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MINI-REVIEW

The Phase Behavior of Lipids in Photosynthetic Membranes

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Abstract

The phase behavior of the main classes of polar lipids found in the photosynthetic membranes of higher plants and algae is reviewed and compared to that of binary lipid mixtures and total lipid extracts of such membranes. Particular interest is paid to the way in which factors such as temperature and acyl chain saturation influence the phase behavior of these lipids and the implications this has in terms of the ability of photosynthetic membranes to resist environmental stress.

Key Words: Membrane lipids; phase behavior; photosynthesis; chloroplast; thylakoid membrane.

1. Introduction

One of the most striking features of biological membranes is the great variety of polar lipids that they contain. Although only relatively few amphipathic lipid classes are found in any given membrane, each class consists of a range of molecular species depending on the type of hydrocarbon substituent. When extracted from biological membranes and dispersed in aqueous systems of composition similar to that surrounding the membrane in its natural environment, the individual molecular species present in the membrane will normally form one of a number of characteristic phases. These phases include bilayer arrangements, with gel, crystal, or liquid-crystal configurations of the hydrocarbon component: an hexagonal-II structure, and a number of cubic phases. In general, the polymorphic behavior of polar lipids is strongly dependent on the activity of water in the dispersion and temperature.

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A considerable amount is known about lipid polymorphism, and excellent reviews of the literature in this field have been published by Luzzati (1968, 1974), Shipley (1973), and Verkleij (1985).

The need for lipid diversity in biological membranes and the relationship of the complex polymorphism exhibited by the isolated lipids to their roles in the intact membrane are unknown. It is clear, however, that the behavior of these lipids is greatly modified both by interactions between the different molecular species of lipid present in such membranes and between the lipids and other membrane constituents, particularly membrane proteins. Current thinking departs considerably from the ideas underlying the original fluid-mosaic model of Singer and Nicolson (1972) in which the membrane lipids were considered to serve simply as a fluid two-dimensional matrix supporting the membrane proteins.

It is now widely accepted that lipids play a much more complex role in membrane organization. In most membranes, the balance of interacting forces results in the formation of a liquid-crystalline bilayer conformation. There are, however, a few notable exceptions of which the most striking are the prolamellar bodies found in etioplasts, the membranes of which appear to be in an organization resembling a bicontinuous cubic phase (Larsson *et al.*, 1980). It is also well established that many, if not all, membranes exhibit an asymmetry in the distribution of lipids between their opposite sides, possibly reflecting the asymmetry of their membrane protein components (Op den Kamp, 1979). Much less is known about the lateral distribution of different membrane lipids although there are a number of cases, of which the chloroplast thylakoid membrane is a particularly good example, of lateral asymmetry in membrane proteins.

In this review we will consider the phase behavior of the main classes of polar lipids found in the photosynthetic membranes of higher plants and algae. In particular, we will describe how such factors as temperature and hydrocarbon chain saturation influence their phase properties and review the implications that this has on the organization of photosynthetic membranes and their ability to resist environmental stress.

2. Phase Behavior of Isolated Lipids

The dominant polar lipid of the photosynthetic membranes of algae and higher plants is monogalactosyldiacylglycerol. It accounts for about 40–50% of the total polar lipid content of such membranes. The other major component is digalactosyldiacylglycerol accounting for a further 20–25%. The remainder is made up of approximately equal proportions of sulfoquinovosyldiacylglycerol and phosphatidylglycerol together with a small amount

of phosphatidylcholine (Douce *et al.*, 1973; Quinn and Williams, 1978, 1983). In higher plant chloroplasts, there is a strong preponderance of polyunsaturated fatty acyl residues particularly those of (18:3) linolenic and (18:2) linoleic acids. The fatty acid composition of algae, however, is much more variable, varying from that of the red and brown marine algae which often contain appreciable proportions of polyunsaturated C_{20} and C_{22} fatty acids to the thermophilic blue-green algae in which palmitic (16:0) and palmitoleic (16:1) acids predominate (Hitchcock and Nicholls, 1971).

2.1. Nonbilayer-Forming Lipids

The phase behavior of monogalactosyldiacylglycerol has been extensively studied using X-ray diffraction, differential scanning calorimetry, fluorescence probe, and freeze-fracture techniques (Shipley *et al.*, 1973; Sen *et al.*, 1981a, 1983; Gounaris *et al.*, 1983a; Mannock *et al.*, 1985a, b). Monogalactosyldiacylglycerol is unusual in that it normally forms nonbilayer structures when dispersed in aqueous media. Monogalactosyldiacylglycerols with polyunsaturated fatty acyl chains, extracted from higher plant chloroplasts, normally form hexagonal-II phases in distilled water or dilute salt solutions. The shorter-chain more saturated derivatives extracted from thermophilic blue-green algae tend to form sheetlike bilayer structures at room temperature. X-ray diffraction studies indicate, however, that they are in gel phase under these conditions (Mannock *et al.*, 1985a). Freeze-fracture electron micrographs of samples, thermally quenched from higher temperatures, show clear evidence of nonbilayer behavior. Typical freeze-fracture electron micrographs of monogalactosyldiacylglycerols thermally quenched from the gel and the liquid-crystalline states are presented in Fig. 1 together with an electron micrograph of a partially hydrogenated sample in which the lipid appears to be in the process of undergoing a transition between the two states. Monogalactosyldiacylglycerols appear to go direct from the lamellar gel state (L_β) to an hexagonal-II liquid-crystalline state. There are no indications of the existence of a stable lamellar liquid-crystalline phase (L_α) of the type found for phosphatidylethanolamines (Seddon *et al.*, 1983).

