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Hypothesis

A model for chloroplast thylakoid membranes involving orderly arrangements of negatively charged lipidic particles containing sulphoquinovosyldiacylglycerol

William S. Sakai[+], Harry Y. Yamamoto, Tatsuo Miyazaki* and James W. Ross

Department of Botany, University of Hawaii, Honolulu, HI 96822, [+]*College of Agriculture, University of Hawaii, Hilo, HI 96720, USA* and **Institute of Biological Sciences, University of Tsukuba, Ibaraki 305, Japan*

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Addition of sulphoquinivosyldiacylglycerol (SL) to mixtures of monogalactosyldiacylglycerol (MG) and digalactosyldiacylglycerol (DG) induces the appearance of paracrystalline arrays of 80–100 Å lipidic particles. The hypothesis is presented that SL occupies the position in the leaflet opposite the micelle of MG or the position in the leaflet on the convex side of the bulge or cusp in the corresponding model for lipidic particles, and that these orderly arranged lipidic particles containing negatively charged SL may form part of the basis for the orderly arrangement of other molecules functioning in photosyntesis in the thylakoid membrane.

Lipidic particle *Liposome Chloroplast structure* *Monogalactosyldiacylglycerol* *Digalactosyldiacylglycerol* *Sulphoquinivosyldiacylglycerol*

1. INTRODUCTION

Particles on thylakoid surfaces were first postulated in [1] and later described in [2–8]. Two sizes of particles have been described; large 150–175 Å particles and smaller, 80–110 Å particles. The larger particles have been reported to be two different molecules; carboxydismutase [9,10] and CF_1 [11]. In [12], about 30% of the larger particles were removed under conditions which selectively removed carboxydismutase [13] and sequential removal of carboxydismutase and CF_1 left a thylakoid membrane with only 90 Å particles.

The smaller, 80–110 Å particles have not been adequately described and have sometimes been referred to as a structural protein. Here, we suggest a relationship between these 80–110 Å particles and the similar sized particles produced in mixtures of the chloroplast lipids digalactosyldiacylglycerol (DG), monogalactosyldiacylglycerol (MG), and sulphoquinivosyldiacylglycerol (SL). Further, we suggest that the paracrystalline arrangement of the lipidic particles containing negatively charged SL provides the basis for the orderly arrangement of other molecules functioning in photosynthesis in the thylakoid membrane.

2. FORMATION OF LARGE PARACRYSTALLINE ARRANGEMENTS OF LIPIDIC PARTICLES IN MODEL SYSTEMS

Lattice-like arrangements of particles in artificial membranes composed of a mixture of the chloroplast lipids DG, MG and SL were reported in [14,15] without an explanation of their formation. Particles were visible as electron opaque dots in negatively stained preparations [14] while those in [15] appeared as a paracrystalline arrangement of bumps in freeze fracture preparation. Particles in both [14,15] were of similar size (80–100 Å) and apparently represented the same type of particle prepared for electron microscopy by different

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methods. Subsequently, these particles were described as inverted lipid micelles sandwiched between leaflets of a lipid bilayer [16], similar to the model proposed in [17–21]. The bulged bilayer model [17] or the cusp model [22,23] have not been suggested for these particles, but are discussed below.

In [24] small paracrystalline arrangements of particles in mixtures of DG and MG were reported. We have found that the addition of SL to mixtures of DG and MG is required to develop large paracrystalline arrangements of 80–100 Å particles as seen with negative staining. Fig. 1 shows electron micrographs of purified chloroplast lipids in various mixtures. Whereas pure DG forms liposomes (fig. 1a), DG–MG in 6:4 mole ratio formed predominantly large complex structures (fig. 1b). Paracrystalline arrays were not apparent in either preparation. Addition of SL to these galactolipid mixtures resulted in appearance of paracrystalline arrays (fig. 1c) which increased in number with increasing SL ratio (fig. 1d). Addition of SL to these galactolipid mixtures resulted in appearance of paracrystalline arrays (fig. 1c) which increased in number with increasing SL ratio (fig. 1d).

3. STRUCTURAL MODELS OF DG, MG AND SL PARTICLES

Several molecular species of DG, MG and SL have been reported [25]. These species differ in the number of carbon atoms and double bonds of the fatty acids attached to the C-1 and C-2 carbon atoms of the glycerol. Because of the *cis* configuration of the carbon–carbon double bonds, unsaturated fatty acids are of shorter length, but occupy a larger cross-sectional area than saturated fatty acids of similar carbon length [26]. In [10], naturally occurring DG and MG with mostly unsaturated fatty acids may have had the sugar groups parallel and adjacent to the fatty acids. However, in [6,27,28], the sugar groups were suggested to be exposed on the surface and oriented perpendicularly to the fatty acids. This orientation of the sugar groups would affect the head space of each lipid. Monolayer studies have shown MG to occupy a smaller head space than DG or SL [29]. The predominance of unsaturated fatty acids in MG [25] gives it a cone-shape with the head group at the smaller end of the cone [17]. Thus, pure MG forms hexagonal phase structures [30,31] characteristic of cone-shaped molecules [17]. In the inverted micelle model the MG would occur in the inverted micelle (fig. 2a). In the bulged-bilayer model and the cusp model the MG would occur in the concave side of the bulge (fig. 2b and 2c, respectively).

