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LATERAL HETEROGENEITY IN THE DISTRIBUTION OF THYLAKOID MEMBRANE LIPID AND PROTEIN COMPONENTS AND ITS IMPLICATIONS FOR THE MOLECULAR ORGANISATION OF PHOTOSYNTHETIC MEMBRANES

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The lateral distribution of all the major components of photosynthetic thylakoid membranes, i.e., acyl lipids, chlorophylls and proteins, has been examined by comparing the composition of whole thylakoids with that of appressed membrane regions derived therefrom. Separations were effected by means of French-press treatment followed by aqueous polymer two-phase partition. Considerable lateral heterogeneities in the distribution of all major membrane components were found. Membrane preparations highly enriched in appressed (granal) thylakoids contained less than 10% of the total thylakoid Photosystem I complex, but contained twice the amount of total chlorophyll relative to protein (mg/ml or mol/mol) and only 50–60% of the amount of total lipid relative to protein compared with whole thylakoids. Appressed thylakoids were substantially depleted in both major galactolipids in comparison with both whole thylakoids and non-appressed thylakoids. The ratio of diacylglycerophosphoglycerol: protein showed little variation between membrane fractions. A molar ratio of lipid: protein in appressed regions of only 13.5: 1 was deduced, which implies a very considerable protein-protein proximity in chloroplast granal membranes. The results are discussed in terms of their implications for the molecular organisation of photosynthetic membranes.

Introduction

The fluid-mosaic model of biological membrane organisation was published in its original form by Singer [1] in 1971. This model adequately describes planar or slightly curved membranes with high ratios of lipid: protein, but cannot fully represent more complex membranous structures, such as endoplasmic reticulum, mitochondrial inner membranes and chloroplast thylakoids [2]. It has been proposed that factors such as lipid packing properties and relative hydrophobicity/hydrophilicity may lead to a spontaneous thermodynamically stable transverse membrane lipid asymmetry [3–8]. Such an asymmetry is now well documented in both animal and plant membranes [9–12]. Lateral asymmetry of protein components is also well established in red blood cell membranes [13] and chloroplast membranes [14–16]. The possibility that there may also be a lateral asymmetry of acyl lipids has been raised by a number of investigators but direct measurements of large-scale lateral acyl lipid heterogeneities have

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Abbreviations: PS, photosystem; Chl, chlorophyll.

hitherto not been possible [2]. Indirect methods, such as the use of non-penetrating cross-linking probes [17], fluorescent probes [18] or ferritin/lectin/antibody capping studies [2], imply that separate lipid domains, not necessarily associated with protein molecules, may exist in certain biological membranes. It has been very recently reported that the lateral redistribution of specific erythrocyte membrane lipids into rod-shaped protuberences could be induced by the addition of saturated long-chain (C_{16}-C_{18}) alcohols [19]. The authors concluded that the lateral asymmetry may be due to simple lipid-lipid interactions and that these may impart a degree of mechanical stability to non-spherical biological membrane structures. An analagous proposal, that exclusively lipidic galactolipid domains may stabilise the tightly curved margins of chloroplast thylakoids, has also been recently put forward [8].

With the advent of techniques to fractionate and separate appressed and non-appressed regions of thylakoid membranes, it is now possible to study structurally differentiated regions of the same contiguous biological membrane. Such studies have already demonstrated pronounced lateral asymmetries in protein distribution [14–16]. In this report we present evidence for large-scale lateral heterogeneities in the distribution of all of the major components of photosynthetic membranes: acyl lipids, chlorophylls and proteins. The implications of these findings for the molecular organisation of thylakoid membranes are then explored.

Materials and Methods

Dextran T500 was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden) and poly(ethylene glycol) 4000 from Union Carbide (New York, U.S.A.). Other biochemicals were of the best obtainable reagent grade. All organic solvents were distilled prior to use. *Spinacea oleracea* L was grown in a glasshouse under natural illumination. Intact chloroplasts were isolated from 100 g of 5-week-old leaves according to the method of Walker [20]. The chloroplast pellets were resuspended in 30 ml of 50 mM sodium phosphate buffer, pH 7.4, 10 mM NaCl, 50 mM sucrose and incubated on ice for 30 min. Class II chloroplasts were sedimented from this suspension by centrifugation at $1000 \times g$ for 10 min. The resultant pellet was washed twice with resuspension medium and finally resuspended at a concentration of 400 μg chlorophyll/ml. An aliquot of Class II chloroplasts was retained for lipid, protein and chlorophyll analyses. The washed chloroplasts were passed twice through a French press. The remaining procedures were carried out essentially according to the method of Andersson and Anderson [14] and a similar nomenclature for describing membrane preparations is employed here.

