Biochemical Studies on the Iojap Mutant of Maize

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ABSTRACT

The white leaf tissue of seedlings of Zea mays L. affected by the recessive nuclear gene iojap shows no photosynthetic activity; it contains about 1.4% of carotenoid and less than 0.1% of chlorophyll a content of normal green tissue. Neither fraction I protein nor chloroplast adenosine triphosphatase (EC 3.6.1.4) (CF₁) is detectable. This confirms earlier observations that plastids of white sectors of iojap maize do not contain ribosomes. About 40% of the activity of phosphoenolpyruvate carboxylase (EC 4.1.1.31) in green leaves could be found in white leaves indicating that the phosphoenolpyruvate carboxylase EC 4.1.1.31 is made on cytoplasmic ribosomes. The oxygen consumption of iojap-affected leaves is decreased.

The nuclear gene ij₂ of Zea mays L. induces in homozygous condition plastid alterations (7) which are inherited in a uniparental mode and independently of the genetic constitution of the nucleus (13). The leaves of the affected plants are green, green-white striped, and white. Cytological investigations (13) showed morphological changes in the plastids of white tissue which have been studied in more detail with electron microscopy by Shumway and Weier (14).

The plastids of the white tissue are much smaller than the normal chloroplasts in green mesophyll or bundle sheath cells. Their matrix contains DNA fibrils and membranous structures, but grana thylakoids are not formed. Interestingly, no ribosomes have been observed in the affected plastids (14). The absence of ribosomes in altered plastids was recently confirmed by Walbot and Coe (15). They assume that the nuclear ij gene does not induce the loss of plastid ribosomes via a mutation of plastid DNA since no major changes in the plastid DNA of white tissue could be observed by restriction enzyme analysis (15).

The aim of the biochemical investigations reported in this communication is a further attempt to find out what physiological changes are caused by the ij gene.

MATERIALS AND METHODS

Seedlings of +/ij maize were grown in a greenhouse. The seeds were obtained from E. H. Coe, Jr., Columbia, Missouri. For most of the experiments we used green and pure white leaf tissue of +/ij seedlings which are phenotypically green, pure white, or cleanly striped. The white plastids are the result of the action of the homozygous ij gene during the previous generation.

In the measurement of PEP carboxylase activity, white (ij/ij) and green (ij/ij) tissue were used carrying genetic modifiers which lead to extreme expression of the ij character. The nuclear background of these plants was 50% inbred line Oh51A (cf. 15). Seedlings were harvested for all investigations 7–10 days after sowing. Pure white leaf material used for biochemical studies has been shown to contain no green cells as far as can be seen by light microscopy and to lack photosynthetic activity by measuring the delayed light emission with a phosphoroscope (5).

Fraction I Protein. Soluble proteins were extracted from leaves by homogenization of 1 g leaf material in 3.5 ml 50 mM Tris-glycine buffer (pH 8.9) containing 0.1% sodium ascorbate, 0.05% sodium diethylthiocarbamate and 0.5% PVP 10 (Serva, Heidelberg). The suspension was passed twice through a 80-µm nylon sieve and centrifuged at 4,000 g for 10 min. The supernatant fraction was centrifuged at 20,000g for 45 min. The supernatant fraction of the second centrifugation containing the soluble proteins was subjected to crossed immunoelectrophoresis after a 2-fold concentration by filtration through Diaflo ultrafilters PM 30 (Amicon, Oosterhout, Holland). Crossed immunoelectrophoresis of soluble protein was carried out as described (12).

Chloroplast ATPase (CF₁). The leaf material (2 g) was homogenized in 10 ml phosphate buffer (pH 7.9) containing 0.6 M sucrose and 10 mM MgCl₂. The homogenate was centrifuged at 1,000g for 2 min. Plastids were sedimented from the supernatant fraction by centrifugation at 4,000g for 10 min (1). The resulting pellet consisting of intact (about 40%) and broken plastids was washed three times with twice-distilled H₂O. All steps were at 4° C. The stroma-free plastid membranes were stirred for 20 min with 20 ml EDTA (pH 8.0) at room temperature (cf. 16). After centrifugation at 20,000g for 1 h, the supernatant fraction was concentrated 10-fold by ultrafiltration using Diaflo ultrafilters PM 10 (Amicon, Oosterhout, Holland). Electrophoresis was carried out according to the method of Börner et al. (1). The gels were stained for ATPase activity by incubation in 0.1 mM Tris-glycine, (pH 8.0) 5 mM ATP and 50 mM CaCl₂ for 1 h (6).

PEP Carboxylase. Leaf material (1 g) was homogenized in 5 ml 100 mM Tris-HCl (pH 7.5), 4 mM EDTA, and 0.2 mM cysteine. Homogenate was centrifuged at 40,000g for 20 min (11). PEP carboxylase activity was measured in the supernatant fraction following the oxidation of NADH at 340 nm in a Beckman DB-G recording spectrophotometer. Incubation mixture (3 ml) contained 15 µM KHCO₃, 22.5 µM MgCl₂, 0.6 µM NADH, 12 µM PEP and 6 units malate dehydrogenase (Boehringer, Mannheim) in 0.3 ml 1 M Tris-HCl (pH 7.8) and 25 to 50 µl of the supernatant fraction (11).

