomes, bilayers formed practically lamellar crystal-like structures, in which the dimensions are very similar to patterns of the hexagonal H_{II} phase. These structures are seen clearly in Fig. 5c and d on their cross-fractures in the areas marked by asterisks. In these liposomes, the formation of tightly or randomly disposed point contacts (lipidic intramembrane particles), as well as different polymorphic structures (Fig. 5c and d), were frequently observed.

The transitions from lamellar to hexagonal phase was more frequent in the presence of Chl in the 3 : 2 : 1 mixture of MGDG/DGDG/PG (not shown). The development of point contacts led to the appearance of short-lived lipidic intramembrane particles or so-called inverted lipid micelles [37,43,48]. In our micrographs (Fig. 5b and c), the spherical lipidic intramembrane particles were usually about 5–10 nm in diameter and about 10–16 nm and more for conical and ring-like contacts. According to our observations, the size of the lipidic intramembrane particles depends on the time of their development until quenching. This is consistent with the data published by other authors [20]. The Chl-induced polymorphic behaviour of the MGDG/PG (3 : 1 or 2 : 1) mixtures was quite similar to that of the other mixtures studies. For example, the replica of a 2 : 1 MGDG/PG mixture quenched from 20 °C demonstrates numerous spherical and conical lipidic intramembrane particles (Fig. 6b,No. 1) and pits (not shown), which in some areas fuse into linear contacts (Fig. 6b, No. 3). Fig 6b and c show the lipidic intramembrane particles as crater-like particles (F, black arrowheads) and hexagonal (H_{II}) phase (Fig. 6e, H_{II}) which is clearly seen in the thin-section micrograph (Fig. 6e[1]). We believe these structures (Fig. 6b–e) represent different stages of the bilayer fusion, including the formation of spherical (Fig. 5c, No. 2) and conical (Fig. 6, No. 1; Fig. 6e, F, black and white arrowhead) lipidic intramembrane particles with (Fig. 6e, F, black arrow) or without central holes on the top of particles, as well as the appearance of stalks [49,50] (Figs. 6d and 7a and c, black arrow) and pores (Fig. 6d, black and white arrowhead).

We were not able to observe the hexagonal-H_{II}-) phase transition on replicas of 3 :'1 MGDG/PG mixtures containing Chl and quenched from 20 °C. Nevertheless, this is easily observed when the quenching was from 40 or 50 °C. In the first case, when the samples were quenched from 15 to 20 °C, the replicas often

Fig. 4. Electron micrographs of freeze-fracture replicas (a–g) and thin-section (h and i) prepared from non-sonicated dispersion (without glycerol) of: MGDG incubated for 2 h and thermally quenched from 40 °C (a); and from 20 °C (b); MGDG incubated at 0 °C for 48 h and quenched from 20 °C (c); DGDG incubated for 2 h and quenched from 20 °C (d); depigmented total spinach thylakoid membrane lipids incubated for 2 h and thermally quenched from 20 °C (e); and the latter with chlorophylls (Chl/lipids, 1 : 5) (f, g, h and i). Areas with lipidic intramembrane particles (F), as well as bilayer tubular (BT), complex hexagonal ((CH), f and g negative prints) and hexagonal H_{II} phase (f and i) structures are visible; thin-section image (h) confirms the presence of tightly packed bilayer tubular (or complex hexagonal) structures in the sample; the sample in (i) was etched for 15 s.