Aliquots of fractions B3 and T2 were immediately frozen at -40°C for later electrophoretic and photochemical activity determinations. Chlorophyll was determined according to the method of Bruinsma [21] and membrane proteins as described by Sandermann and Strominger [22].

Freshly prepared class II chloroplasts and T2 and B3 fractions from both 5.7 and 5.8% (w/w) dextran:poly(ethylene glycol) phase systems were subject to lipid analysis by extraction into 20 vol. chloroform/methanol (2 : 1, v/v) to produce a monophasic extract. Water-soluble constituents were partitioned into 7.0% NaCl. The lower (lipid) layer was quantitatively recovered and the solvent removed under nitrogen.

Complex lipids were fractionated by thin-layer chromatography on silica gel H using acetone/benzene/water (91 : 30 : 8, v/v) as developing solvents [23]. Purified chloroplast membrane lipid standards were also applied to each chromatoplate. The lipids were located by spraying with anilinonaphthalenesulphonic acid (0.2%, w/w in methanol) and visualised under ultraviolet light. Separated lipid classes were recovered from chromatoplasts into 5 ml benzene/methanol/sulphuric acid (10 : 84 : 4, v/v/v). A known quantity of heptadecanoic acid was added as an internal standard. The lipids were transmethylated by incubation at 80°C for 2 h. Methyl esters were recovered in hexane and either stored under nitrogen at 40°C, or in some cases, purified further on layers of silica gel H using hexane/diethyl ether (70 : 30, v/v) as developing solvent and water-saturated ether for eluting from chromatoplates.

Methyl esters were analysed by gas-liquid chromatography in a Perkin-Elmer F-22 instrument

equipped with flame-ionisation detectors. Separations were effected by means of a 1.8 m × 4 mm column packed with 10% silar 5CP on Gas-Chrom Q, 100/120 mesh. Methyl ester peaks were quantitated with respect to the heptadecanoic acid internal standard. Complex lipid classes were quantitated using the quantitative methyl ester data and known molecular weight values for the various acyl, glycerol and head group moieties (see Table I).

SDS-polyacrylamide gel electrophoresis of chloroplast and subchloroplast membrane fractions was carried out essentially according to the procedure used by Andersson and Anderson [14]. The distribution of chlorophyll in the chlorophyll-protein complexes separated in gels was measured as described in Ref. 24. Proton-translocation studies with either inside-out or normal-sided thylakoid vesicles were carried out by monitoring external pH changes according to the method of Andersson and Akerlund [25].

Results

The passage of chloroplast thylakoids through a mechanical press, such as a French press or a Yeda press, causes a shearing of the membranes along the edges of granal appressions [26,27]. The two French-press fractions separated in the present study exhibited opposite light-induced proton fluxes in the presence of phenyl-*p*-benzoquinone.

TABLE I

ABSOLUTE DISTRIBUTION OF ACYL LIPID, CHLOROPHYLL AND PROTEIN IN FRENCH-PRESSED SPINACH THYLAKOID MEMBRANE FRACTIONS AND WHOLE THYLAKOIDS

All quantities are relative to a standard 1 mg total chlorophyll. Nomenclature of membrane fractions is given in Ref. 14. The lower, dextran, phase (B3) was enriched in inside-out vesicles derived from appressed thylakoids (grana). Membrane fractions equivalent to approx. 1 mg chlorophyll were analysed. Total lipids were determined by quantitative gas-liquid chromatography of purified methyl esters. Details are given in the text. Chlorophyll values were measured as described in Ref. 21 and proteins as in Ref. 22. Results are average value from three separate experiments. Average lipid molecular weight values for each membrane fraction were calculated from the lipid and fatty acid composition data presented in Tables IV and V. The following average lipid molecular weights were used: 757, whole thylakoids; 753, lower, dextran, phase (B3).