According to [32], lipids with the larger head space would migrate to the outer monolayer during the process of self assembly of lipid bilayers. In the inverted micelle model this would correspond to the area of the leaflet opposite the micelle. In the cusp model this would correspond to the convex side of the bulge. In the mixture of DG, MG and SL, the SL would have the greater polar head group, though similar in molecular size to DG [29]. Furthermore, due to the saturated nature of at least one fatty acid [25], SL would have a smaller hydrophobic cross section than DG, and an inverted cone-shape. SL would thus occupy the area of the leaflet opposite the micelle (fig. 2a) or in the convex side of the bulge (fig. 2b,c).

4. ORDERLY SEPARATION OF CHARGES

Besides DG, MG and SL, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, and several other lipids may be found in thylakoid membranes [25,33]. However, SL is the major charged lipid and thus probably occupies the majority of the positions in the leaflets opposite the micelle or the convex side of the cusp. Because of the repulsion of like charges, probably only a few molecules of SL per lipidic particle would occur at each site, with DG or other lipids forming the rest of the leaflet. The result would be an orderly arrangement of negative charges on the thylakoid membrane. Perhaps, this would explain the preponderance of uncharged DG and MG in chloroplast membranes.

The bulges in the leaflet around the micelle or in the bulge or cusp would produce monolayers with different degrees of curvature. Lipids with similar head space and similar fatty acids would thus migrate to areas of the monolayers with specific head space and fatty acid length and area requirements. Thus, similar molecular species of each lipid type would concentrate in specific areas and monolayers of the bilayer. Orderly separation