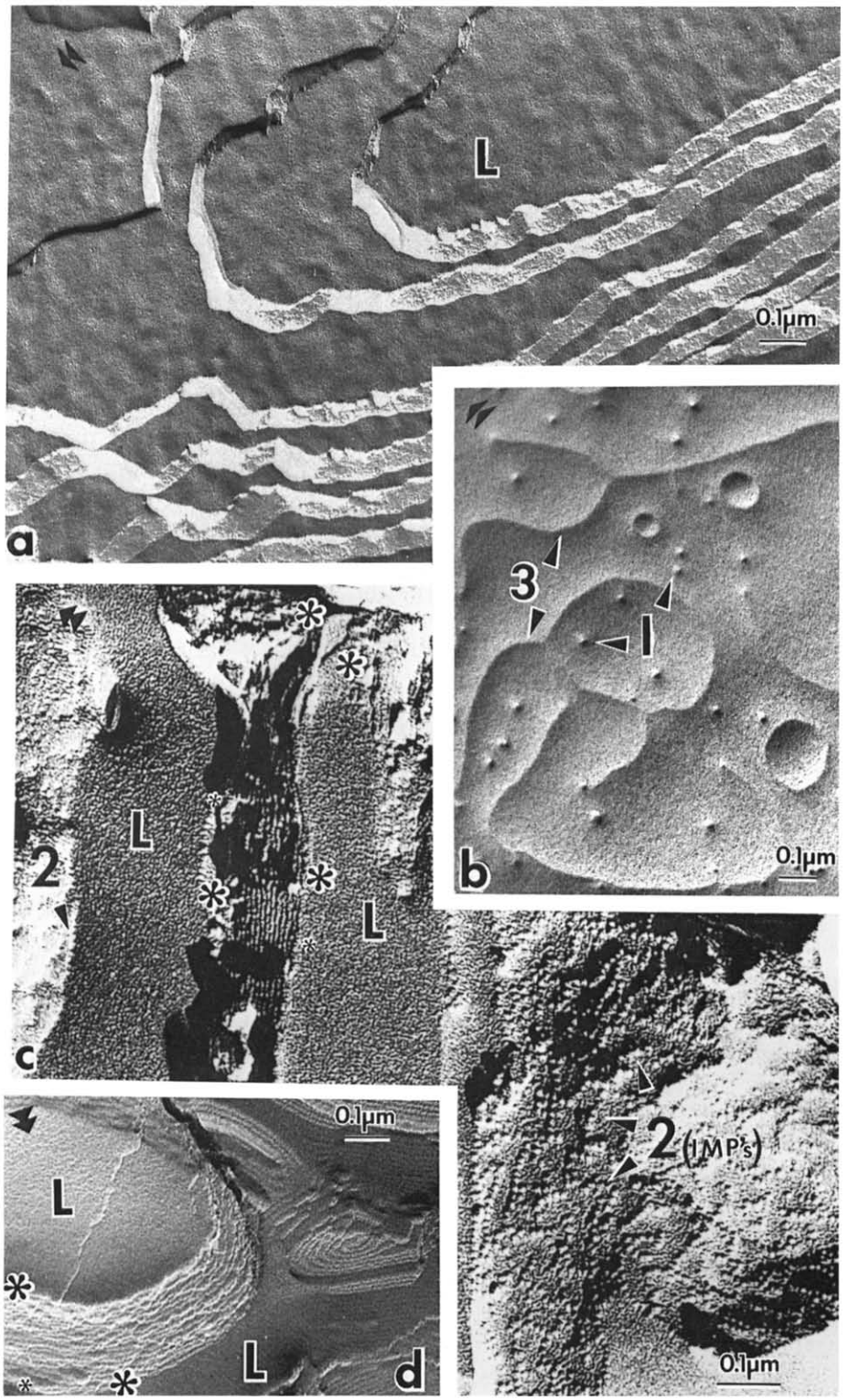


Fig. 5. Freeze-fracture images of suspensions of MGDG/DGDG/PG (2:2:1) in 20% glycerol incubated for 1 h and thermally quenched from 20 °C without chlorophylls (a) and after addition of the pigments with lipid/Chl molar ratio of 5:1 (b,c, negative prints); the micrographs (b) show point (No. 1), crater-like or ring-like and linear (No. 3) intermembrane contacts, as well as (c) lipidic intramembrane particles (No. 2), different polymorphic structures and areas where tightly packed bilayers (L) formed practically crystal-like structures (asterisks), of which the cross-fracture dimensions are very similar to patterns of hexagonal H_{II} phase.