Membrane constituent	Fraction			
	Whole thylakoids		Lower, dextran phase (B3)	
	μg	%	μg	%
Acyl lipid	3254	24.4	1002	13.8
Protein	9103	68.1	5275	72.5
Total chlorophyll	1000	7.5	1000	13.7
Chl *a*	706		590	
Chl *b*	294		410	
	ratio			
Chl *a*/*b*	2.41		1.44	
Acyl lipid/protein	0.36		0.19	
Acyl lipid/chlorophyll	3.84		1.19	

TABLE II

CHLOROPHYLL DISTRIBUTION BETWEEN CHLOROPHYLL-PROTEIN COMPLEXES FROM FRENCH-PRESSED SPINACH THYLAKOID MEMBRANE FRACTIONS AND WHOLE THYLAKOIDS

Nomenclature of chlorophyll-protein complexes is according to Ref. 14. Chlorophyll values were determined from 80% acetone extracts of gel slices according to Ref. 24. See Table I for nomenclature of membrane fractions

Fraction	Average percentage of total chlorophyll			
	PS I reaction centre complex CP 1 + CP Ia	PS II reaction centre complex CP a	Light-harvesting complex LHCP[1-3]	Free chlorophyll
Whole thylakoids	29	11	48	12
Upper, poly(ethylene glycol), phase (T2)	31	11	46	12
Lower, dextran, phase (B3)	9	12	65	14

TABLE III

ACYL LIPID COMPOSITION OF FRENCH-PRESSED SPINACH THYLAKOID MEMBRANE FRACTIONS AND WHOLE THYLAKOIDS

Acyl lipids were fractionated by thin-layer chromatography on silica gel H. The individual lipid classes were transmethylated and the methyl ester were quantitatively analysed by gas-liquid chromatography with reference to a methylheptadecanoate internal standard. Details are given in the text. Nomenclature of membrane fractions is given in Table I. Values are expressed as mol%.

Acyl lipid class	Fraction	
	Whole thylakoids	Lower, dextran phase (B3)
Diacylgalactosylglycerol	38.0	44.0
Diacylgalactosylglycerol	29.1	18.2
Diacylsulphoquinovosylglycerol	12.5	9.5
Diacylglycerophosphoglycerol	14.3	33.3
Diacylglycerophosphocholine	5.1	1.9

Vesicles partitioning into the upper, poly(ethylene glycol), phase (T2) showed a light-induced, reversible proton uptake similar to that of whole thylakoid preparations. The material in the lower, dextran, phase (B3) contained vesicles which exhibited a light-induced, reversible proton extrusion. This material, which is derived mainly from appressed thylakoids, is henceforth referred to as inside-out vesicles or appressed regions [28,29].

The distribution of the three major thylakoid constituents – acyl lipid, chlorophyll and protein – between whole thylakoids and inside-out vesicles is shown in Table I. Together these three constituents accounted for over 98% by weight of the photosynthetic thylakoids membranes. The most notable results here are the relative depletion of acyl lipid and Chl *a* in the inside-out vesicle (B3) fraction. The relative amount of protein is unchanged in both membrane populations, but the inside-out vesicles are severely depleted in acyl lipid and enriched in total chlorophyll. Chlorophyll-protein complexes, which are known to contain all the thylakoid chlorophyll [30], and pigment-free proteins together account for 75.6% of the total weight of thylakoid membranes but are 86.2% of the total weight of inside-out vesicles. The lipid/protein ratio (by weight) of inside-out vesicles is only about half that of whole thylakoids.

The asymmetric distribution of the major chlorophyll protein complexes between the thylakoid fractions is shown in Table II. Appressed thylakoids are very depleted in Photosystem I complex and enriched in light-harvesting complex. It has been estimated that 75% of the total B3 fraction is composed of inside-out vesicles [16]. Andersson and Anderson [14] have reported that, assuming that 30% of the total chlorophyll is in non-appressed regions, the amount of PS I com-

TABLE IV

FATTY ACID COMPOSITION OF THE ACYL LIPIDS OF FRENCH-PRESSED SPINACH THYLAKOID MEMBRANE FRACTIONS AND WHOLE THYLAKOIDS

Fatty acids were estimated as methyl esters prepared by transmethylation of separated acyl lipid classes. The methyl esters were quantitatively analysed by gas-liquid chromatography as outlined in the text. See Table I for nomenclature of membrane fractions. WT, whole thylakoids; B3, lower, dextran, phase. tr., trace.

