PRELIMINARY NOTES

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Reversible changes in the conformation of thylakoid membranes accompanying chloroplast contraction or expansion

It has been established that chloroplasts incubated in weak-acid anion solutions[1-4] manifest a shrinkage upon illumination, which is reversed by turning off the light. A reversible photo-shrinkage of isolated chloroplasts suspended in strongly dissociated ions like NaCl can also occur, if phenylmercuric acetate (PMA) is present, as reported by SIEGENTHALER[5]. In these instances the decrease in chloroplast volume is associated with a tightly packed grana lamellar membrane structure[6]. In this paper new evidence is presented from analysis of thickness, spacing, and electron density changes of the thylakoid membrane, that reversible changes in the conformation of the membrane accompany contraction or expansion of the thylakoid membrane-bounded compartment.

The effect of light, phenazine methosulfate (PMS) and PMA on the light scattering and transmission of a chloroplast suspension is shown in Fig. 1. The basic light scattering was increased on illumination by 5 % and was further increased to 10 % by adding PMS. The simultaneous record of transmission showed a small decrease followed by a gradual increase. The decrease in transmission corresponded to an increase in light scattering. The increase in transmission continued somewhat even after extinguishing the red light but ceased on the addition of PMA. In the presence

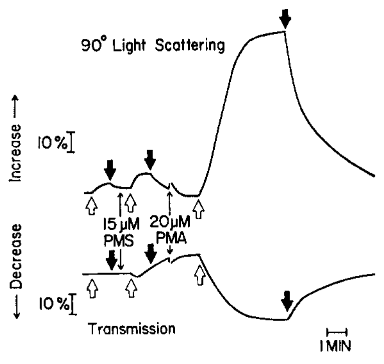

Fig. 1. Stimulation of light-induced light scattering and transmission changes of chloroplast suspension by PMA. Chloroplasts were isolated from spinach leaves in 50 mM Tris–HCl buffer (pH 8.0) containing 175 mM NaCl. Light scattering at 90° and transmission of the suspension were measured at 546 mμ in the Brice–Phoenix light scattering photometer[1], to which a cuvette with a circulating water (25°) jacket was fitted. The 546-mμ light scattering and transmission level of the chloroplasts in the dark was adjusted as 100%. Then illumination by red light (600–700 mμ) from a side tungsten lamp was made to induce chloroplast conformational changes. Chloroplasts (18 μg chlorophyll per ml) were suspended in 175 mM NaCl–50 mM Tris–HCl at pH 8.0.

Abbreviations: PMA, phenylmercuric acetate; PMS, phenazine methosulfate.

of both PMS and PMA, the light scattering increase and transmission decrease induced by illumination were remarkably enhanced; an 80 % scattering increase and 24 % transmission decrease were observed.

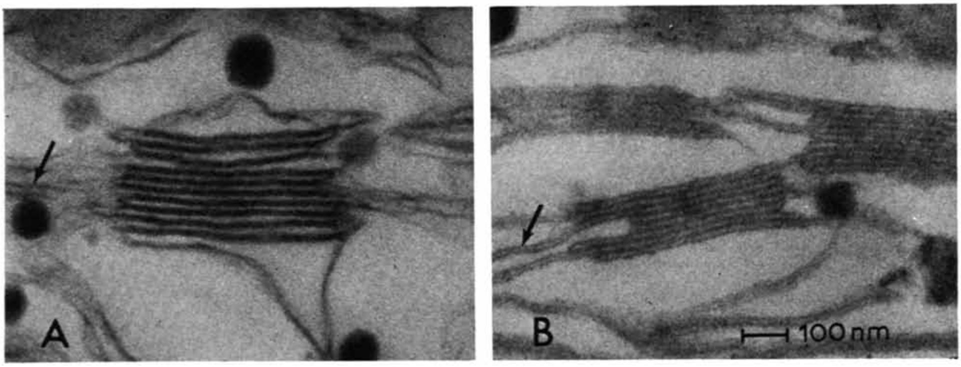

Fig. 2. Changes in the structure of the chloroplast membrane system upon illumination. (A) Dark, before illumination and (B) light. × 57150.