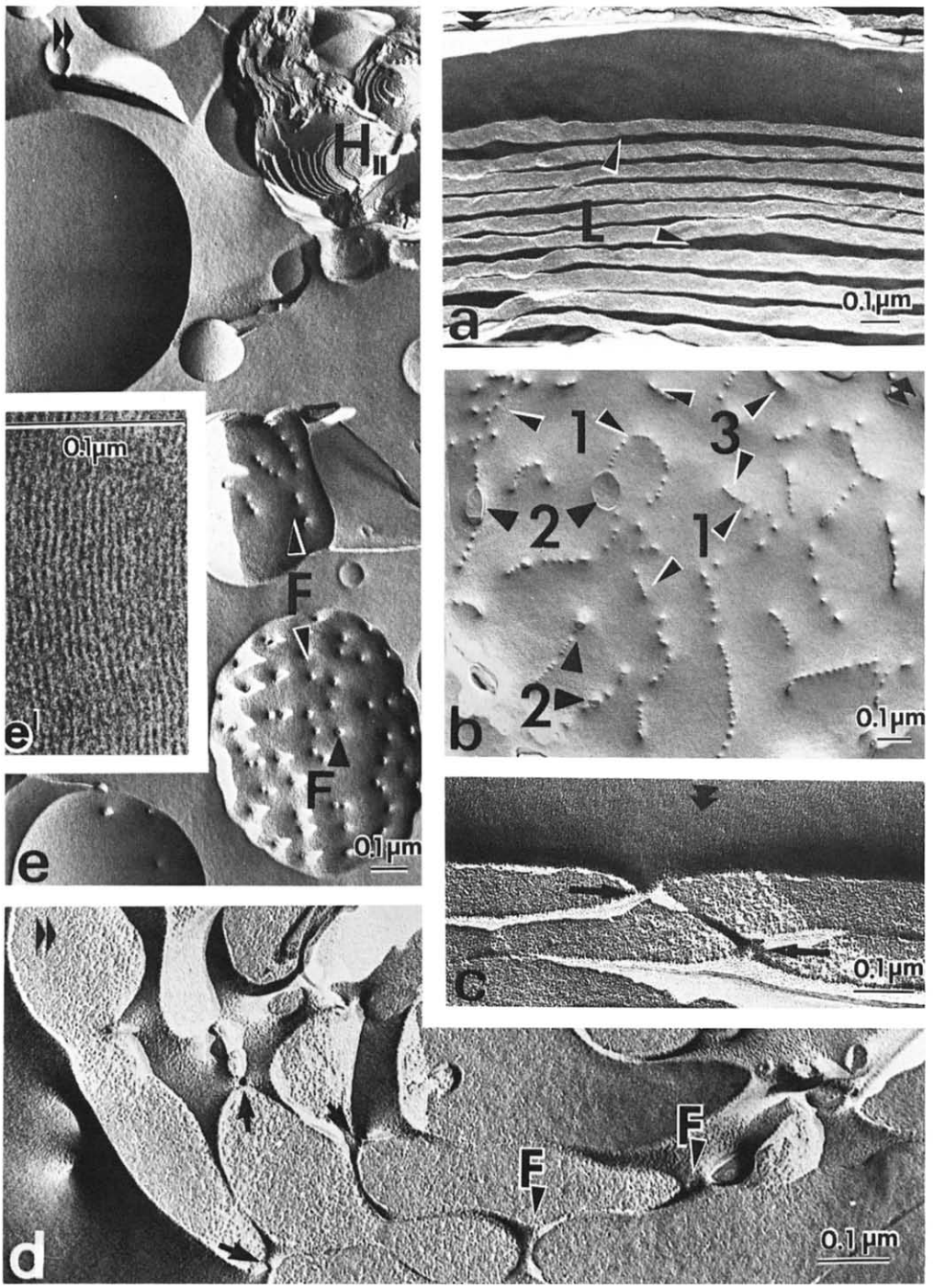


Fig. 6. Electron micrographs of dispersions of a molar ratio of 2:1 MGDG/PG mixture incubated for 1 h and without glycerol thermally quenched from 20 °C without (a) and after the addition of pigments (b, c, d, e and e^1); freeze-fracture replicas show different crater-like (b, No. 2) or conical (b, No. 1) contacts without (e, F, black and white arrowhead) or with (e, F, black arrowheads) central hole point or conical contacts in some areas fused into linear ridges (b, No. 3); stalk (c and d, black arrow), pore (e, F, black arrowhead and d, F, black and white arrowhead) and hexagonal H_{II}-phase structures (in e and e^1) are also visible; samples in (c) and (d) were etched for 30 s; the thin-section micrograph (e^1) confirms the presence of hexagonal H_{II} phase in the MGDG/PG/Chl mixture.

