

Light-induced transformation of amyloplasts into chloroplasts

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Plant Physiol. (1984) 75, 142-145 0032-0889/84/75/0 142/04/\$01.00/0 Light-Induced Transformation of Amyloplasts into Chloroplasts in Potato Tubers' Received for publication November 9, 1983 and in revised form January 19, 1984 YU S. ZHU, DENISE L. MERKLE-LEHMAN, SHAIN D. KUNG* Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, Maryland 21228 ABSTRACT The transformation of amyloplast into chloroplasts in potato (*Solanum tuberosum* L.) tuber tissue can be induced by light.

Excised potato tuber discs illuminated with white light of 3000 lux began to synthesize chlorophyll after a lag period of 1 day, and continued to synthesize chlorophyll for 3 weeks. In this paper we present evidence, based on ultracentrifugal sedimentation and immunoprecipitation, that the light-mediated synthesis of Ribulose-1, 5-bisphosphate carboxylase began 1 day after illumination with white light. When illuminated the chloroplasts isolated from light-grown potato tuber tissue incorporated ¹³S-methionine into polypeptides, one of which has been identified as the large subunit of Ribulose-1, 5-bisphosphate carboxylase.

These chloroplasts are functional as determined by O₂ evolution in the Hill reaction. A great deal of data is now available on the biochemistry of the development of chloroplasts from etioplasts (6). Much less is known about the development of chloroplasts from amyloplasts, a starch storing organelle, which exists in storage tissue, roots, and some callus. It is well known that potatoes turn green on exposure to light for several days.

Not only does the study of the organelle transformation and light-regulated gene expression stimulate theoretical interest, but the greening of potato also has some practical importance, since the greening of the potato is accompanied by the formation of the poisonous alkaloid, solanine (5). The morphological and ultrastructural changes in the development of chloroplasts from amyloplasts were described in several electron microscopic studies (3, 11). In this communication we present some biochemical evidence to show the function of chloroplasts from potato tuber based on the biosynthesis of Chl, RuBPCase, 2 and Hill activity. Tuber discs in sterile Petri dishes (9 cm), 40 discs per dish. The discs were illuminated at room temperature by fluorescent tubes with intensity of 3000 lux. Chlorophyll and RuBPCase Determination. Chl was determined according to Arnon (2). RuBPCase was detected with a Model E analytical ultracentrifuge, as previously described (9). Schlieren pictures were taken at 44, 770 rpm, 10 min after attaining this speed. An Ouchterlony double diffusion test was employed to determine the RuBPCase specifically. The antiserum was prepared against RuBPCase from tobacco (15).

Light-Driven Protein Synthesis in Chloroplasts. Ten g of green potato tuber discs which had been exposed to white light for 7 d were homogenized in a Waring Blendor with cold isolation buffer (sucrose 0.35 M, HEPES-NaCl 25 mm, EDTA 2 mM, isoascorbateNa 2 mm, pH 7.6) and filtered through 2 layers of Miracloth. The resultant filtrate was centrifuged at 30g for 1 min to remove starch granules, and the supernatant was then centrifuged at 2500g for 1 min. The pellet was resuspended in 1 ml of KCl suspension (KCl 0.2 M, Tricine-KOH 66 mm, MgCl₂ 6.6 mM).

The chloroplast suspension was transferred to a Petri dish (3 cm in diameter) and incubated with [¹³⁵S]methionine (10 , uCi) at room temperature under white light (4000 lux). During a 2 h incubation, the incorporation of [³⁵S]methionine into proteins was measured. After the 2 h incubation the radioactive polypeptides were separated on an SDS-polyacrylamide gradient gel (815%), followed by fluorography as described by Blair and Ellis (4). 02 Evolution Assay. The ability of chloroplasts from potato tuber discs to evolve 02 was measured in the Hill reaction according to the procedure of Marsho et al. (12). The incubation system consisted of ferricyanide 1. 7 mm, methylamine 3 mm, glyceraldehyde 10 mm, and chloroplasts corresponding to 15 yg of Chl in a total volume of 0. 6 ml. The reaction was initiated by irradiation with red light (22. 4 mw/cm²). The 02 evolution was measured and recorded with a polarograph. RESULTS MATERIALS AND METHODS Chlorophyll Synthesis in Light-Illuminated Potato Tuber. Our Preparation, Culture, and Light Treatment of Potato Tuber experiments showed that the greening of potato tuber depended Discs.