In addition to the conventional lamellar gel state, in which the acyl chains are packed in a hexagonal arrangement, monogalactosyldiacylglycerol slowly relaxes on standing at low temperatures into one of two possible lamellar crystal phases, in which the chains are packed on an orthorhombic lattice (Sen *et al.*, 1983). These crystalline phases are not formed in the presence of other lipids (Mannock *et al.*, 1985b) and as such have little direct relevance to the *in vivo* phase behavior of photosynthetic membranes and will not be discussed here.

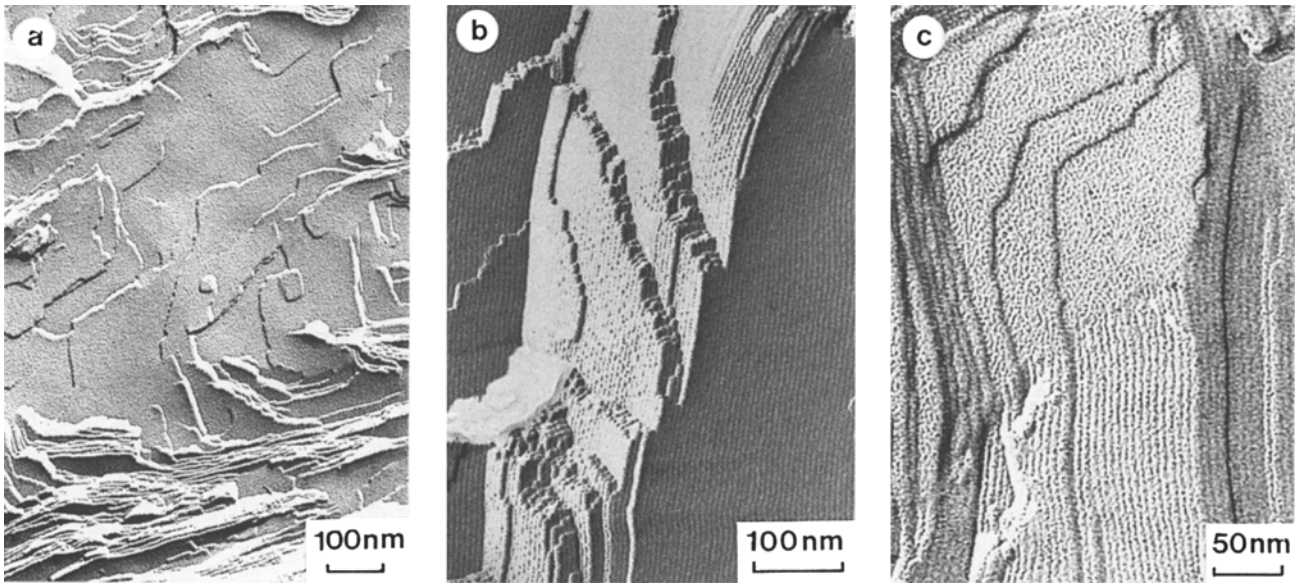

Fig. 1. Electron micrographs of freeze-fracture replicas prepared from aqueous dispersions of monogalactosyldiacylglycerol extracted from bean chloroplasts (a and b) and of a partially hydrogenated sample of this lipid (c). Samples (a) and (b) were thermally quenched from −28 and 20°C respectively, and are in the gel and liquid-crystal phases. Sample (c), thermally quenched from 20°C, is undergoing a transition between the two states. Data from Gounaris *et al.* (1983a).

A detailed study of the effects of unsaturated fatty acyl residues on the phase behavior of monogalactosyldiacylglycerol extracted from higher plant chloroplasts has been reported by Gounaris *et al.* (1983a). The ability of this lipid to form nonbilayer structures when dispersed in excess water was shown to depend on the extent of unsaturation of the associated fatty acyl chains. Highly unsaturated lipids extracted from the membranes of broad bean (*Vicia faba*) chloroplasts, containing more than five double bonds per molecule, formed inverted hexagonal structures at 20°C. More saturated lipids, prepared by catalytic hydrogenation of the native lipid, in which there were less than 4.5 double bonds per molecule, tended to form gel-phase lamellar structures under the same conditions.

More recent measurements comparing the gel-to-liquid crystal phase transition temperatures of monogalactosyldiacylglycerol samples isolated from the blue-green alga *Anacystis nidulans*, which consist predominantly of the 16 : 1/16 : 0 derivative, with that of the corresponding fully hydrogenated sample suggests that the introduction of a single double bond into the molecule decreases the phase-transition temperature by about 40°C (Mannock *et al.*, 1985a). This observation is difficult to reconcile with the earlier observations on hydrogenated lipid and the possibility that the hydrogenation procedure used in these experiments could have led to *cis–trans* isomerization and/or double bond migration cannot be excluded.

2.2. Bilayer-Forming Lipids

The polar lipid constituents of photosynthetic membranes other than monogalactosyldiacyglycerol tend to form bilayer structures, in both the gel and liquid-crystalline states, when dispersed in excess aqueous medium. The phase behavior of digalactosyldiacylglycerol, the second most abundant polar lipid of the photosynthetic membranes of algae and chloroplasts of higher plants, has been investigated by X-ray diffraction, differential scanning calorimetry, and freeze-fracture methods (Shipley *et al.*, 1973; Sen *et al.*, 1981a). Of the other lipid classes represented in photosynthetic membranes, the phospholipids phosphatidylcholine and phosphatidylglycerol have been the subject of innumerable studies. A detailed account of the properties of these lipids, which are in any case only minor components of the photosynthetic membrane, is beyond the scope of this review. Suffice it to say that while the fatty acyl chains of the phosphatidylcholine components tend to be unsaturated, those of the phosphatidylglycerols are often fully saturated. The presence of these saturated residues, as discussed in more detail in Section 3.1.2, has been suggested to be of particular significance in terms of the susceptibility of higher plant chloroplasts to chilling damage.