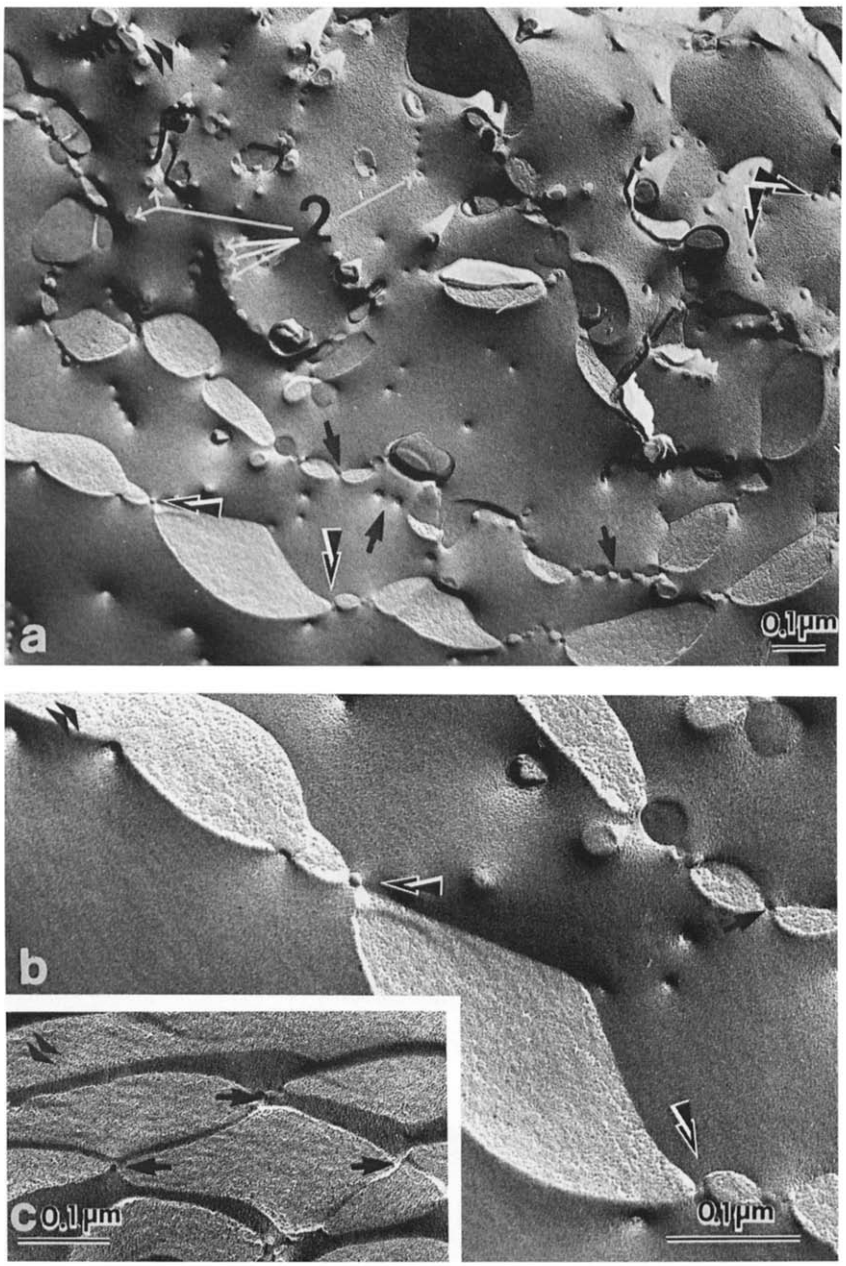


Fig. 7. Freeze-fracture electron micrographs of MGDG/PG/Chl mixture (molar ratio, 3:1) incubated for 1 h and thermally quenched without glycerol from 20 °C; the sample was etched for 30 s; the micrographs show numerous point or ring-like (double arrowheads) contacts which produce lipidic intramembrane particles of different size, stalks (black arrow) and pores (No. 2).

demonstrated one and the same striking picture (see Fig. 6c and d): a lot of attachment sites situated quite far from each other and located between widely dispersed bilayers. Fig. 7 shows images of numerous cone-shaped particles and inverted micelles of different sizes (Fig. 7a and b, double arrowheads). The stalk and pore structures became distinguishable on the bilayer fracture

faces only when fractured samples were slightly etched. After etching, the central part of the pore became concave (Fig. 7a, No. 2), whereas the stalks preserved a truncated cone shape (Fig. 7a and c, black arrow).