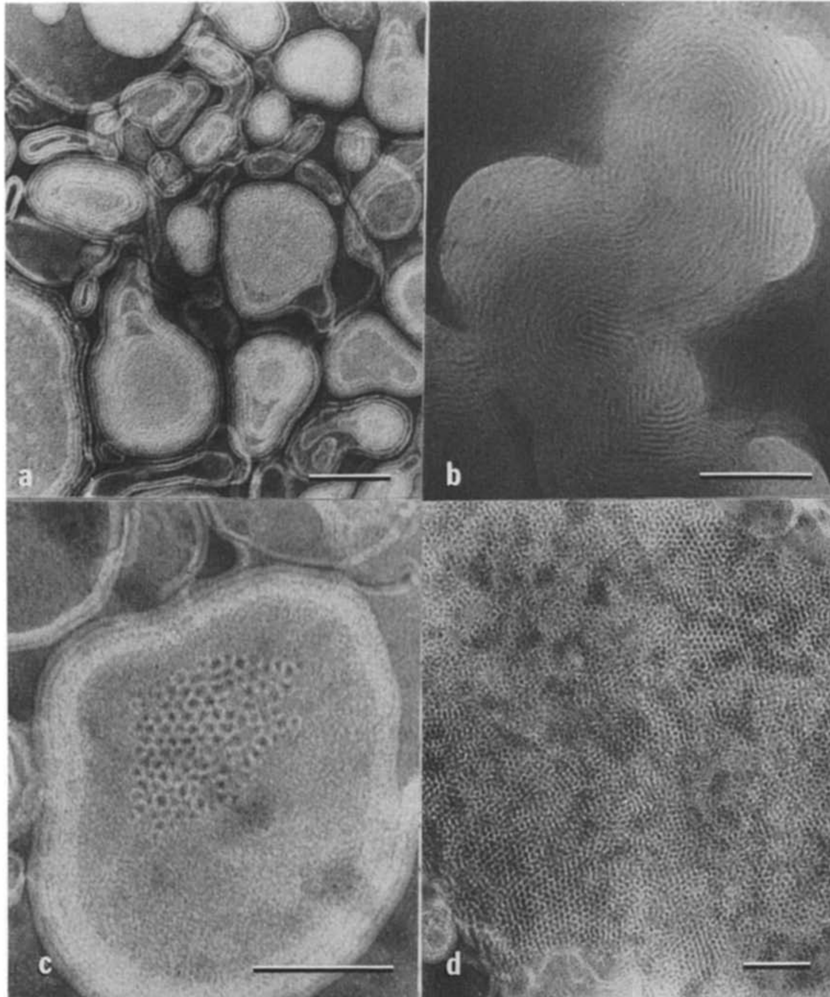

Fig. 1. Negatively stained chloroplast lipid suspensions of DG (a), DG–MG (6:4) (b), DG–MG–SL (6:4:2) (c), and DG–MG–SL (6:5:3) (d). Scale: each bar equals 1000 Å. DG and MG were purified from *Spinacia oleracea* L., while SL was extracted from *Ipomoea aquaticus* Forsk. DG, MG and SL were isolated by column chromatography on acid-washed florisil. SL was purified further by anion exchange chromatography on DEAE-cellulose. All lipids were homogenous by thin-layer chromatography in chloroform–methanol–H_2O (65:25:4, by vol.). All lipids, after being thoroughly dried of solvents were suspended in 10 mM Na-phosphate buffer (pH 7.0), with 1% ammonium molybdate under nitrogen on a vortex for 1 min. The suspension was nebulized onto carbon support films and viewed with a Hitachi HS-8-1 electron microscope operated at 50 kV, of the H. St. John Plant Science Laboratory Electron Microscope Facility.

of the charged phospholipids could also thus occur. The reported probable concentration of phosphatidylglycerol in the outer monolayer of thylakoid membranes [34,35] and the concentration of phosphatidylcholine in the inner monolayer [35] supports this hypothesis. Independence of lipid type and quantity in inner and outer monolayers of lipid bilayers has also been reported [36]. In addition, studies using X-ray diffraction [35] and extraction with media of different pH [37] appear to have localized SL on the surface of the thylakoids. Antibodies against SL have also shown the determinant groups of the sulpholipid to be accessible in the lamellar system [38].

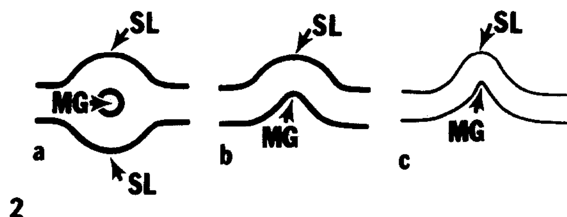

Fig. 2. Location of MG and SL in the inverted micelle (a), bulged bilayer (b), and cusp (c) models of lipidic particles. The micelle is simplified as a circle and each monolayer of lipids is shown as a line; (a,b) redrawn from [17]; (c) redrawn from [22].

5. EVIDENCE FOR THE INTERACTION OF COUPLING FACTOR (CF_1) AND SL AND LOCATION OF FERREDOXIN-NADP REDUCTASE BETWEEN KNOBS OF CF_1

A lipid component of the chloroplast thylakoid membrane interacts with CF_1. This lipid component protects CF_1 against cold inactivation. Galactolipids and phospholipids are effective in this respect. However, sulfolipids [SL] are the most effective [39]. Thus, possible interaction of a CF_1 particle with SL has been reported.

Using antisera, ferredoxin-NADP reductase was located on the surface of the outer membranes of the thylakoids between knobs of coupling factor [40,41]. Antisera against ferredoxin-NADP reductase inhibited photosynthetic NADP-reduction and $NADPH_2$ diaphorase activity of the enzyme, but did not agglutinate the thylakoid membrane. Indirect agglutination could be obtained by incubation with antisera against the reductase followed by antisera against rabbit-α-globulin or soluble reductase. It was concluded [40, 41] that the thylakoids could not be agglutinated directly by antibodies against reductase, because the antibody molecules were too short to connect reductase located in deepenings between knobs of CF_1. These deepenings were estimated to be >60 Å but <200 Å. Removal of CF_1 by treatment with EDTA allowed direct agglutination of the thylakoids by antisera against the reductase.

Although we have only related CF_1 to SL and ferredoxin-NADP reductase to CF_1, interaction of other molecules functioning in photosynthesis with SL or phospholipids could account for the orderly structure of the thylakoid membrane.

6. SUMMARY

We have shown that the addition of SL to DG–MG mixtures induces the appearance of paracrystalline arrays of 80–100 Å lipidic particles. Our hypothesis is that SL occupies the position in the leaflet opposite the micelle or the position on the convex side of the bulge or cusp in the corresponding model for lipidic particles. Only a few SL molecules would occur per lipidic particle and thus the orderly arrangement of particles would result in an orderly arrangement of separated negative charges on the chloroplast thylakoid membrane. Separation of charged phospholipids may also occur due to the different degrees of curvature of the monolayers caused by the particles. This orderly arrangement of separate charged particles forms the basis for the orderly attachment and arrangement of other molecules functioning in photosynthesis in the thylakoid membrane.

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NOTE ADDED

The hypothesis in [42] differs from ours in that it relates to the 'tightly curved thylakoid margins', bilayer deformities, or hydrophobic proteins associated with inverted micelles. Ours relates to uniform particles, orderly arranged, and with orderly separation of charged SL or phospholipids. Although presently cited relationships of SL with other molecules functioning in photosynthesis [43] show a non-appressed region location for our particles, it does not preclude occurrence of our particles in appressed regions. Perhaps in these appressed regions phospholipid may form the orderly arrangement of charges.

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