Fatty acid	Acyl lipid class									
	Diacylgalactosyl-glycerol		Diacyldigalactosyl-glycerol		Diacylsulphoquino-vosylglycerol		Diacylglycero-phosphoglycerol		Diacylglycero-phosphocholine	
	WT	B3	WT	B3	WT	B3	WT	B3	WT	B3
16:0	0.6	2.0	7.5	8.5	34.2	54.3	19.3	12.4	19.0	23.8
16:1 [a]	0.1	0.6	0.2	0.1	0.5	0.6	30.2	38.9	1.1	0.8
16:3	25.9	19.5	6.2	4.9	0.9	0.1	01.1	tr	0.9	5.2
18:0	tr.	tr.	0.1	2.6	0.1	0.2	0.2	0.6	1.9	1.1
18:1	0.8	0.6	1.7	1.7	0.4	tr	5.2	1.3	8.6	8.1
18:2	1.0	1.0	1.8	1.2	4.1	6.1	10.0	4.1	20.0	15.8
18:3	71.5	76.2	82.6	80.2	59.8	38.3	33.9	37.3	48.4	45.8

[a] *trans*-3-Hexadeceanoate.

plex in appressed regions is about 9% of total chlorophyll. From our data we find that, assuming that either 30 or 35% of total chlorophyll is in non-appressed regions, then the amount of PS I complex in the appressed regions is 8.3 or 5.8%, respectively. Inside-out vesicles derived from appressed thylakoids in this study contained 9% of total chlorophyll as PS I complex (Table II). Correcting for the contaminating by non-appressed regions, we deduce that a maximum of 4% of the total chlorophyll in appressed thylakoids is actually derived from PS I complexes in a mature spinach chloroplast. It has recently been reported that the primary plastoquinone acceptor of PS II, 'Q', is present in appressed spinach thylakoids in a ratio Q/P-700 of 10 [16]. This also suggests that only about 10% of the total PS I complexes are present in appressed regions.

Total acyl lipids accounted for about 25% of the total mass of undisrupted thylakoid membranes but only 14% of the mass of appressed regions. In addition to this gross asymmetry, there are also considerable inhomogeneities in acyl lipid class distribution between the various membrane fractions as outlined in Table III. The most striking difference is in the relative enrichment of the appressed regions in diacylglycerol phosphoglycerol. In overall terms, the appressed regions contain 60% more anionic lipids (as a proportion of total lipid) than do whole thylakoids. There are also significant differences in the galactolipid content of the membrane fractions. Diacylgalactosylglycerol is the major galactolipid in all cases but the ratio of the monogalactolipid to the digalactolipid is 1.31 in whole thylakoids, 2.42 in appressed regions, and only 1.11 in non-appressed regions. This trend is similar to, although considerably more pronounced than, the reported galactolipid ratios found in whole thylakoids and granal and stromal lamellae isolated by density gradient centrifugation [31,32].

The fatty acid compositions of the individual acyl lipids from the various thylakoid regions are given in Table IV. The two galactolipids show remarkably little variation in their acyl residues. Both fractions contain about 75% α-linolenate (18:3) plus 25% hexadecatrienate (16:3) in the monogalactolipid class, and over 80% α-linolenate plus 6–8% palmitate (16:0) in the digalactolipid class. The anionic lipids have more heterogeneous fatty acid distributions. The diacylsulphoquinovosylglycerol from appressed regions is specifically depleted in α-linolenate and enriched in palmitate. The ratio of unsaturated: saturated acyl residues in the sulpholipid class is 1.9 in whole thylakoids, 2.4 in non-appressed regions, and only 0.8 in appressed regions. Diacylglycerolphosphoglycerol, from appressed regions is slightly enriched in *trans*-3-hexadecenoate relative to whole thylakoids. This is of interest in the light of the controversy surrounding the role, if any, of this fatty acid in thylakoid membranes [33–36].

TABLE V

MOLAR RATIOS BETWEEN INDIVIDUAL ACYL LIPID CLASSES, CHLOROPHYLLS AND TOTAL PROTEIN IN FRENCH-PRESSED SPINACH THYLAKOID MEMBRANE FRACTIONS AND WHOLE THYLAKOIDS

The ratios are expressed relative to 1 mol protein. The ratios are deduced from the lipid, chlorophyll and protein composition of the respective membrane fractions (see Tables I and III). An average molecular weight for the five major thylakoid intrinsic membrane-spanning complexes of 250000 has been assumed. See text for details and Table I for nomenclature of membrane fractions.