Discussion

The concept that MGDG may play a basic role in the structural interconversions of galactolipid mixtures is supported by Refs. 21, 22 and 38 and confirmed in this paper by spectroscopic and freeze-fracture data. However, as previously mentioned, according to the NMR data [40,41], all lipid components in mixtures are in the liquid crystalline state. This logically suggests that all mixture constituents should participate in polymorphic transition from lamellar to hexagonal (H_{II}) phase. In other words, the structural properties of individual lipids may be significantly changed when they are in binate or in a more complex mixture. This means that the concentration of MGDG in bulk lipid mixture is not be considered as a direct indication of proportional aptitude of the mixture for polymorphism. For instance, our freeze-fracture micrographs demonstrated that, in the presence of Chl, the hexagonal (H_{II}) phase in 3:1 MGDG/PG mixtures was developed only when the samples were thermally quenched from temperatures of 40 °C or higher. At the same time, in MGDG/PG (2:1) as well as in MGDG/DGDG/PG (3:2:1) mixtures or in the total lipid fraction, where the relative concentration of MGDG was lower, a range of structural interconversions took place in the samples quenched even from 15 °C to 20 °C.

Thus, the present morphological results confirm our suggestion (see the NMR section) that in a lipid mixture, the structural properties of MGDG, and perhaps of other lipids with a tendency to polymorphism, may be changed due to the presence of other lipids or components. It is not essential that the rather different structural organization frequently observed on electron micrographs obtained from lipid mixtures is due to a phase separation of pure and/or mixed lipids [38]. This might be explained by the heterogeneous distribution of loosely and tightly packed multiwalled liposomes and lipid aggregates as well as by unequally dispersed factors such as the degree of dehydration, cations, and others.

At present, the molecular mechanism of how Chl influences the structure of MGDG-containing plant-lipid mixtures is unknown. There are no data available concerning this problem, except for those of Sprague and Staehelin [21], in which no evidence for the effect of Chl on chloroplast total lipid fraction has been found. This result might be affected by the presence of other pigments and substances in the bulk lipid extract.

It may be suggested that the polar moiety of the pigment molecule affects mainly the configuration mobility and/or redistribution of charges of the hydrophilic lipid region as well as the hydration of the lipid molecules. It is also possible that the phitol chain of Chl interacting with the hydrocarbon chain of lipid [9] changes motility and alters the lipid–lipid interaction, which leads to the destabilization of the bilayer structure. This raises the possibility that Chl may affect the structural properties of thylakoid membranes in vivo. Recently, Quinn and Williams [38] have discussed the experimental data, suggesting that in light-harvesting thylakoid membranes, the lipid bilayer is in general stabilized by interaction with proteins. The formation of grana in higher plant chloroplasts is believed to be associated with the presence of the Chl *a/b* light-harvesting protein [51]. Mutants lacking this protein usually show little or no tendency to form grana [7]. There is also some experimental evidence that factors determining grana membrane stacking are primarily electrostatic [52,53].

Thus, it is likely that Chl in vivo participates directly or indirectly in the formation of grana intermembrane contacts. For example, Carter and Staehelin [51] have shown that in unstacked pronase-digested grana membranes in which the light-harvesting Chl *a/b*-protein complex is removed, the ability of the membrane for restacking is abolished. It has also been shown that, in the same regions of cation-induced restacked grana membranes, different polymorphic structures are present [51].

Conclusions

Based on the evidence presented here and the findings of Carter and Staehelin [51], we conclude

that Chl and divalent cations have a similar mechanism of triggering the intermembrane contacts, at least in thylakoid membranes.

However, further investigations are needed to give a more definite answer to the intriguing questions concerning the structural influence of Chl in vivo.

We believe that this study is the first in which the structural effect of Chl on MGDG-containing plant-lipid mixtures has been analyzed.

We wish to emphasize that the different structural stages of contact development and bilayer fusion described here represent the first polymorphic transition steps which are practically ubiquitous, not only for any lipid systems with a tendency for polymorphism, but even for the process of cell membrane fusion[54–57].

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