Freeze-Etched Membrane Faces and Photosynthetic Activity in Normal and Mutant Tradescantia Chloroplasts

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Summary
Membrane structure and photosynthetic activity was investigated in normal and mutant plastids of Tradescantia albiflora cv. aureo-vittata. In the stacked membrane regions (the macrograna) of mutant plastids, the B fracture faces lack both 170 Å particles and photosystem II (PS II) activity. The C face has the normal 110 Å particles, and photosystem I (PS I) activity is also similar to that in normal chloroplasts. In dilated macrograna the particle size on the C face significantly decreases, and as progressive plastid destruction occurs so PS I activity also disappears. It has been concluded that the integrity of B face particles is related to PS II activity, rather than for membrane stacking. A similar correlation seems to be valid for C face particles and PS I activity.

1. Introduction
The fine structure of photosynthetic membranes, particularly the arrangement of membrane particles, is still a subject of controversy in the literature. Arntzen, Dilley, and Crane (1969) considered the 170 Å particles belonging to the inner leaflet of the thylakoid membrane as “PS II markers”, and the 110 Å particles of the outer leaflet as “PS I markers”. Sane, Goodchild, and Park (1970) demonstrated that fret membranes as well as end granal membranes contained only 110 Å particles, while membranes of partitions in the grana possessed both 170 Å and 110 Å particles. The latter authors confirmed the results of Arntzen and co-workers, reporting that both photosystems were functioning in the partitions, while in the fret membranes and end granal membranes only PS I, performing mainly cyclic photophosphorylation was operational.

In contrast with this data, Hall, Edge, and Kalina (1971) presented results with spinach, the same material as that used in the above investigations. Using an electron stain assay (ferricyanide photoreduction) they concluded that...
PS II activity was not restricted to the grana. Goodenough and Staehelein (1971) found that lack of the $160 \pm 10$ Å particles in Chlamydomonas mutants did not result in the disappearance of PS II activity, but absence of the particles was connected with inhibition of the membrane stacking process. The aim of the present study was to contribute to solving the controversy of particle function by investigating membrane structure and photosynthesis in mutant plastids of Tradescantia.

2. Material and Methods

2.1. Material and Sampling

Investigations were carried out on variegated leaves and on plastids isolated from normal (green) and mutant (light green/cream) strips of Tradescantia albiflora cv. aureo-vittata Kunth em. Brünn (Encke 1958). When isolated plastids were examined by freeze-etching, only young leaves were used as a source of organelles, since their mutant segments contain plastids both with and without macrograna (Keresztes 1971, Keresztes and Faludi-Daniel 1973). In the case of examination of intact leaf pieces or homogenates by low temperature fluorescence, oxygen evolution, P$_{700}$ photo-oxidation, and $^{14}$CO$_2$ incorporation, old mutant segments with plastids lacking macrograna were also used for comparison.

2.2. Freeze-Etching

Normal chloroplasts were isolated by grinding the leaf pieces with a chilled pestle and mortar in a small volume of cold 0.01 M Tris-HCl buffer (pH 7.9) containing 0.25 M sucrose, according to the rapid method of Nobel (1974). Mutant plastids were isolated in 0.5 M Tris-HCl buffer (pH 8.0) containing 0.5 M sucrose, 0.001 M MgCl$_2$ and 0.2% w/v bovine serum albumin (Jacobson 1968). The homogenates were fractionated using a bench-top centrifuge. Plastid pellets were resuspended in media containing the same ingredients as the isolation media but with sodium phosphate buffer replacing Tris (De Petrocellis, Sikeevitz, and Palade 1970). Plastids were fixed by addition of an equal volume of 2% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.9) adjusted in composition to the above media, for at least 30 minutes at room temperature. Plastids were washed in 2 changes of 0.1 M sodium phosphate buffer (pH 7.9), followed by addition of an equal volume of 50% glycerol as cryoprotectant.

Freeze-etch replicas were made according to Moor and Mühlenthaler (1963) using a Balzers BA 360 M unit. Specimens were etched for 1 minute at $-100\, ^\circ$C. The thickness of the platinum-carbon layer was 20 Å, controlled by a quartz-crystal monitor QSG 201. Replicas were cleaned using 5% sodium hypochlorite solution followed by 70% sulphuric acid (3 hours each), and examined in an AEI Corinth 275 electron microscope. Measurements were carried out on enlarged micrographs using a micrometer slide with a 2 mm scale in a Zeiss stereomicroscope. The width of the particles was measured at the shadow line.

2.3. Low Temperature Fluorescence

The light source was a high pressure mercury lamp (HBO 200) with cut-off filters BG 7 and BG 12 transmitting the 435 nm line for fluorescence excitation. The leaves were placed in a plexiglass sample holder and immersed in a transparent Dewar flask filled with liquid nitrogen. The fluorescent light was focussed on the entrance slit of a Hilger and Watts D 330...