Centennial and Katahdin, two varieties of potato tubers on varieties, storage temperature, light intensity, and wavelength supplied by the United States Department of Agriculture were of light. Out of more than ten varieties of potatoes tested, two used in this study. The tubers were peeled and sterilized with varieties, Centennial and Katahdin, which turn green more Amphyl (National Laboratories, Lehn and Fink Industrial Prod- quickly under light, were selected in this study. Storage of potato ucts Division of Sterling Drug Inc. , New Jersey).

Discs (10 x 2 tuber below 4°C retarded or inhibited the transformation of mm) were made with a sterile cork borer and a gel slicer. These amyloplasts into chloroplasts. Blue light was most effective in discs, after washing in sterile water, were placed on 1% agar inducing greening of potato tubers. No red light stimulation was observed. Potato tuber discs illuminated with white light began to green ' Supported by National Institutes of Health grant CM22746-01 and with a lag period of about 1 d. The greening continued for 3 United States Department of Agriculture agreement 58-32044157. 2

Abbreviations: RuBPCase, ribulose-1, 5-bisphosphate carboxylase; weeks after culture (Fig. 1). After illumination for 3 weeks, the potato tuber tissue contained 10 Ag Chl/g of fresh tissue, about LS, large subunit. 142 TRANSFORMATION OF AMYLOPLASTS IN POTATO TUBERS 143 14 oct[L] 15 - a) 121 10 101 5 C-) 8 x 0 > ct [D] Days After Illumination FIG. 1. Time course of the synthesis of chlorophyll in potato tuber discs during continuous illumination with white light. IAt [D] By 0 15 I AAt [L] i _x- 60 120 90 Time (min) FIG. 3. Incorporation of [35S]methionine into proteins in the lightdriven chloroplast protein synthesis system. -M1 samples were added to 20% TCA containing 10 tg/ml unlabeled methionine. Proteins were precipitated with 5% TCA containing 10 Mg/ml unlabeled methionine, and counted with a Mark I scintillation counter. Ct, chloroplasts; At, amyloplasts (control); L, light-incubated; D, dark-incubated. FIG. 2. Biosynthesis of RuBPCase in potato tuber tissue as assayed by Ouchterlony double diffusion 0 h (1), 6 h (2), 12 h (3), 24 h (4), 48 h (5), and 72 h (6) after illumination. The central well contained antibody to LS. One g of potato tuber discs was homogenized with 0. 1 ml of Tris 80 mm, MgCl₂ 20 mm, KCl 40 mM, pH 8. . After centrifugation

at 12, 000g for 10 min, 20 ml of the supernatant were used for assay of RuBPCase. hundredth of the Chl content in normal leaves. This is primarily due to fewer chloroplasts per cell. The chloroplasts were not distributed uniformly in discs and were probably linked to some specific ultrastructure in the cortex. Microscopic observation revealed that the amyloplasts were comprised of two types: large (55 x 80 Mm) and small (5-20 Mum). Most of them were stainable with I₂-KI. Upon exposure of the discs to light the small amyloplasts only turned greenish.

RuBPCase Biosynthesis in Potato Tuber during Greening. Since RuBPCase is the most abundant soluble protein in the chloroplasts (10), it can be used as an important biochemical marker of chloroplasts. The de novo synthesis of RuBPCase was clearly demonstrated by ultracentrifugal sedimentation (data not shown) and specific immunoprecipitation (Fig. 2). The Schlieren pattern of extracts from normal leaves consists of four peaks representing 80S cytoplasmic ribosomes, 70S chloroplast ribosomes, 18S Fraction I protein (RuBPCase), and 4 to 6S Fraction II proteins (8, 9).

The extract from potato tuber stored in the dark lacked the peak of RuBPCase, whereas a small peak was observed in discs exposed to 3 d of light, indicating the light one initiated the synthesis of RuBPCase (data not shown). The biosynthesis of RuBPCase was also demonstrated by an Ouchterlony double diffusion assay (Fig. 2). The light-mediated synthesis of RuBPCase started at 1 d after illumination and increased during greening. The RuBPCase content was estimated to be -6 , ug/g fresh potato tuber

tissue, whereas the RuBPCase content of a typical green leaf is 5 to 10 mg/g fresh tissue (10).

Further evidence for the de novo synthesis of RuBPCase in light-treated potato tuber was obtained from the experiment on light-driven protein synthesis in chloroplasts. The chloroplasts, isolated from potato tuber discs after illumination for 7 d, exhibited a higher activity of protein synthesis, as demonstrated by the incorporation of [35S]methionine into proteins (Fig. 3). In contrast to light-driven protein synthesis in chloroplasts, the chloroplasts in the dark and especially amyloplasts, either in the light or dark, exhibited a very low protein synthesis activity.

