Distribution of Chloroplast Coupling Factor \((\text{CF}_1)\) Particles on Plastid Membranes during Development

A.R. Wellburn

Department of Developmental Biology, Research School of Biological Sciences, Australian National University, P.O. Box 475, Canberra, A.C.T. 2601, Australia

Abstract. Samples of internal membrane systems separated from lysates of intact plastids from dark grown Avena sativa L. (vars, Cooba and Mostyn) and Hordeum vulgare L. (vars, Himalaya and Deba Abed) given different periods of illumination before isolation were assayed for trypsin-activated Ca\(^{2+}\)-dependent ATPase activities and also examined in the electron microscope after treatment in the manner described by Oleszko and Moudrianakis (1974) which assists the visualization of the chloroplast coupling factor \((\text{CF}_1)\) particles. Concentrations of membrane-attached CF\(_1\) particles were not observed on the membrane surfaces of the prolamellar bodies (PLBs) proper but only on the attached extruded lamellar membranes. Increasing lengths of illumination followed by plastid isolation and subsequent membrane separation had the effect of progressively increasing the mean distance between these individual lamellar-attached CF\(_1\) particles. Measurements of trypsin-activated Ca\(^{2+}\)-dependent ATPase activities during similar developmental regimes indicated that functions associated with CE\(_1\) particles are relative constant and largely independent of the period of illumination if the values were expressed on a per plastid basis indicating that assembly of CF\(_1\) particles may take place in either etioplasts, etiochloroplasts or mature chloroplasts.

Key words: Avena — CF\(_1\) particles — Etioplasts — Hordeum — Prolamellar bodies — Trypsin-activated Ca\(^{2+}\)-dependent ATPase.

Introduction

Unlike the mitochondrial coupling factor \((\text{F}_1)\), the chloroplast coupling factor \((\text{CF}_1)\) only exhibits high ATPase activity after treatment with trypsin (Vambutas and Racker, 1965) and is a cold labile Ca\(^{2+}\)-dependent protein which can be removed from membranes by 1 mM EDTA and is inhibited by the antibiotic Dio-9 (McCarty, Guillory and Racker, 1965). It has been purified and its physicochemical properties determined (for the most recent review see Nelson, 1976). The in vivo location of CF\(_1\) in the plastid membrane system is of interest. On the basis of correlation between loss of particles and concomitant loss of photophosphorylating activity, a useful procedure for the electron microscope involving visualization by staining with aqueous uranyl acetate has been developed (Oleszko and Moudrianakis, 1974) to complement previous negative stain and freeze-etch procedures.

By comparison with mature chloroplasts, little study has been devoted to etioplasts and etio-chloroplasts with respect to CF\(_1\) location and synthesis. Coupling factor may be isolated from etioplasts which when added to CF\(_1\) depleted chloroplast membranes will restore phosphorylation (Lockshin et al., 1971; Horak and Hill, 1971) and inhibitor experiments suggest that both the cytoplasmic and chloroplastic ribosomes are involved in the synthesis of CF\(_1\) (Horak and Hill, 1972) during chloroplast morphogenesis. By contrast, Gregory and Bradbeer (1975) in a study of light-triggered dithiothreitol-activated Mg\(^{2+}\)-dependent ATPase of Phaseolus plastids concluded that most or all of the chloroplast ATPase was also present in the etioplast (i.e. light-independent synthesis) and this is in complete agreement with the observations of Lockshin et al. (1971) for trypsin-activated Ca\(^{2+}\)-dependent ATPase formation in Zea plastids.
In an attempt to elucidate these discrepancies a parallel study of the trypsin-activated Ca\(^{2+}\)-dependent ATPase activities in relation to an ultrastructural survey employing the CF\(_1\) visualization procedure of Oleszko and Moudrianakis (1974) was carried out and the results are reported below.