The ultrastructure of the chloroplasts in these different scattering–transmission states was examined by the electron microscope. Chloroplasts suspended in a medium containing 50 mM Tris–HCl, 175 mM NaCl, 15 μM PMS and 20 μM PMA at pH 8 were fixed for at least 1 h by adding an 8 % glutaraldehyde solution to the suspension in the dark state before or after illumination, or at the maximum response in the light. The final concentration of glutaraldehyde was 1 %. The chloroplasts were suspended for 2 h in 1 % osmium tetroxide (50 mM sodium phosphate buffer at pH 7.4) and then embedded in an Araldite–Epon mixture. Chloroplasts examined in the dark, either before or after an interval of illumination, show normal morphology, though the membrane system, especially the outermost thylakoids of the grana, and intergrana thylakoids are slightly swollen. This is probably due to the slightly hypotonic conditions employed in these *in vitro* experiments. Upon illumination a flattening of the membrane system was induced, and both grana and intergrana thylakoids became tightly packed (Fig. 2). The thickness, spacing and electron density of the membrane in a grana stack as well as intergrana region (arrows) are reduced by illumination. Thickness of the grana membrane measured on enlarged photographs (20000 × 10) was 135 ± 8, 104 ± 5 and 133 ± 8 Å, respectively, for the dark condition before illumination, in continuous light and in the dark period after illumination.

Thickness and spacing of the grana membrane were measured by photo-densitometric analysis of the original negatives (Table I). Illumination caused the thickness of the grana membrane to be reduced by 23 %, and a 32 % reduction was measured in the spacing. These changes were reversed completely when chloroplasts were permitted to swell again in the dark. When photo-shrinkage was induced by illumination in weak-acid anion solution (150 mM sodium acetate *plus* 15 μM PMS at pH 6.5), similar results were obtained, although the amplitude of the changes was smaller.

The time course of light scattering and transmission changes induced by red light shows discrepancies when these two responses are compared. For example, in Fig. 1, chloroplasts in NaCl medium containing PMS and Tris–HCl buffer show an initial small transmission decrease followed by a gradual increase while light scattering

TABLE I

THE EFFECT OF LIGHT ON THE THICKNESS AND SPACING OF GRANA MEMBRANES

Conditions of incubation were: NaCl–PMA (175 mM NaCl, 50 mM Tris–HCl, 15 μM PMS and 20 μM PMA at pH 8.0). Sodium acetate (150 mM sodium acetate and 15 μM PMS at pH 6.5). Mean values (± S.D.) of half-width and peak-to-peak distance of density distribution curves on photo-densitometric records (finally magnified at 1000000 = 20000 × 50) were designated, respectively, as the thickness and spacing of grana thylakoid membrane.

Medium	*Thickness (Å)*			*Spacing (Å)*		
	Dark →	*Light* →	*Dark*	*Dark* →	*Light* →	*Dark*
NaCl–PMA	131 ± 10	101 ± 9	130 ± 9	212 ± 8	144 ± 9	214 ± 4
Sodium acetate	129 ± 9	112 ± 10		196 ± 4	144 ± 3	

shows the normal response (increase). The phases of light scattering increase and small transmission decrease appear perfectly synchronized. In NaCl medium illumination is known to cause a swelling of the chloroplast as a whole, *viz.* an expansion of inner space of thylakoid[7] which is accompanied by transmission increase[6]. Therefore, transmission changes are apparently correlated with the spacing change or flattening of the membrane system. This conclusion is also supported by studies made by YAMASHITA *et al.*[8] of absorbance changes of chloroplasts upon illumination.

Flattening of the membrane system has been recognized as the ultrastructural basis of photo-shrinkage of chloroplasts[3,4,6,9–11]. This investigation now established that at least two conformational changes of the membrane system itself, *viz.* decrease of thickness and spacing, are involved in this flattening effect. We have noted that these changes are widely observed in chloroplasts in which shrinkage or contraction is induced by different means, such as illumination in either a NaCl–PMA or sodium acetate medium, or by acidification of NaCl medium in the dark. Hence, membrane thickness changes should be taken into account in considering mechanisms of conformational change in chloroplasts, since reversible thickness and electron density changes are a morphological expression of tertiary and/or quaternary structural changes of macromolecules and their organization. It is relevant in this connection to consider refractive index changes of the membrane suggested by MUKOHATA[12] and DEAMER *et al.*[13] to result in light scattering changes, *viz.* the thickness changes cause refractive index and hence light scattering changes. In support of this view we have found that the time course of thickness changes follows exactly the kinetics of light scattering change. Further studies of conformational changes of thylakoid membranes induced by PMA and other means are in progress.

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