Another interesting feature of the phosphatidylglycerols found in photosynthetic membranes is the presence of molecular species containing *trans*-Δ^3-16:1 fatty acyl chains. These particular derivatives appear to be unique to the photosynthetic membranes of higher plants and green algae. The reasons for this are not well understood. It has been suggested that they are associated with the formation of oligomeric complexes of chlorophyll *a*/*b* light-harvesting protein in chloroplast membranes (Trémolières *et al.*, 1981). See Quinn and Williams (1985) for a critical discussion of this and other possible lipid–protein interactions in photosynthetic membranes.

The only other polar lipid class found in the chloroplast membrane is a sulfonated derivative of monoglucosyldiacylglycerol, referred to as sulfoquinovosyldiacylglycerol. This lipid, like the phosphatidylglycerols of chloroplast membranes, tends to be fairly highly saturated. Its phase properties were also investigated by Shipley *et al.* (1973). It again forms liquid-crystalline bilayers over a wide range of temperatures and water contents.

2.3. Mixed Lipid Systems

As discussed above, monogalactosyldiacylglycerol forms nonbilayer structures in the liquid-crystalline state while the remaining lipid classes found in photosynthetic membranes form bilayer structures under such conditions. A number of studies have been undertaken to examine the phase behavior both of binary mixtures of the bilayer- and nonbilayer-forming lipids and of total polar lipid extracts of photosynthetic membranes.

2.3.1. Binary Lipid Mixtures

The phase properties of aqueous dispersions of binary mixtures of monogalactosyl and digalactosyldiacylglycerol have been examined in considerable detail using ^{2}H-NMR and low-angle X-ray diffraction methods (Brentel *et al.*, 1985) and by freeze-fracture electron microscopy (Sen *et al.*, 1981b, 1982a, b; Sprague and Staehelin, 1984). The freeze-fracture studies revealed that the two galactolipid components do not mix ideally but tend to phase-separate to form mixed lipid phases. Sen and his co-workers investigated the organization of monogalactosyl and digalactosyl lipids codispersed in a similar molar ratio (2:1) to that found in the native chloroplast membranes. A characteristic feature of such dispersions is the formation of spherical inverted lipid micelles (presumably consisting predominantly of the monogalactolipid component) either sandwiched within lipid bilayers (presumably formed predominantly from the digalactosyldiacylglycerol) or arranged in three-dimensional aggregates. Typical examples of such structures are shown in Fig. 2.

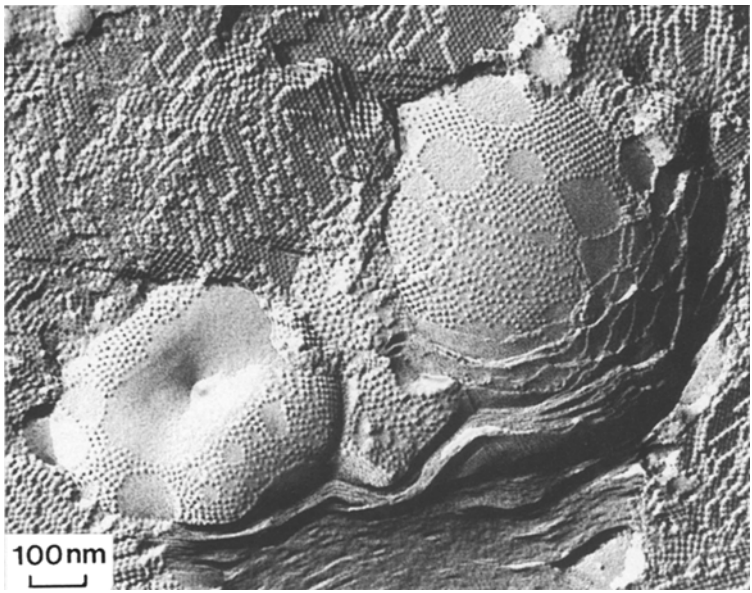

Fig. 2. Electron micrograph of a freeze-fracture replica prepared from an aqueous dispersion of a binary mixture of monogalactosyl and digalactosyldiacylglycerols (2 : 1 mole ratio) showing spherical inverted micelles sandwiched within lipid bilayers and organized in three-dimensional quasi-crystalline arrays. Data from Sen *et al.* (1982b).

Sprague and Staehelin (1984) have attempted to classify the different structures formed in such mixtures on the basis of the relative proportions of the two lipids present in the initial mixture. It must, however, be borne in mind that such classifications, although useful, are of necessity only rough guidelines as the mixtures are not in phase equilibrium and tend to phase-separate on standing. The structures formed are also dependent on the distribution of molecular species, and hence the source, of the lipids.

Samples thermally quenched from higher temperatures and/or samples containing high concentrations of cryoprotectants show increased phase separation involving three-dimensional aggregates of inverted micelles apparently arranged on cubic or orthorhombic lattices (Sen *et al.*, 1982b; Williams *et al.*, 1982). Sprague and Staehelin (1984) have questioned the effects of both temperature and cryoprotectants on the tendency of the nonbilayer component to phase-separate in such mixtures. There is, however, clear evidence from X-ray and NMR studies to indicate that such phase separations are favored by high temperatures and low water activities. Brentel *et al.* (1985) have used ^{2}H-NMR data to construct phase diagrams of monogalactosyl and digalactosyl lipids codispersed in water in molar ratios of 1 : 2,

1.2 : 1, and 2 : 1 over the temperature range 10–40°C and water contents up to 14 moles of water per mole of lipid. Their results confirm that phase separation increased with increasing temperature and decreasing water activity.

