Rapid Report

Destabilisation of lamellar dispersion of thylakoid membrane lipids by sucrose

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Abstract

Employing negative-staining electron microscopy, convincing evidence has been obtained for the destabilisation of multi-lamellar organisation of aqueous dispersions of chloroplast thylakoid membrane lipids, to an inverted micellar structure under the influence of sucrose solution. The present study provides a new insight into the phase behaviour of a naturally existing galactolipid-rich lipid mixture consequent to interaction with a kosmotropic reagent.

Key words: Thylakoid membrane; Lipid; Sucrose; Inverted micelle; Electron microscopy

Kosmotropic reagents such as sucrose, glucose and trehalose are known to stabilize the structure of bulk water and therefore influence the lamellar organisation formed by aqueous dispersions of phospholipids [1-3]. The phenomenon has a bearing on the survival of small organisms in 'dry' state in the presence of large amounts of sugars [4]. Recently, it has been shown that lamellar structure formed by aqueous phospholipids undergoes a transition to hexagonal phase II (HII) organisation under the influence of sucrose solution [2]. Despite mounting interest in such investigations, there is, as yet, no report in the literature on this aspect involving lipid mixtures containing glycolipids. It may well be, that the presence of a sugar moiety in the headgroup of glycolipids may elicit somewhat altered interactions vis à vis those found for phospholipids. An added motivation for the present investigation was that, in nature too, the galactolipid-rich thylakoid membranes are likely to be exposed to sugars produced in the process of photosynthesis.

Lipids extracted from thylakoid membranes, which are naturally abundant in galactolipids [5] are, therefore, the system of choice for the present investigation. Although the largest fraction of these lipids, namely, monogalactosyl diacylglycerol (MGDG) forms an aqueous hexagonal HII structure [6], there are reports to suggest [7-11] that lipid organisation in chloroplast thylakoid membranes is largely a bilayer. In the present investigation, effect of sucrose solution on the lamellar structure of lipid mixture extracted from spinach thylakoid membranes is being reported employing transmission electron microscopy. In this study, 0.35 M sucrose was employed for experimental work keeping in view the fact that this concentration of sucrose is routinely employed for the isolation of chloroplasts [12].

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plast membranes [8,9]. This technique has one definite advantage over the freeze-fracture and etch techniques in that it does not entail any cold-stress on the system under study which may destabilize this system [8]. Furthermore, the multiplicity of lipid structures that may be formed from aqueous dispersions of total lipids extracted from the chloroplast membranes, have been more clearly characterized and better described after negative-negative electron microscopy [8] rather than by freeze-etch electron microscopy [13].

$^{31}$P-NMR technique, which is normally employed for studying bilayer, hexagonal and micellar phases of phospholipids [14] is not suitable for studying the chloroplast thylakoid membrane lipid mixtures, as the latter are markedly deficient in phospholipids. Negative-negative electron microscopy was, therefore, found to be the suitable technique for the present investigation especially in light of the useful information it has already yielded on the kind of lipid system under study [8,9].

Chloroplasts were obtained from fresh spinach leaves [15] and thylakoid membranes were obtained from them according to the Mackender and Leech [16]. Lipids from these membranes were extracted [17] and their aqueous dispersions were prepared as already described [8]. For electron microscope studies, a thin film of dilute lipid-extract was laid on a formvar-coated copper grid and dried under N$_2$ gas. It was then dipped in distilled water for 2 min before processing for staining with phosphotungstic acid [8]. For treatment with sucrose solution, the lipid-coated grids (immediately after dipping in distilled water for 2 min as described above) were obtained on a flat surface and a drop of 0.35 M sucrose was placed gently on its coated side. The sucrose solution was soaked away after 1 min by touching the filter paper strip on the edge of the grid which was then stained with phosphotungstic acid [8]. For comparison, the control grids were treated with

![Fig. 1. Electron micrograph of aqueous dispersion of lipids extracted from spinach thylakoid membranes stained with phosphotungstic acid. Multilamellar organisation is clearly noticeable. Original magnification: $\times 190000$.](image1)

![Fig. 2. Electron micrograph of the aqueous dispersion of lipids extracted from the spinach thylakoid membranes, treated with 0.35 M sucrose solution and stained with phosphotungstic acid. Micellar (m) as well as some multilamellar (f) structures are observable. Arrows show single lamellar structures. Original magnification: $\times 190000$.](image2)
0.175 M NaCl solution in the same was for treatment with sucrose solution. The grids were examined under Siemens Elmiskop I following standard procedures [18].

