The lateral displacement of intramembraneous particles in chloroplast membranes as a function of light intensity

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1. INTRODUCTION

Lateral diffusion of proteins and lipids in cell membranes has now been well documented [1–4]. It can result for a variety of reasons including such chemical and physical conditions as temperature, pH and ionic strength. Diffusion coefficients have been calculated in some cases [1–3].

In chloroplasts, light intensity causes structural changes in the thylakoid membranes; increasing the intensity results in shrinking of thylakoid grana stacks [5] and the thinning of individual membranes [6,7]. Ionic strength effects are also evident. In a low salt environment there is a loss of grana structure. At the same time particles observed on the EF freeze-fracture face that show lateral heterogeneity by being concentrated in the membrane-appressed regions of grana, undergo a long range displacement to produce a uniform distribution on the unstacked EF fracture face [8,9].

Particles observed on the EF_s fracture face (membrane-appressed) are thought to contain elements of the oxygen-evolving photosystem II and are in close association with light-harvesting complex (LHCP) [10,11]. In certain buffers, large changes in EF_s face particle density and average diameter could be induced by illumination [5]. To sharpen a quantitative description of these and other effects we have devised a method for measuring the radial distribution function (RDF) for particles seen in freeze-fracture electron micrographs [12]. The method is applicable to the comparatively small areas of fracture face that result from the random fracturing process. The RDF measured provides quantitive information about even small displacements of intramembraneous particles with respect to each other. The method has been used here to investigate the distribution of EF_s face particles in isolated chloroplasts incubated at low and high light intensity.

2. METHODS

Chloroplasts were prepared from spinach plants (*Spinacia oleracia* L.) grown in water culture. Chopped young leaves were homogenised in 50 mM K-phosphate buffer (pH 7.2) with 0.3 M sucrose and 0.1 M KCl (5 ml buffer/g fresh wt of leaves) in a Servall Omnimix for 4 s at 75% line voltage. The brei was filtered through 2 layers of Miracloth and the starch removed by centrifugation at $1000 \times g$ for 1 min. Intact chloroplasts were sedimented from the supernatant at $5000 \times g$ for 1 min.

For oxygen evolution measurements, chloroplasts (100 μg chl) were suspended in 2 ml 50 mM Tris-HCl buffer (pH 8.0) containing 70 mM NaCl and 4 mM potassium ferricyanide (Hill reaction buffer). The oxygen evolved was

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measured polarographically at 20°C in suspensions incubated in 50 000 lux from photoflood lamps or at ~500 lux of laboratory illumination.

For freeze-fracturing, chloroplast suspensions in the Hill reaction buffer were sedimented for 15 s in a microfuge (Beckman Instruments) and a small aliquot of the pellet, on a copper disk, was quench-frozen in melting freon 12 without any cryoprotectant. One sample was illuminated at ~50 000 lux from a series of photoflood lamps suspended above the centrifuge and other equipment. The second sample was processed under laboratory illumination of ~500 lux. The freeze-fracturing was accomplished without etching, at −20°C in a Balzers BA 360 M freeze-etch. About 2.5 nm of platinum was deposited from an electron beam gun followed by carbon. The replicas were floated on water, cleaned with sulphuric acid and sodium hypochlorite then picked up on 400 mesh uncoated grids and photographed in a JEOL 100S electron microscope at 18 000×. The magnification was calibrated for each series of micrographs with a grating replica of 2160 lines/mm. Enlargements of ~11× were made but the final magnification was calibrated with the appropriate grating replica for each series of pictures and was ~200 000×.

EF_s face particle positions were determined directly from these micrographs using a Summagraphics digitising tablet (Anderson Digital, Melbourne, VIC) having a resolution of 0.1 mm which produced a resolution of 0.5 nm on the 200 000× micrographs. Radial (RDF) and angular (ADF) distribution functions for EF_s face particles on the fracture faces from the two light treatments were determined as in [8]. Briefly, polygons are drawn on the fracture faces and the positions of their vertices determined using the digitiser. The positions of each particle within the polygon is also digitised and the data stored. When all the data for each sample were accumulated the RDF and ADF were computed using a Digital Equipment PDP 11/34 computer system. The results were plotted on a Hewlett Packard 77221B plotter. The RDF was computed for a range of interparticle distances at 1 nm steps. Particle diameters of 500 EF_s particles from within the polygons used for the RDF and ADF calculations, were measured using a 10× measuring magnifier. From these measurements particle size distributions were constructed for EF_s face particles from the two light treatments.

3. RESULTS

Oxygen evolution rates are shown in table 1. There was an ~20-fold stimulation in rate between 500–50 000 lux. The addition of the uncoupling agent methylamine hydrochloride resulted in a 5-fold stimulation in rate, demonstrating that the chloroplasts suspended in Hill reaction buffer were indeed tightly coupled.