In the case of the binary mixture consisting of a 2 : 1 molar ratio of the two lipids, they showed that an hexagonal-II phase dominates the system at low water content. With increasing water concentration a reversed cubic liquid-crystalline phase gradually replaces the hexagonal-II phase which, in turn, is replaced by a liquid-crystalline lamellar phase in excess water. With increasing proportions of a monogalactolipid in the mixture the nonlamellar regions of the phase diagram increase. The reversed cubic liquid-crystalline phase, which was characterized by X-ray diffraction methods, was shown to belong to the *Ia3d* space group. ^{2}H-NMR measurements of translational diffusion rates indicated that the structure of the cubic phase was bicontinuous.

2.3.2. Total Polar Lipid Extracts

Total polar lipid extracts of the chloroplasts of higher plants form small unilamellar vesicles when dispersed in distilled water (Gounaris *et al.*, 1983b). Dispersion in chloroplast assay medium (Quinn *et al.*, 1982), however, leads to the phase separation of nonbilayer lipid. The structural organization of such dispersions is, as discussed below, strongly dependent on such factors as temperature, lipid saturation, and the ionic strength and pH of the dispersing medium.

2.3.2.1. Effect of Temperature

As might be expected from studies on the effects of temperature on the phase behavior of aqueous dispersions of binary mixtures of bilayer- and nonbilayer-forming lipids (Sen *et al.*, 1982a; Brentel *et al.*, 1985), the tendency of the nonbilayer components to separate from bilayer-forming constituents in total polar lipid extracts of higher plant chloroplast membranes increases with increasing temperature. At temperatures below zero, homogeneous bilayer arrangements tend to dominate. With increasing temperature, increasing proportions of phase-separated nonbilayer lipids are seen. These effects are strongly influenced by the presence of cations; much more marked changes are seen in samples suspended in dilute salt solutions than in the corresponding samples dispersed in distilled water (Gounaris *et al.*, 1983a).

2.3.2.2. Effects of Chain Length and Saturation

One of the principal factors determining the phase of polar membrane lipids is the number and position of *cis* unsaturated double bonds in the

hydrocarbon chains (Phillips *et al.*, 1972; Barton and Gunstone, 1975; van Dijk *et al.*, 1976). This determines the temperature both of the gel-to-liquid-crystalline phase transitions of lipids in a bilayer configuration and, if the lipids undergo such transitions, the corresponding temperatures for the formation of nonlamellar phases.

Murata and Yamaya (1984) have shown that with the exception of the phosphatidylglycerol fraction, and to a lesser extent the sulfoquinovosyldiacylglycerol fraction, all the membrane lipids found in the leaf tissue of higher plants are in a liquid-crystalline state at temperatures above 5°C (see also Raison and Wright, 1983). In the case of the thermophillic blue-green alga *Anacystis* in which, as pointed out above, the lipid chains are both shorter and more saturated, the gel-to-liquid-crystal phase transitions of all the different fractions occur, or at least partially occur, above this temperature (Mannock *et al.*, 1985a, b). As a consequence, while there is little or no evidence to indicate the existence of any appreciable amounts of gel-phase lipid in total polar lipid extracts of higher plant chloroplasts at above 0°C, there is abundant evidence that the main transition in the corresponding extracts of *Anacystis* occurs well above 0°C.

Studies performed on mixed lipid dispersions prepared by combining hydrogenated samples of monogalactosyldiacylglycerol of increasing degrees of saturation with nonhydrogenated samples of the other polar lipids of higher plant chloroplast membranes in their native form show that the ability of the monogalactolipid to phase-separate into nonbilayer phases decreases as the hydrocarbon chains become progressively more saturated (Gounaris *et al.*, 1983a). While there must be some reservations about the precise interpretation of experiments involving the use of hydrogenated lipids for the reasons outlined in Section 2.1, the general thesis that the extent of nonbilayer lipid phase separation occurring in total polar lipid extracts depends on the degree of saturation of the nonbilayer component seems undeniable.

2.3.3. Effects of Charge Shielding

The effects of screening the negative charges of the headgroups of the acidic lipids of polar lipid extracts of higher plant chloroplast membranes by the addition of cations, and of their neutralization by exposure to low pH, have been investigated by Gounaris *et al.* (1983b). Dispersion of polar lipid extracts in excess water results in the formation of single bilayer vesicles ranging in diameter from 30–50 nm. Following the addition of monovalent or divalent cations, these vesicles fuse to form larger multilayered vesicles made up of bilayers containing spherical inverted micelles. With increasing ionic strength, particularly of divalent cations, larger and larger aggregates