Membrane constituent	Fraction	
	Whole thylakoids	Lower, dextran, phase (B3)
(a) Acyl lipids		
Diacylgalactosylglycerol	44.8	27.5
Diacyldigalactosylglycerol	34.3	11.5
Diacylsulphoquinovosylglycerol	14.8	6.0
Diacylglycerophosphoglycerol	16.8	16.5
Diacylglycerophosphocholine	6.0	1.3
Total acyl lipid	116.7	62.8
(b) Chlorophylls		
Chl *a*	21.8	31.0
Chl *b*	9.0	21.8
Total chlorophyll	30.8	52.8
(c) Total protein	1.0	1.0

Discussion

The present study demonstrates the existence of an extensive lateral heterogeneity in the distribution of the acyl lipid components of photosynthetic membranes from spinach chloroplasts. This is the first time that such a large-scale heterogene-

ity of acyl lipid distribution has been observed contiguous regions of a single biological membrane. The more general implications of these findings are explored in greater detail elsewhere [37]. In addition to lateral heterogeneity of acyl lipid distribution, chlorophylls and proteins were also found to exhibit heterogeneous lateral distributions broadly, similar to those noted by earlier investigators [14–16,38]. Since the acyl lipids, chlorophylls and proteins together account for over 99% of the mass of higher plant photosynthetic membranes, their respective molar ratios can shed considerable light on thylakoid organisation at the molecular level. For this reason, an average protein molecular weight must be derived. The great majority of the 30–50 thylakoid polypeptides are arranged in five large membrane-spanning complexes, i.e., the PS I, PS II, light-harvesting, cytochrome *b-f* and coupling factor complexes (15). Most of the light-harvesting complex is associated with either PS I or PS II in vivo [15] and therefore there are effectively only four supramolecular protein complexes. Using the known molecular weights of the various polypeptides that make up these four protein complexes and adjusting for non-integral membrane proteins a final average integral protein molecular weight of 250 000 was deduced. It is valid to compare directly the molar ratios of lipids relative to protein in the different membrane fractions, since in all cases the protein made up a similar proportion of the total membrane constituents (Table I). The molar ratios of the total acyl lipid and total chlorophyll, with respect to protein, are shown in Table V. This gives a much clearer picture of the organisation of the lipophilic membrane components at the molecular level. For example, the apparent enrichment of appressed regions in diacylglycerophosphoglycerol, while true in terms of percent total acyl lipid, is not found when molar ratios relative to protein are considered.

The similarity in the ratio of this anionic phospholipid to protein in the different membrane fractions raises the interesting possibility that it may have some specific interaction with one or more of the thylakoid proteins. The appressed thylakoids contain only half of the molar ratio of total galactolipid found in whole thylakoids and only one third of the digalactolipid. This is consistent with a recent hypothesis in which it was predicted that much of the thylakoid galactolipid should be located in the tightly curved marginal membranes rather than in the planar lamellar regions. There are about 53 chlorophyll molecules per protein in the appressed thylakoid regions, contrasting with 31 in the whole thylakoids. This indicates that the lateral distribution of total chlorophyll in photosynthetic membranes may not be as uniform as was hitherto believed. The molar ratio of acyl lipid : protein is about 118 in whole thylakoids, but only 63 in appressed regions. The latter figure is particularly low, since it would not even provide a monomolecular lipid annulus around each protein complex.

The four principal supramolecular protein complexes of thylakoid membranes display a pronounced lateral heterogeneity [14–16,29]. The PS II plus light-harvesting complex is localised in appressed membrane regions (Table II and Refs. 14, 15 and 29), while the PS I plus light-harvesting complex (Table IV and Refs. 15 and 16) and the ATP synthetase [39] are confined to non-appressed regions. The cytochrome *b-f* complex is present in similar amounts, on a chlorophyll basis, in both membrane regions [40,41]. However, in the present study it is demonstrated that the proportion of total protein (on a % (w/w) basis) is similar in both appressed and non-appressed thylakoids to the proportion in whole thylakoids (all are about 70%, w/w)). This is similar to the proportion of protein in other energy-transducing membranes such as inner mitochondrial membranes [42] and *Escherichia coli* membranes [43], both of which contain about 75% (w/w) protein. But there is a heterogeneity of combined [chlorophyll + protein] distribution in thylakoids (Table I).

Appressed thylakoid membranes are relatively depleted in total acyl lipid and enriched in [chlorophyll + protein] (Table V). This would lead to an apparent increase in density of protein particles as has been observed in ultrastructural studies [44]. Assuming an average integral protein-complex diameter of 100 Å [31] and an acyl lipid diameter of 8.7 Å [45], the average area of each protein complex and of each acyl lipid molecule is 7850 and 60 $Å^2$, respectively. There are 63 acyl lipids per protein (Table V), or 31.5 diacyl lipids, since most of the lipids are believed to be arranged in a bimolec-

ular leaflet. The relative areas occupied by one protein molecule and 31.5 diacyl lipids are 7850 and 1890 $Å^2$, respectively, and thus protein accounts for about 80% of the total surface area of appressed regions of thylakoids, which is similar to the figure deduced from ultrastructural studies [8,31].