Absorption Spectra. Absorption spectra of intact leaves were measured with a recording spectrophotometer using the opal glass method (9).

Oxygen Consumption. Mitochondrial activity was determined by measurement of the O₂ consumption of intact leaf pieces. Leaf tissue (50 mg) was cut into small pieces with a razor blade. The pieces were incubated at room temperature in darkness for 3.5 h in 10 ml 0.25 mM NaHCO₃ (pH 6.6), 5 mM KCl and 0.1 mM

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2 Abbreviations: iojap: ij, iojap-affected; +/ij; PEP carboxylase: phosphoenolpyruvate carboxylase (EC 4.1.1.31).
CaSO₄. The leaf pieces were infiltrated with this buffer 0.5 h before measurement. Assay mixture (3 ml) contained 0.65 nmol NaHCO₃, 0.26 nmol CaSO₄, and 13 nmol KCl. Reaction was started after raising the NaHCO₃ concentration to 1.05 nmol in order to inhibit photorespiration. Temporal change of the O₂ concentration was determined by a Clark type electrode. Measurement was carried out first in darkness and then in light (effective 40,000 lux) at 25 C for 20 min until O₂ consumption became linear.

RESULTS AND DISCUSSION

Green and white tissue of +/ij maize were assayed for the presence of fraction I protein and coupling factor CF₁ since subunits of both proteins are made on chloroplast ribosomes (2, 10). Therefore, these proteins can be used as indicators of the function of the protein-synthesizing system of plastids. Fraction I protein can be detected by means of reaction with antibodies against fraction I protein from barley in crossed immunoelectrophoresis (Fig. 1a). Extracts of soluble proteins from barley leaves contain more fraction I protein (Fig. 1c). The comparable low content of fraction I protein in maize leaves was expected, because the gene for the large subunit of fraction I protein which is localized in plastid DNA is expressed only in the bundle sheath cells since maize is a C₄ plant (8). The possibility of bad solubilization of fraction I protein from bundle sheath cells was not tested. No fraction I protein could be detected in the white tissue of +/ij maize (Fig. 1b). The method applied would allow us to find 1% or less of the normal content of fraction I protein (12).

The plastid coupling factor CF₁ was removed from prewashed plastid membranes by treatment with EDTA, and the EDTA extract was concentrated about 10-fold by ultrafiltration before electrophoresis (1). After electrophoresis, the gels were stained for ATPase activity (6).

In the case of green +/ij plastids, an intense white band indicative of ATPase was observed whereas no ATPase activity was found in EDTA extracts from white +/ij tissue (Fig. 2). According to the sensitivity of the method we can assume that the +/ij tissue contains less than 5% of normal content of ATPase activity. This ATPase shows exactly the same electrophoretic mobility as purified coupling factor CF₁ from Vicia faba, and the EDTA extract from maize chloroplast membranes reacts with antiserum against purified coupling factor CF₁ from V. faba in immunoelectrophoresis (1). The ATPase accounts for 80% of the total protein in the EDTA extract. We concluded that the ATPase isolated from the maize plastid membranes is identical with the coupling factor CF₁. The absence or, at least, the very low content of fraction I protein and of plastid coupling factor CF₁ in the white tissue of +/ij maize confirms an earlier electron microscopic observation that the white plastids of this mutant contain no ribosomes (14). White plastids of +/ij maize were reported to contain no rRNA and to have no ability to incorporate amino acids into protein (15).

In contrast to coupling factor CF₁ and fraction I protein, activity of PEP carboxylase could be found easily in +/ij (ij/ij) white leaves. The activity assayed in extracts of green (ij/ij) leaves was 1.45 ± 0.16 μmol/min·g tissue (on fresh weight base). Extracts of white leaves show an activity of 0.57 ± 0.1 μmol/min·g tissue corresponding to about 40% of the activity measured in green tissue. (An equivalent weight of leaves per ml was used to prepare each extract.) Since the plastid ribosomes are obviously missing in the altered plastids, this enzyme must be synthesized on cytoplasmic ribosomes.

The absorption spectra of white +/ij leaves show a very low amount of carotenoids and of Chl as compared with green +/ij leaves (Fig. 3). The height of the peaks at 482 nm and 670 nm corresponds to 1–2% of carotenoid content and to less than 0.1% of Chl a content of the control. Chl b could not be detected by

![Fig. 1. Crossed immunoelectrophoresis of soluble protein extracts from green +/ij (a), white +/ij leaf tissue of maize (b), and from barley (c) with antibodies against fraction I protein from barley. Approximately 10-μl extracts from equal amounts of leaves were loaded on each gel.](image-url)
h was measured in the dark. Due to photosynthetic O₂ development a lower value of 5.1 ± 0.02 nmol/50 mg tissue-h was observed in the light. Such a dark-light difference could not be found in white tissue. The measured O₂ consumption of mutant tissue was 3.3 ± 0.2 nmol/50 mg tissue-h in dark and light. Thus the mitochondria of the white tissue are much less active than those of green tissue. We are currently investigating whether this is a further direct effect of the mutation or a secondary effect caused by a shortage of substrate.

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LITERATURE CITED
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