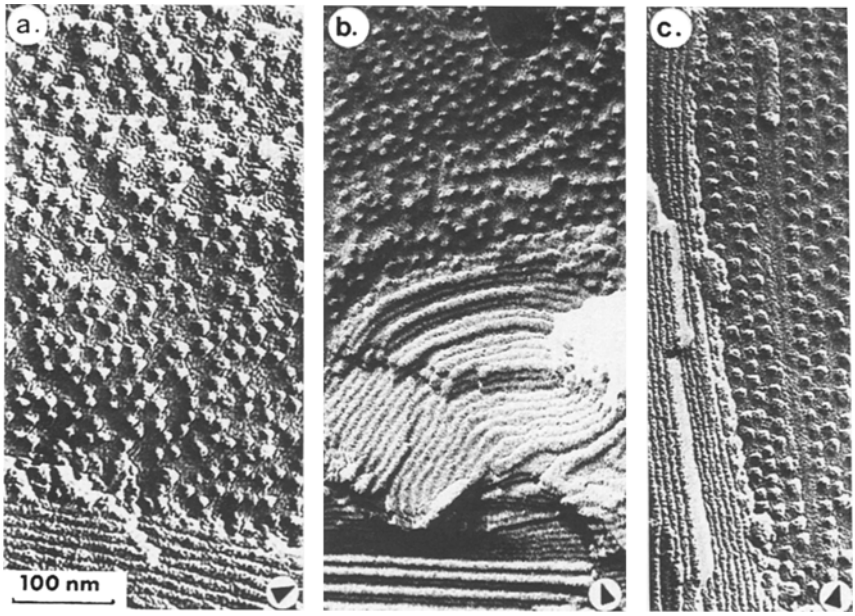

Fig. 3. Electron micrographs showing the main features present in freeze-fracture replicas prepared from total polar lipid extracts of chloroplasts sonicated in 30 mM $MgCl_2$. Data from Gounaris *et al.* (1983b).

are formed containing increasing proportions of nonbilayer structures. The nonlamellar structures formed in such aggregates, as illustrated in Fig. 3, consist mainly of isolated spherical inverted micelles and three-dimensional aggregates of tubular inverted micelles. Three-dimensional aggregates of spherical inverted micelles of the type commonly seen in binary mixtures of monogalactosyl and digalactosyldiacylglycerol are not normally observed in total polar lipid extracts of chloroplast membranes.

Similar changes are seen in samples in which the pH has been reduced to below 5.0. In both cases, it is presumably the acidic lipids rather than the neutral galactolipids that are directly affected. However, neutralization of the charges on these lipids appears to lead to the formation of sufficiently large aggregates to allow phase separation of monogalactosyldiacylglycerol to occur. Related changes are also observed on adding Mg^{2+} to total polar lipid extracts of *Anacystis* membranes. In this case, however, the phase-separated lipid forms gel phase lamellae rather than nonbilayer structures, reflecting the much higher transition temperature of the monogalactosyldiacylglycerol fraction present in thermophilic algae (Mannock *et al.*, 1985b).

3. Phase Separations in Photosynthetic Membranes

There are two basic types of phase separations that might be expected to occur in photosynthetic membranes. These are gel-to-liquid-crystal phase separations, of the type associated with all the main classes of polar lipid present in such membranes, and nonbilayer lipid phase separations of the type specifically associated with monogalactosyldiacylglycerol. The evidence for the occurrence of such phase separations, and their implications in terms of membrane stability, are discussed below.

3.1. Gel Phase Separations

Discussion of gel phase separations in photosynthetic membranes is conveniently divided into two parts: one relating to the thermophilic blue-green algae, where there is unequivocal evidence for the occurrence of such transitions, and the other to high plants and nonthermophilic algae, where the occurrence of such transitions, at temperatures above 0°C is dubious.

3.1.1. Thermophilic Algae

Most of the available data relating to the phase behavior of thermophilic algae relates directly to the blue-green alga *Anacystis*. The occurrence of gel-to-liquid-crystal phase transitions in *Anacystis* was first inferred from measurements on the temperature dependence of chlorophyll *a* fluorescence (Murata and Fork, 1975; Murata *et al.*, 1975) and spin probe studies (Murata *et al.*, 1975). More direct evidence for the transition between gel and liquid-crystalline states has since been reported from X-ray diffraction measurements (Tsukamoto *et al.*, 1980), differential scanning calorimetry (Furtado *et al.*, 1979; Ono *et al.*, 1983; Mannock *et al.*, 1985b), and freeze-fracture electron microscopy studies (Armond and Staehelin, 1979; Brand *et al.*, 1979; Furtado *et al.*, 1979; Verwer *et al.*, 1979; Ono and Murata, 1982). The freeze-fracture evidence in particular, as illustrated in Fig. 4, indicates the creation of gel-phase domains from which the protein is excluded.

The temperature at which the gel phase appears depends on the growth temperature of the organism. Differential calorimetry measurements, of the type presented in Fig. 5, indicate that the gel phase first appears on cooling at about 16°C for cells grown at 28°C whereas for cells grown at 38°C it first appears at about 26°C. The bulk of the lipid, however, remains in a liquid-crystalline configuration until much lower temperatures and it is into this domain that the protein components of the photosynthetic membrane are segregated.

Detailed analyses of the phase properties of the thylakoid and cytoplasmic membranes of *Anacystis* cells indicate that the onset of phase separation in

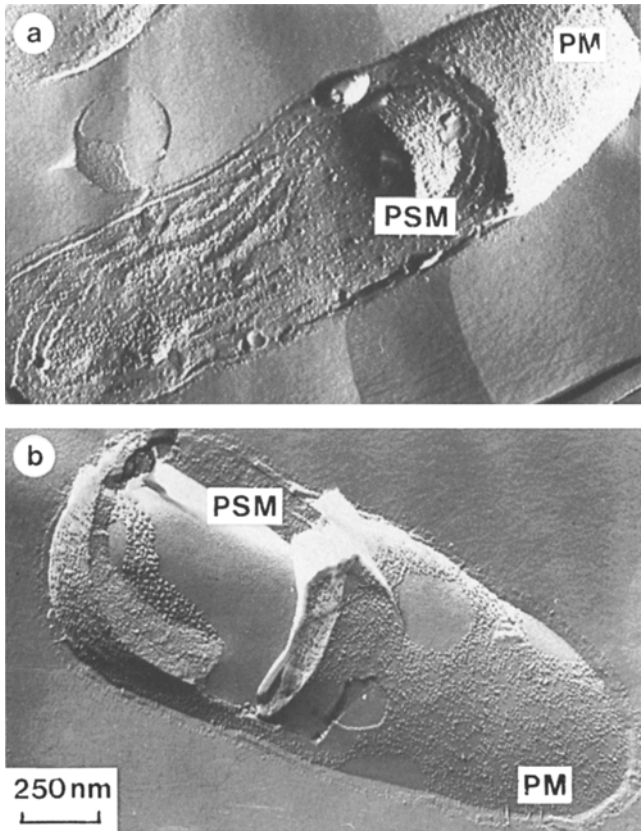

Fig. 4. Electron micrographs of freeze-fracture replicas of *Anacystis* cells grown at 38°C and pre-equilibrated at (a) 35°C and (b) 15°C just before thermal quenching. Note the random distribution of freeze-fracture particles in the plasma membrane (PM) and photosynthetic membranes (PSM) of the sample equilibrated at the higher temperature and the lateral separation of these particles in the sample equilibrated at the lower temperature.