Plots of the RDFs from the two light treatments are shown in fig. 1. The RDF expresses the probability density of finding a particle at a given distance from another particle. Thus at 500 lux the maximum probability is for finding two particles separated by 25 nm and at 50 000 lux this distance is reduced to 22 nm. The steep slope of the initial peak indicates that relatively few particles were closer than the most probable distance. For a homogeneous fluid at larger distances this probability density theoretically becomes 1 as the plot is beginning to show.

Table 1
Hill reaction activity of chloroplasts incubated at high and low light intensity

Light intensity	Hill reaction (μM ferricyanide reduced.mg $chl^{-1}.h^{-1}$)	
(lux)	−MA[a]	+MA[a]
50 000	198	1150
500	<10	50

[a] Methylamine hydrochloride (150 μM).

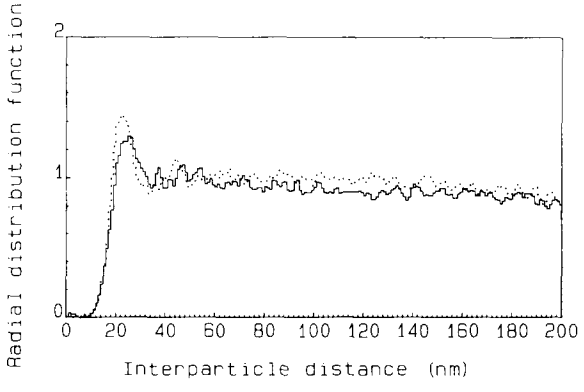

Fig. 1. Radial distribution function (RDF) plots for EF_s fracture-face particles from isolated chloroplasts incubated at 50 000 lux ((···) and 500 lux (——).

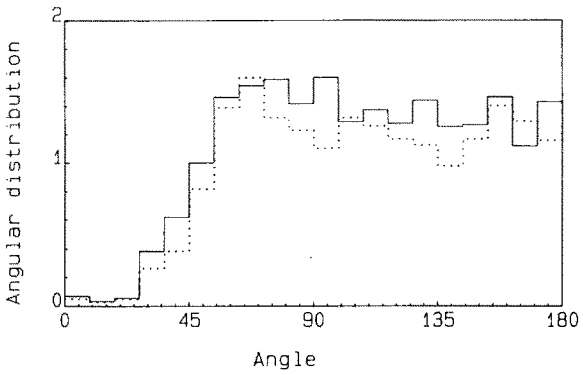

Fig. 2. Angular distribution function (ADF) plots for EF_s fracture face particles from isolated chloroplasts incubated at 50 000 lux (···) and 500 lux (——).

The ADF's for the EF_s fracture-face particles are shown in fig. 2 and indicate that the commonest angle between a particle and its two nearest neighbours is 60°. The shape of the ADF from 60°–180° is slightly different for the two treatments and possibly indicates more particle order for the higher light intensity.

The particle size distributions for the two light treatments were similar: 31% of all particles from chloroplasts incubated at 500 lux had 17.5 nm diam.; and 36% from the chloroplasts incubated at 50 000 lux. This distribution agrees with [8–11].

4. DISCUSSION

The concept of membranes as two-dimensional fluids introduced by Singer and Nicholson [13], has permitted their description in terms developed in statistical physics and the physics of fluids. The RDF is fundamental to the characterisation of fluids and when applied to the study of particles on freeze-fracture faces of membranes provides a more subtle description than the traditional statistical methods that treat these particles as infinitesimal points in a matrix [12]. Thus it might be expected that small but important changes in the disposition of membrane components can be detected by changes in the RDF.

Increased rates of energy transduction in membranes might be expected to affect components. The results reported here suggest that in chloroplast membranes higher rates of energy transduction, exemplified by higher oxygen evolution rates, are accompanied by the closer association of the intramembraneous particles, in the membrane-appressed regions of the chloroplast grana. These regions contain at least the PS II complex and the LHC associated with it, as well as the cytochrome *b–f* complex [10,15]. These intrinsic protein complexes have comparatively high M_r-values and must contribute to the intramembraneous particles seen in freeze-fracture faces.

The particle movements described here in tightly coupled chloroplasts are considerably less than reported for isolated chloroplasts incubated in light and dark [5]. However, the buffer conditions used and the presence of cryoprotectant appear to have produced unusually small particles (8.5 nm av. diam.) on the EF_s face that also changed in diameter on going from the light to dark conditions [5]. The comparatively small short range particle movement we have observed may be due to the absence of cryoprotectant, which causes changes in the RDF (unpublished) and the attempt made to preserve conditions as close to physiological as possible. Thus it is likely that such micro-adjustments in inter-particle distances take place in vivo under differing light conditions.

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