**Materials and Methods**

Preparations of prolamellar bodies, with their associated stromal lamellae, were made from lysed isolated Arena sativa L. (var. Cooba) and Hordeum vulgare L. (var. Himalaya) etioplasts according to the differential centrifugation method described earlier (Wellburn et al., 1977). Exactly the same procedure was adopted to prepare complete internal plastid membrane systems from lysed 1–72 h etio-chloroplasts from appropriate oat and barley laminae. Routine electron microscopy was carried out to monitor the various preparations (also as described earlier) with the inclusion of the pre-stain procedure of Oleszko and Moudrianakis (1974) to enhance the visualization of membrane-bound coupling factor (CF\(_1\)) particles. Micrographs (magnified 60,000 times when printed) were taken of these membrane systems external to any prolamellar bodies for each developmental stage. Lengths of membranes shown to have been sectioned at right angles to the normal plane were measured using a suitably calibrated map measuring device and the number of particles (not < 500) within the size range 8–12 nm were counted along the length of these membranes. From such measurements a value for the mean distance between CF\(_1\) particles was established for each developmental stage in both species.

Repeated estimations of trypsin-activated Ca\(^{2+}\)-dependent ATPase activities of similar preparations, the equivalent supernatant fractions and also of total plastid lysates from Arena sativa L. (var. Mostyn) and Hordeum vulgare L. (var. Deba Abed) were carried out using the McCarty method as described by Ryrie and Jagendorf (1971) but with 6 mM EDTA instead of 2 mM EDTA in the initial incubation medium and trypsin inhibitor from soya bean (12,000 units of activity raph-1, Calbiochem) to stop the trypsin activity after 15 min incubation at 20°C. Released inorganic phosphate was determined by the method of Taussky and Short (1953) and total protein by the Lowry et al. (1951) method. Control estimations of released inorganic phosphate by these preparations in the absence of trypsin, Ca\(^{2+}\) or ATP, plus combinations of these values of released phosphate (less ATP) were subtracted from estimations of the complete system to give the values quoted below.

**Results**

Earlier studies (Wellburn et al., 1977) have already indicated the remarkable concentration of coupling factor (CF\(_1\)) particles on the stromal membranes of detached PLBs. Figures 1–3 show representative micrographs of a study of the changes in the distribution of CF\(_1\) particles over the whole process of chloroplast development. When low centrifugal forces (3000 x g, 10 min) were employed in the isolation procedure, subsequent micrographs show that whole interconnected membrane systems containing several developing granal systems, rather than fragments of separated membrane systems, were isolated from lysed etioplasts and 1–72 h etio-chloroplasts from both Arena and Hordeum laminae. The majority of these stromal lamellae systems up to and including those from 8 h etio-chloroplasts show evidence of dispersing prolamellar bodies, albeit of a progressively vestigial nature.

Figures 1A and 1C demonstrate the concentration of CF\(_1\) particles of *Arena* and *Hordeum* on the extruded stromal membranes attached to PLBs. Usually these lamellar systems appear in the form of separated vesicles around the periphery of prolamellar bodies but most sections also show a continuation of this membrane with that of the prolamellar body proper. The vesicular appearance, rather than that of two appressed membranes, is much commoner and is probably due to a deficit of external osmotic pressure and a natural tendency to assume the shape of lowest entropy. Nevertheless, appressed membranes are often observed and illustrate more dramatically the concentration of CF\(_1\) particles; although usually they appear to be in the process of conversion to the vesicular form. This phenomenon is well illustrated by Figure 1B. At the transition points from the appressed to the vesicular form there is a constriction of the membranes implying a pinching off of membranous material to release smaller detached spherical vesicles studded with CF\(_1\) particles.

Examination of prolamellar body structure, at high magnification, shows little evidence for the presence of attachment of CF\(_1\) particles to lattice membranes. Observation of such particles is hampered by the presence of larger ribosome-like particles within the prolamellar bodies. Treatment of such preparations with EDTA at concentrations greater than 0.6 mM or ribonuclease causes the loss of these larger particles (Wellburn et al., 1977) and a micrograph of the latter treatment is shown in Figure 1D. No evidence for CF\(_1\) particles is to be found on the lattice membranes although the adjacent lamellar membranes still retain CF\(_1\) particles after such digestions. An interesting additional observation is that the osmiophilic globules, even when they occur deep in prolamellar bodies, are always studded with attached particles (Figs. 1A, C and D; Fig. 2A) of similar size to the CF\(_1\) particles but the relationship or significance is unknown.

Progressive increase in distance between CF\(_1\) particles on stromal membranes during development was observed. Figures 2 and 3 showing material from 1–72 h etio-chloroplasts, demonstrate this dispersion for *Arena* but Table 1 summarizes more accurately this phenomenon for both *Arena* and *Hordeum*. So close together are the particles in the etioplast stage that it was extremely difficult to establish these mea-