the cytoplasmic membrane occurs at a temperature about ten degrees lower than in the photosynthetic membrane (Ono and Murata, 1982). The cells do not appear to undergo permanent damage as a result of phase separations occurring in the photosynthetic membrane, but phase separations in the cytoplasmic membrane result in a leakage of ions and small molecules and cell death (Ono and Murata, 1981a, b).

3.1.2. Higher Plant Chloroplasts

Phase separations of gel-phase domains of lipid of the type seen in thermophilic algae are not observed in the membranes of higher plant chloroplasts,

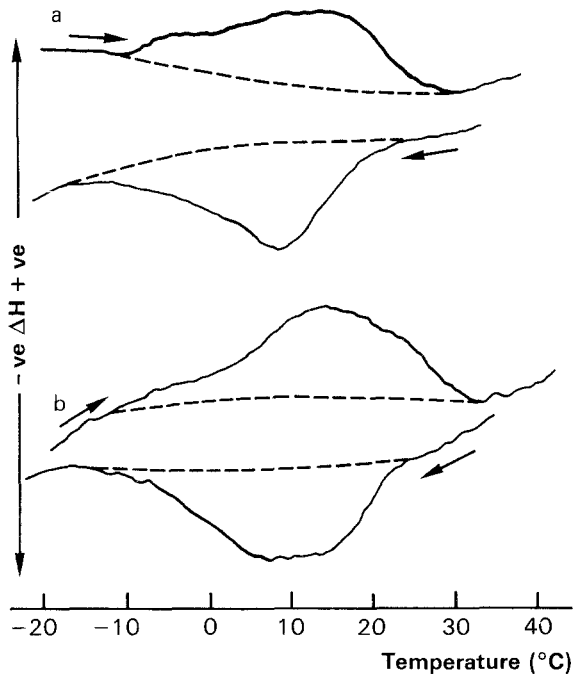

Fig. 5. Thermograms of cells of *Anacystis* grown at (a) 28°C and (b) at 38°C. Data from Mannock *et al.* (1985b).

presumably because the main gel-to-liquid-crystal phase transitions of the polyunsaturated lipids that predominate in such membranes tend to be at temperatures less than −30°C (Shipley *et al.*, 1973). As pointed out above, some of the minor lipid components, particularly the phosphatidylglycerols, are capable of undergoing gel-phase transitions at much higher temperatures. The proportions of these lipids in the membrane are, however, insufficient to create conspicuous regions of membrane from which membrane-associated particles are excluded. The possible existence of microdomains of gel-phase lipid cannot be excluded as such domains would be extremely difficult to detect using freeze-fracture, wide-angle X-ray diffraction methods, or differential scanning calorimetry.

Murata and his co-workers (Murata, 1982, 1983; Murata *et al.*, 1982) have reported that the relative proportions of the dipalmitoyl and 1-palmitoyl-2-(*trans*-Δ^3-hexadecanoyl) molecular species of phosphatidylglycerol present in chilling sensitive plants is significantly higher than in chilling resistant species. This suggests the possibility that the chloroplasts of chilling sensitive plants might be more at risk from membrane lesions associated with the phase separation of these particular lipids. While this is undoubtedly an

attractive possibility, it must be emphasized that the fact that such transitions can occur in lipid fractions isolated from chloroplast membranes does not mean that such phase separations necessarily occur *in vivo* and that the presence, or absence, of these species might be governed by other quite different criteria.

Thomas *et al.* (1986a) have recently demonstrated the existence of particle-free areas, resembling those seen in the membranes of *Anacystis* cells thermally quenched from temperatures below their gel-to-liquid-crystal phase transition, in the thylakoid membranes of pea chloroplasts that have been subjected to hydrogenation using the water-soluble catalyst palladium di-(sodium alizarine monosulfonate). This suggests that the hydrogenation process has led to a decrease in the saturation of the membrane lipids to a level at which they are able to phase-separate at room temperature, a view that is supported by direct measurements of fatty acid saturation levels of the hydrogenated samples.

3.1.2. Nonbilayer Lipid Phase Separations

Phase separations of nonbilayer-forming lipids, in contrast to gel-phase lipid separations, have been detected in the membranes of higher plant chloroplasts but not in thermophilic algae. Phase separations of this type can be induced by exposure of isolated chloroplasts to a variety of stresses. In our experience, the factors that favor such phase separations may be predicted from the effects of temperature, charge shielding and water activity, etc., on the phase behavior of total polar lipid extracts of these membranes. The changes that occur are consistent with the idea that the different forms of stress lead to a disturbance of the interactions between the polar membrane lipids and the membrane components which under normal conditions impose a bilayer configuration on these lipids and prevent their segregation into separate domains. The effects of different types of stress on the phase behavior of chloroplast membranes are examined in the following sections.

