Changes in Envelope Permeability during Chloroplast Development *

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Summary. The permeability of the plastid envelope during the development of *Avena sativa* plasts was investigated by light scattering and uptake of various labelled compounds (malate, succinate, glutamate, α-ketoglutarate, citrate, glycine, sucrose). The results presented show that a primary event during greening is a change in permeability, thereby allowing an increased transport of metabolites across the membranes of very early etio-chloroplast stages. The results are discussed in view of an adaption of the plastid envelope permeability to the changing requirements of externally synthesized precursors and intermediates during development.

Introduction

Chloroplasts possess two membrane systems; the bounding double-membrane system of the plastid envelope, and the inner chlorophyll-containing lamellae. Although plastid lamellae have been investigated in detail much less is known of the structure and function of the plastid envelope.

The plastid envelope, separating the stroma from the cytosol, appears as two distinct membranes in electron micrographs. Freeze-etch studies have shown morphological differences between the two membranes (Bisalputra and Bailey, 1973). The outer membrane is thought to be freely permeable to metabolites of low molecular weight whereas the inner membrane functions as the metabolic barrier between the cytoplasm and the chloroplast (Heldt and Sauer, 1971).

In a recent study Cobb and Wellburn (1974) observed a primary light-dependent and cytoplasm-dependent increase in both total plastid protein and envelope associated protein during greening of *Avena sativa* laminae. The authors suggest from this that the envelope structure is altered during the process of greening.

This paper demonstrates that the alteration of the envelope structure during development is also expressed by an alteration of the permeability of the plastid envelope due to the changing requirements of externally synthesized precursors and intermediates of the plastid throughout its development.

Methods

Preparation of Etioplasts and Etio-chloroplasts

Nine-day old *Avena sativa* L. seedlings were grown in the dark or partially illuminated (metal halide lamp, Osram, 400 W, providing 6,000 lx) in the later stages of growth in a moist peat/permutite mixture at 25°C. Intact isolated etioplasts or etio-chloroplasts were prepared from the laminae by a loosely-packed Sephadex method (Wellburn and Wellburn, 1971), applying the following modifications: The laminae were homogenized in a medium containing 0.45 M sorbitol, 0.05 M MES, pH 6.1, 0.001 M NaNO₃, 0.001 M MgCl₂, and 0.001 M KH₂PO₄. The column medium contained 0.05 M HEPES, pH 7.3, instead of MES. The complete isolation was carried out within 45 min.

Measurement of Chloroplast "Transmission" Changes

Changes in "transmission" at 535 nm of plastid suspensions were measured at intervals from 1 s to 10 s. The transmission of a sample [2.5 ml of isolation medium with an osmolarity of about 0.5, containing plasts corresponding to a protein content of about 1 mg or to a plastid number from 0.8 × 10⁶ (dark grown) to 0.3 × 10⁶ (72 h light)] was recorded in a 1 cm cuvette. Small amounts of 2.0 M solutions of the substances tested were added to give a final concentration of 0.2 M and the change in transmission recorded in comparison to a control to which the same amount of aqua dest. was added.

Measurement of Penetration of Labelled Compounds across the Plastid Envelopes

a) Condition of Incubation. The incubations were carried out at 4°C in the same medium as that used for the Sephadex column,
containing plastids corresponding to about 2 mg protein/ml. The final concentration of the substances tested was 5 mM.

b) Filtering Centrifugation. The penetration of labelled compounds across the plastid envelope was measured by filtering centrifugation (Klingenberg and Pfaff, 1967). The uptake of labelled compounds was determined by counting the sediment and 100 μl aliquots of the supernatant after rapid centrifugation of the plastids through an inert layer of silicone oil. The centrifugation was carried out with the Beckman minifuge 152 (15 s). Centrifuge tubes (0.5 ml) contained 50 μl HClO₄, 10%, 100 μl silicone oil (Type AR 150, Wacker Chemie, München, F.R.G.), and 200 μl suspension of plastids. Plastid spaces in the sediment were calculated from the amounts of tritiated water (water space) and of the non-permeating (14C) sorbitol (sorbitol space) to correct unspecific permeation into the inner membrane space. Dextran was used (2 mg/ml) to correct for medium adhering to the outer surface of the plastid (see Heldt and Sauer, 1971).

The amount of protein was measured according to Lowry et al. (1951).

The degree of integrity of the plastids was determined by phase contrast microscopy. About 70-90% of the plastids were found to be intact at the end of the different incubation times.

![Fig. 1a-f. Kinetics of the changes in absorbance at 535 nm, called forth by the addition of glutamate (a), α-ketoglutarate (b), succinate (c), citrate (d), malate (e), and glycine (f) (final concentration 0.25 M) to intact plastids. The osmolarity before addition was 0.5; x-x etioplasts; ○-○ etio-chloroplasts (24 h light); ●-● etio-chloroplasts (72 h light)](image)