A number of radioactive polypeptides synthesized in the chloroplasts in the light were recognized on SDS-polyacrylamide gels followed by fluorography (Fig. 4). It was observed that some chloroplast polypeptides (mol wt 39, 000, 50, 000, 52, 000, 55, 000, 94, 000, 96, 000) were synthesized more readily in the light than in the dark. One of these polypeptides co-migrated with purified unlabeled LS of RuBPCase (mol wt 52, 000), and was identified as the LS, based on this and results presented in Figure 2.

There is also one strongly light-initiated polypeptide (mol wt 64, 000) which does not appear in the dark. 02 Evolution of Chloroplasts from Light-Induced Potato Tubers. Upon illumination with red light, the chloroplasts, isolated from light-treated potato tuber discs after 3 weeks of light treat- 144 Plant Physiol. Vol. 75, 1984 ZHU ET AL. sure to light, the amyloplasts are transformed into chloroplasts, which have a different function. The changes

in ultrastructure of potato tuber amyloplasts during greening were investigated by electron microscope.

It was shown that the main developmental features were elongation of vesicles into thylakoids, the differentiation of grana and the appearance of ribosomes in the stroma (3), although the ultrastructure is generally less well developed compared to that for normal leaves. On the other hand, there is very little information on biochemical alterations in potato tuber during greening. To confirm the transformation of amyloplasts into chloroplasts, this study provides some biochemical evidence: (a) the synthesis of photosynthetic pigments, (b) the synthesis of RuBPCase and other proteins, and (c) Hill reaction activity.

Before illumination the potato tubers contain no Chl or Pchl, but do contain carotenoid which increases during greening (1). Very low light intensity (400 lux) was required to initiate greening. The light may penetrate into the potato tuber discs and evoke the development of chloroplasts from amyloplasts. During the development of chloroplasts from amyloplasts, assembly of Chl into the newly synthesized membranes occurs. Cold storage may cause the breakage of membranes (13); however, some studies suggested that amyloplast membranes remain intact in cold storage (14).

It was frequently observed that the chloroplasts were formed as streaks in the potato tuber discs, suggesting a special structure is linked to the chloroplast development. The Schlieren pattern of the extracts from light-induced potato tuber tissue as well as its immunoprecipitation reaction with antiserum to RuBPCase demonstrated that RuBPCase, an important enzyme

in the photosynthetic carbon cycle, was de novo synthesized during greening. This result was further confirmed by the active light-dependent protein synthesis in the isolated chloroplasts.

The biosynthesis of RuBPCase during greening suggests the involvement and operation of CO₂ assimilation in the chloroplasts. Furthermore, the photosynthetic function of electron transport and O₂ evolution in isolated chloroplasts from potato tuber was shown by the high Hill reaction activity. The transformation of amyloplasts into chloroplasts is absolutely light-dependent. The fact that red light did not stimulate this transformation indicates that phytochrome may not participate in this regulation. This coincides with the result that potato tuber does not have phytochrome (7).

What is the photoreceptor in this light-induced organelle transformation? How does light turn on the genes for the development of chloroplasts, and turn off the genes for the development of amyloplasts? Undoubtedly, this system provides an attractive and challenging model for investigations into the molecular mechanisms underlying the photoregulation of development and gene expression. FIG. 4. SDS-PAGE of light- (1) and dark- (d) initiated polypeptides synthesized in chloroplasts isolated from light-treated potato tuber discs. (R) photograph of stained gel. L) fluorograph of (R). Arrows indicate the mol wt of light-stimulated polypeptides and band corresponding to LS. Although the dark-incubated sample was more concentrated than the light-incubated sample (R), the fluorograph (L) indicates there was more incorporation of [35S]methionine into the light-induced polypeptides than into the dark-initiated polypeptides, demonstrating that more polypeptides

were synthesized in the light than in the dark. The samples were incubated with [³⁵S]methionine for 120 min. ment, evolved O₂ using ferricyanide as an electron acceptor.

This Hill reaction activity is comparably high (177 Amol O₂/mg Chl h), indicating that an active electron transport reaction took place in this chloroplast preparation. DISCUSSION The cells of potato tuber contain a large number of amyloplasts, whose function is to accumulate and store starch in the form of reserve starch granules. It is of interest that upon expo-

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