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Mobility of membrane particles in chloroplasts

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SUMMARY

A unique structural feature of chloroplasts is the association of the energy transduction system with fused pairs of membranes in the grana. Type II spinach chloroplasts examined by freeze–fracture electron microscopy reveal that particles located in the hydrophobic core of the grana region of the thylakoid membrane system change their orientation in response to:

(a) bivalent cations, at concentrations which are known to increase efficiency of coupling between Photosystems II and I, and (b) illumination, which causes a reversible change in the density and size of particles. These results are consistent with a previously reported reversible 25% thinning of a fused pair of membranes in illuminated chloroplasts, observed by conventional electron microscopy.

It is concluded that the particulate (protein or lipoprotein) components of the grana membrane system of chloroplasts are mobile, *i.e.* change their orientation within the membrane in response to functional states.

The energy transducing membrane system in chloroplasts of eucaryotic plant cells contains a system for light and dark stages of energy transduction, electron transfer and energy conservation. This membrane system has unique structural features for the investigation of the relation of structural organization to function. It is composed of a network of single unapposed membranes (stroma lamellae) connected to a series of membranes which adhere to one another to form the stacked membranes of the grana region. The stacked membranes may be fused to one another over large areas.

This membrane fusion is of considerable importance for the efficient coupling of energy flow between Photosystems II and I. More efficient coupling between these photosystems has been reported by Sun and Sauer[1] to be brought about by Mg^{2+}. Furthermore,

conventional electron micrographs of spinach chloroplasts reveal that the structure of the stacked area of the grana membranes are markedly affected by bivalent ions. Ions like Mg^{2+} cause close packing, stacking and reordering of the fused membranes of the grana after distilled water treatment[2]. Murakami and Packer[3] also found that the thickness of a pair of fused grana membranes decreases by 25% after illumination and the effect is reversible. This structural change was related to the capacity of illuminated chloroplasts to carry out a light-dependent protonation of the membrane and formation of a pH gradient. In addition, the thinning of the dimensions of a fused membrane pair was accompanied by changes in the intensity of internal (chlorophyll) and externally added fluorescent probes[3].

Hence, large changes in internal structural organization of grana membranes occur upon illumination or treatment by divalent cations. To clarify the nature of these changes we have used the technique of freeze–fracture electron microscopy to investigate the influence of light and ionic environment on membrane organization. It has been found that particles seen in the hydrophobic core of grana membranes can change their orientation within the membrane.

Typical fracture faces of spinach chloroplast grana membranes are shown in Fig. 1. Here we have used the designation of B_s, B_u, C_s and C_u faces of Goodenaugh and Staehelin[4]; these correspond to the B, D, A and C faces, respectively, of Branton and Park[5]. Two types of fracture faces are shown. The B and C faces appear either in unstacked (B_u,C_u) or

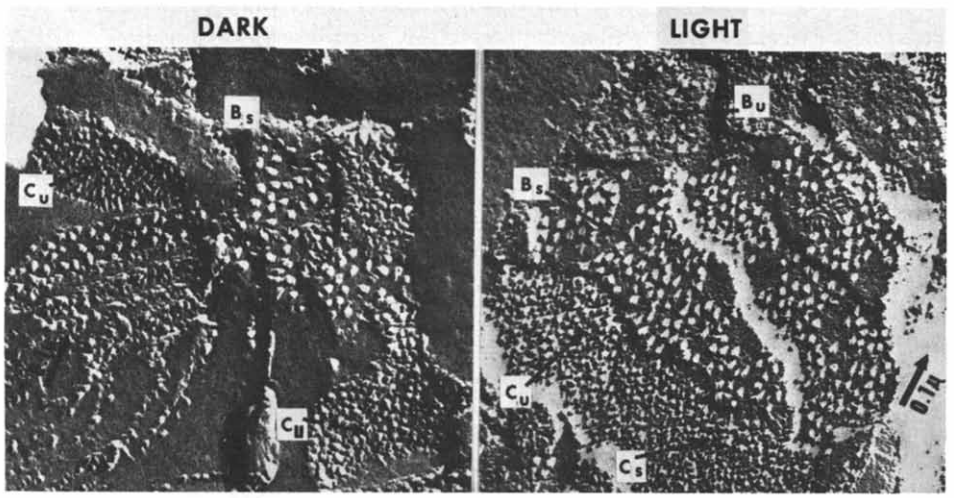

Fig. 1. Membrane fracture faces of spinach chloroplasts. Chloroplasts were prepared from spinach leaves and incubated as follows. Leaves were homogenized in a medium containing 50 mM Tris–HCl buffer, pH 8.0, 175 mM NaCl, centrifuged at 250 × *g* for 2 min. The supernatant was recentrifuged at 1500 × *g* for 5 min, and the pellet was collected and resuspended in 0.1 M sodium acetate, 50 μM phenazine methosulfate at pH 6.8. Glycerol (20%) was added to protect samples from ice crystal damage during subsequent freezing. Chloroplasts were incubated in the dark or illuminated for 2 min. Samples were quickly frozen by transferring a drop of the reaction mixture to a copper disc, which was then quickly frozen in liquid freon. Frozen discs were transferred to liquid nitrogen and replicas for freeze- fracture electron microscopy were made in a Balzer's apparatus and examined in Siemens I electron microscope.

stacked (B_S,C_S) regions of the membrane. Both C faces contain numerous small particles, about 4000–5000/μm^2, and thus it is difficult to analyze their size and distribution. However, the B faces contain a smaller number of larger particles, thus facilitating an analysis of their size and density in the unstacked and stacked membrane areas.