3.2.1. Thermal Stress

Exposure of leaves and suspensions of higher plant chloroplasts to elevated temperatures leads to loss of photosynthetic electron transport, photophosphorylation, and the ability to fix carbon dioxide (Berry and Björkman, 1980; Stidham *et al.*, 1982). One of the most striking changes is the occurrence of a sharp decline in rates of photosystem II-mediated electron transport. This decrease, which usually occurs over the temperature range 35–45°C, is accompanied by an increase in the fluorescence yield of chlorophyll *a* that is believed to be a reflection of the physical dissociation of the light-harvesting apparatus of photosystem II (Schreiber and Berry, 1977;

Armond *et al.*, 1978; Schreiber and Armond, 1978). These functional changes are accompanied by characteristic changes in chloroplast membrane organization. Armond *et al.* (1980), working with chloroplasts isolated from oleander leaves, and Gounaris *et al.* (1983c, 1984), working with chloroplasts isolated from broad beans, reported the occurrence of extensive destacking of chloroplast thylakoid membranes following heat treatment. In the latter case, at least, this was accompanied by a bulk phase separation of nonbilayer lipids in the chloroplast membrane.

Gounaris *et al.* (1984) have examined these structural changes in considerable detail. Following destacking, the normal regions of appression between the membranes of the grana stacks of the chloroplasts appear to be replaced by isolated attachment sites between membrane surfaces. An analysis of the size and packing densities of the freeze-fracture particles present in different membrane fracture faces suggested that this rearrangement reflected the dissociation of the core complex of photosystem II from its light-harvesting chlorophyll–protein antennae complexes. The antennae complexes of photosystem II then appear to cluster together in the regions of the attachment sites, maintaining regions of membrane adhesion, while at the same time excluding the core complexes of photosystem II, and the light-harvesting units of photosystem I, from these regions of membrane contact.

In order to induce bulk phase separations of nonbilayer-forming lipids, exposure to higher temperatures (45–55°C) is usually required. Exposure to temperatures above 55°C normally leads to membrane vesiculation. The extent of phase separation varies from sample to sample. Experiments performed on chloroplasts destacked by suspension in media free of divalent ions suggest that some degree of membrane–membrane interaction is necessary if nonbilayer phase separation is to occur (unpublished results). This possibly accounts for the variability seen in phase separation in heat-stressed chloroplasts.

A typical example of the type of nonbilayer structure observed in heat-stressed chloroplast membranes is shown in Fig. 6. The nonbilayer lipid tends to be arranged in whorled structures consisting of bundles of tubular inverted micelles. The organization, and the dimensions, of the micelles are very similar to those seen in total polar lipid extracts of chloroplast membranes (cf. Fig. 3b). There appears to be no indication of any association of membrane proteins with the structures formed in the native membranes.

Experiments of this type suggest that the ability of nonbilayer lipids to form hexagonal-II phases allows them to interact with the photosynthetic complexes in some way that stabilizes the interaction between the various components of the photosystem II light-harvesting units (Williams *et al.*, 1984; Quinn and Williams, 1985). When conditions are altered in such a way that the tendency of these lipids to form hexagonal-II structures is enhanced,

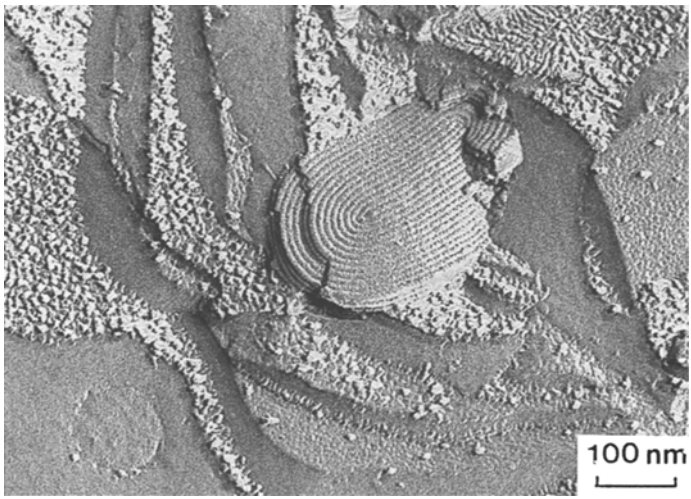

Fig. 6. Electron micrograph of a freeze-fracture replica of a sample of broad bean chloroplasts incubated for 5 min at 50°C prior to thermal quenching showing phase separation of nonbilayer lipids to form tubular inverted lipid micelles. Data from Gounaris *et al.* (1983c).

they appear to move away from these protein complexes and form nonbilayer domains elsewhere in the membrane. Loss of photosystem II-mediated electron transport activity can, on this basis, be thought of as a direct consequence of phase separation.

This view is strongly supported by measurements performed on hydrogenated chloroplast membranes. The occurrence of nonbilayer lipid phase separation is greatly suppressed in hydrogenated membranes (Thomas *et al.*, 1986a). At the same time, the functional stability of the photosystem II light-harvesting apparatus, as reflected in measurements of chlorophyll *a* fluorescence, appears to be increased. Differential calorimetry measurements performed on hydrogenated and nonhydrogenated thylakoid preparations suggest that this, in turn, is reflected in an increase in structural stability. Higher plant chloroplasts show a characteristic endotherm when heated that is thought to reflect the dissociation of the light-harvesting apparatus of photosystem II. The temperature corresponding to the onset of this endotherm, as illustrated in Fig. 7, increases by about 10°C on hydrogenation.