Fig. 1 shows the electron micrograph of the aqueous dispersion of lipids extracted from the thylakoid membranes, clearly demonstrating their multilamellar structure. Earlier studies from this laboratory employing NMR, ESR and electron microscope techniques have also revealed such structures to be multi-bilayers [8,9].

Fig. 2 shows the effect of 0.35 M sucrose solution on the multilamellar structures as shown in Fig. 1. It shows the presence of a large number of spherical vesicular structures (m) of size varying from approx. 100–200 Å in diameter. Similar structures have earlier been identified as spherical micelles [8]. These structures are interpreted as inverted micelles with polar headgroups in contact with the aqueous core in the interior which appears electron-dense after negative-negative (Fig. 3). Also seen in Fig. 2 are remnant multilamellar structures (l). The effect of 0.175 M NaCl solution was also studied on the thylakoid-lipid multilamellar structures as an experimental control (Fig. 4). It is clear that the multilamellar structure formed by the aqueous dispersion of thylakoid membrane lipids is preserved in the presence of salt solution.

It will be desirable to discuss the interpretation of the new structures formed (Fig. 2) as inverted spherical micelles (Fig. 3) vis à vis ‘similar’ structures, formed by phospholipids observed by negative-negative electron microscopy.
Fig. 4. Electron micrograph of the aqueous dispersion of lipids extracted from the spinach thylakoid membranes, treated with 0.175 M NaCl solution and stained with phosphotungstic acid. Only multilamellar structures are observable. Original magnification: ×190000.

microscopy [1]. Crowe et al. [1] have restricted their interpretation only to a relatively small part of their electron micrograph (Fig. 8b), describing therein, only some 'tubular' structures as hexagonal cylindrical phase HII. However, the remaining part (larger view) of the same electron-micrograph (Fig. 8b) was not discussed by the authors. 'Round' structure which predominate the field-of-view of this electron micrograph [1] can be interpreted as spherical micellar structures on the basis of interpretation of Fig. 2 in the present report. In addition, it may not be irrelevant to mention that the 'tubular' structures formed by the hexagonal cylindrical phase II may be seen more clearly and interpreted rather unequivocally by negative staining as shown in Fig. 5 (modified from YashRoy [8]). This figure is an example of the three important lipid phases – lamellar, micellar and cylindrical hexagonal – co-existing in the same view, thus making their interpretation easy.

The results reported here on the galactolipid-rich lipid mixtures of thylakoid membranes, in principle corroborate the observations recorded earlier on aqueous phospholipid systems. For phospholipids, sucrose markedly decreased the temperature of lamellar liquid-crystalline to hexagonal HII phase transition in hydrated dihexadecyl phosphatidylethanolamine and distearoyl phosphatidylethanolamine [2]. The observations in this communication, therefore, are also considered to be another manifestation of Hofmeister effect in which sugar acts as a kosmotropic reagent stabilizing the structure of bulk water. In general, kosmotropic reagents (sugars and other cryoprotectants) stabilize the water structure in favour of small lipid-headgroup areas. This tends to decrease the area of contact between the lipid and the aqueous phases. This tendency leads to a preference for the formation of hexagonal HII phase relative to liquid-crystalline lamellar phase organisation, as observed for phospholipids [2] and, on the same principle, preference for inverted micellar phase relative to lamellar phase organisation as observed in the studies under report. The lipid head-groups, being packed in a minimal space in the interior of the sphere (spherical micelle) minimises their area of contact with the aqueous phase (Fig. 3) vis à vis the situation in the lamellar organisation. The present study shows that sucrose exerts, in principle, a 'similar' effect on the aqueous lipid mixtures obtained from thylakoid membranes, as it does on phospholipids [2]. However, formation of the inverted micellar structure under its influence (instead of hexagonal HII organisation for the phospholipids), may be attributed to the unique composition of lipids in the thylakoid membranes which are rich in galactolipids with polyunsaturated fatty-acyl chains conferring on them distinct fluidity [19–21] and other biochemical and biophysical properties [21–23]. This report may, therefore, stimulate further work, throwing more light on this aspect of lipids of the thylakoid membranes, which constitute [24] 60–80% of total cellular membranes in higher plant mesophyll cells and are also the most abundant membranes in the natural world.

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Fig. 5. Electron micrograph of different lipid phases produced on exposure to low-temperature (5°C, 24 h) of a suspension of sonicated aqueous dispersion of lipids extracted from spinach chloroplast membranes. Compare the lamellar (l) and the spherical micellar (s) phase structures with the ‘bundles’ of laterally-apposed long hexagonal phase II (h) cylindrical structures (modified from YashRoy [8]). Original magnification: x225,000.

1. References