Chloroplasts illuminated in the presence of weak acid anion solutions undergo a decrease in volume[6]. The extent of the shrinkage can be assessed by packed volume determinations of samples fixed by glutaraldehyde, which prevents them from undergoing further changes during the course of their packing in a capillary centrifuge. Table I shows that the packed volume of Type II chloroplasts decreases 42% upon illumination in sodium acetate solutions; this is accompanied by increases in the size and density of particles in the B_S face. The particle density per μm^2 increases 2.2-fold and the average particle diameter is increased by about 25 Å. Examination of replicas prepared from chloroplasts incubated in the dark after a short period of illumination shows that these changes in particle distribution size are reversible.

Chloroplasts, illuminated in the presence of strongly dissociated ions, swell upon illumination. Deamer *et al.*[7] have shown that this effect results from the extremely low internal pH caused by light-dependent H^+ uptake into chloroplasts. This decrease in pH results in membrane disorganization: the configuration of the normal grana structure is pulled apart, the spacing between the membranes becomes larger, and the chloroplasts as a whole, undergo an increase in volume due to the uptake of water and solutes[7]. Similar effects occur by lowering the pH of chloroplasts in the dark[3,7]. Table I shows that under these conditions, chloroplast swelling and presumably grana disorganization are accompanied by marked changes in membrane particle organization of the B_S fracture face. The number of particles per μm^2 of membrane is decreased by 32%, and the average particle diameter is decreased by 41 Å.

Bivalent cations also cause marked changes in the organization of particles in the fused region of the membrane. Low concentrations of Mg^{2+} were earlier shown by Gross and Packer[8] to cause a non-osmotic chloroplast shrinkage. A decrease in chloroplast volume with increasing concentration of Mg^{2+}, seen in Table II, is consistent with these earlier

TABLE I

LIGHT-DEPENDENT CHANGES OF PARTICLE DISTRIBUTION IN THE B_S FRACTURE FACE OF SPINACH CHLOROPLAST GRANA MEMBRANES

Type II spinach chloroplasts were suspended in a medium containing 0.1 M NaCl or sodium acetate, 50 μM phenazine methosulfate, at pH 6.8, 20 °C. Other conditions as in Fig. 1.

		Particle density per μm^2	*Average particle diameter (Å)*	*Packed volume (%)*
Sodium acetate	Dark	1473±244	85	12.0
	Light	3200±200	110	7.0
NaCl	Dark	2150±150	134	10.0
	Light	1466±90	93	12.0

TABLE II

EFFECT OF $MgCl_2$ ON PARTICLE DISTRIBUTION IN THE B_S FRACTURE FACES OF SPINACH CHLOROPLAST GRANA MEMBRANES

Type II spinach chloroplasts were suspended in a medium containing 0.1 M sodium acetate, pH 6.8, in the dark. Additions of $MgCl_2$ were made as indicated, other conditions as described in Fig. 1.

$MgCl_2$ (mM)	*Particle density per μm^2*	*Packed volume (%)*
0	1700±150	35.0
5	1850±100	32.2
15	2200±200	28.0

studies. Further investigations by Murakami and Packer[2] revealed this effect was accompanied by an increase in ordering and stacking of grana membranes. Table II shows that $MgCl_2$ increases the density of membrane particles per μm^2 of membrane.

The results of freeze–fracture electron microscopy[4,5] and low-angle X-ray scattering[9] studies indicate that the smooth areas seen in fracture faces are ordered areas of hydrophobic domains containing at least some lipid bilayer configurations. Although hydrophobic domains are a significant structural feature, the presence of particles embedded within the membrane interior suggests that the membrane is a mosaic structure. Our results suggest that it is a fluid mosaic structure, because the particles (presumed to be proteins or lipoproteins) can change their location or position within the membrane. The increases and decreases in particle density and diameter suggest that the protein components are penetrating further into the interior of the membrane in response to illumination or are being withdrawn from the hydrophobic domains of the membrane. We suggest that these effects are responses to changes in the tightening or loosening of the fusion of membranes in the stacked area of the grana.

Changes of particle density may also result from a lateral movement of membrane components into or out of the fused region of the membrane in response to illumination of the appropriate ionic environment.

The results of this investigation indicate that membrane particles are mobile. If there is close interaction between the protein and lipid phases, we would predict that concomitant changes in the organization of lipids should occur. Indeed, recent studies in our laboratory[10] indicate that changes in the partitioning of lipid spin labels occur under the identical conditions described in this investigation for the redistribution of membrane particles in illuminated chloroplasts. Hence, it may be concluded that the energy transducing membrane is a dynamic structure where changes in protein and lipid organization are closely correlated with functional states.

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