In addition to its effects on photosystem II, thermal stress also leads to changes in rates of electron transport mediated by photosystem I. The changes, which in this case reflect a stimulation rather than an inhibition of activity, show an almost identical threshold temperature to those seen for photosystem II. Electron transport measurements in the presence of different inhibitors, however, suggest that stimulation of photosystem I activity is

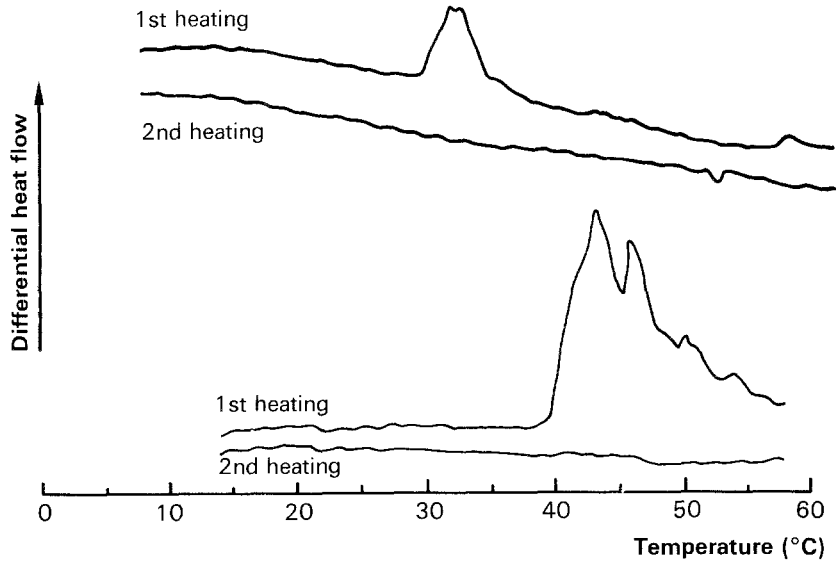

Fig. 7. Thermograms showing the first and second heating scans obtained for (a) nonhydrogenated and (b) hydrogenated pea chloroplasts. The average number of double bonds per lipid molecule before and after hydrogenation was 5.29 and 1.17, respectively. Data from Thomas *et al.* (1986a).

associated with the rearrangement of the thylakoid membrane resulting in the exposure of a new set of electron donor sites within the cytochrome f/b_6 complex of the electron transport chain linking the two photosystems rather than changes in the organization of photosystem I *per se* (Thomas *et al.*, 1986b).

3.2.2. Charge Shielding and pH Effects

Carter and Staehelin (1980) reported that exposure of chloroplast suspensions of high concentrations (20 mM) of Mg^{2+} ions leads to a phase separation of lipids into what were described as myelin figures. Although not recognized at the time, it is likely that these figures were of a nonbilayer form. The formation of such structures probably reflects similar charge shielding effects to those described in Section 2.3.1.3 for total polar lipid extracts of chloroplasts. It is interesting to note that nonbilayer lipid phase separations have also been reported to occur in mitochondrial membranes in response to the addition of Mn^{2+} ions (Van Venetie and Verkleij, 1982). In the case of the mitochondrial system, the ability to trigger such separations appears to be limited to Mn^{2+}. The comparative effects of different divalent cations have not, as yet, been explored for the chloroplast system.

More recently, Thomas *et al.* (1985) have shown that exposure of chloroplasts to pH less than 4.5 leads to the irreversible formation of nonbilayer lipid structures. It is difficult to be certain whether these latter changes are a direct consequence of the neutralization of the acidic lipids present in these membranes or secondary effects associated with the neutralization of charged groups of membrane proteins. Nevertheless, there seems to be good reasons to suspect that charge shielding, as a consequence of high divalent cation concentration, or charge neutralization, by exposure to low pH, result in very similar changes in native chloroplast membranes to those observed for total polar lipid extracts of such membranes. If this is the case, it only serves to emphasize the importance of the relatively small proportion of acidic membrane lipids present in such membranes in preserving overall membrane structure.

The structural changes occurring following a brief exposure to low pH, like those associated with heat stress, are accompanied by an inhibition of photosystem II-mediated electron transport and a stimulation of photosystem I-mediated electron transport (Thomas *et al.*, 1986b). It is tempting to suggest that these reflect similar changes in the arrangement of the light-harvesting units of photosystem II and the cytochrome *f*/*b* complex in the thylakoid membrane to those seen in heat-stressed chloroplasts, but no direct evidence is, as yet, available to support this view.

3.2.3. Phospholipase Treatment

Treatment of chloroplast suspensions with phospholipase enzymes such as porcine pancreatic phospholipase A_2 has often been used as a probe of the arrangement of phospholipids within the chloroplast thylakoid membrane (Shaw *et al.*, 1976; Hirayama and Matsui, 1976; Rawyler and Sigenthaler, 1981; Jordan *et al.*, 1983; Krupa, 1984). Studies performed by Thomas *et al.* (1985) indicate that incubation of chloroplasts in the presence of phospholipase A_2 can lead to phase separation of nonbilayer lipids within the membrane structure. This was interpreted as a reflection of the removal of the negatively charged phosphatidylglycerol leading to similar changes in membrane organization to those occurring following charge shielding or charge neutralization. Again there are losses of photosystem II-mediated electron transport activity. The extent to which these are reflections of structural changes is, however, still a matter of debate.

4. Conclusions

The study of the phase properties of the individual lipid classes, and total polar lipid extracts, of photosynthetic membranes provides valuable insights

into the phase behavior of the native membranes. Comparisons of the properties of the purified lipids with those of the extracts provides information on the role of different lipid classes on the stabilization of the membrane. At the same time, they allow the prediction of the probable response of the membrane to different types of stress. Such studies emphasize the role both of interactions between lipid classes, as illustrated by the part played by the acidic lipids in preventing phase separation of the neutral galactolipids in lipid extracts, and of lipid–protein interactions in the stabilization of key pigment–protein complexes within the membrane bilayer.

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