Thylakoid protein particles visualized in ultrastructural studies have been described as having a roughly globuloid appearance [44] and, with the exception of the CF_1 subunits of the ATP synthetase, they do not protrude extensively into the hydrophilic domain [15]. A globuloid protein complex of 100 Å diameter would require the presence of at least 38 diacyl lipids just to form a monomolecular lipid annulus. Since there are only 31.5 diacyl lipids per protein complex is appressed thylakoids, it can be seen that neighbouring protein complexes must be in close proximity to one another. Following the method of Waksman et al. (46), inter-protein distances may be derived for various lipid/protein ratios. Using the ratio of 31.5 lipids per protein from this study, an average inter-protein distance of 11.5 Å was calculated. Even and edge-to-edge proximity between adjacent protein molecules of 30 Å, as calculated for inner mitochondrial membranes, will lead to a fairly tight contact between projecting parts of proteins [46], and so a proximity of only 11.5 Å would lead to a extremely intimate protein-protein contact. This proximity is of great interest in view of the recent suggestions [47–49] that there may be a direct electron transfer from PS II complexes to cytochrome *b-f* complexes (thus effectively bypassing the mobile quinone pool) due to transient associations between elements of the two protein complexes.

Appressed thylakoid membranes are deficient in both classes of galactolipid (but notably the digalactolipid) and the sulpholipid compared to whole thylakoids (Table V). This observation implies that not all of the acyl lipids are free to diffuse at random in the lateral plane of the thylakoid membrane. The galactolipid depletion in appressed thylakoids is consistent with a recent suggestion by Murphy [8] that the tightly curved marginal regions of the thylakoids are exclusively lipidic, and are stabilised by galactolipids. The presence of the non-bilayer-forming diacylgalactosylglycerol in appressed regions is of interest in the context of recent proposals that this lipid is involved in the stabilisation of intrinsic membrane proteins by means of inverted micelles [8,50]. The important role of diacylgalactosylglycerol is lent credence by a report that this lipid specifically restores inter-complex energy transfer from the light-harvesting to the PS I and PS II complexes, when it is added to lipid-depleted thylakoid fragments [51].

The origin and nature of negative surface charge exhibited by thylakoid membranes (reviewed by Barber [52]) is of considerable current interest due to its possible role in thylakoid stacking. In the present study it was concluded that anionic lipids comprise about 43% of the total acyl lipid in stacked thylakoids. This is equivalent to $1.4 \cdot 10^5$ anionic lipid molecules/nm^2. Since the pK_a values of the charged groups on the anionic lipids are about 2.0 in open solution [52], these lipids will carry a single negative charge each under physiological conditions. The charge density due to anionic lipids works out at 1 per 700 $Å^2$. This is far too high to allow for equilibrium thylakoid stacking [53] and a cationic charge screening must be invoked to account for such stacking. A lateral charge redistribution involving anionic lipids is possible, since stacked thylakoids were strongly depleted in sulpholipids (Table V), but the anionic lipids remaining in stacked regions would still require extensive masking by cations.

In view of the evidence presented here on the lateral distribution and organisation of the major components of photosynthetic membranes, it is felt that our working model of their membrane architecture is in need of reversion. The fluid-mosaic model, as applied to photosynthetic membranes, is commonly depicted as a few iceberg-like proteins floating in a vast ocean of lipid. The extremely high protein density and relative dearth of acyl lipids in the appressed regions of thylakoid membranes give rise to a very different picture of membrane molecular organisation [37]. Appressed regions of thylakoid membranes appear to contain the minimum quantity of acyl lipid to fill in inter-protein packing defects and to allow for the protein complexes to diffuse relative to one another. Modifications to the basic fluid-mosaic model which involve lateral segregation of lipid into

non-bilayer configurations, protein-free curved regions, and planar bilayer regions have recently been proposed [8,38,50]. The findings presented here indicate that lateral segregation of lipid does occur in photosynthetic membranes where it may play an important role in the maintenance of thylakoid stability and the optimisation of its photosynthetic functions.

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