THE ACTION OF LIGHT AT THE STRUCTURAL PROTEINS LEVEL ON ETIOLATED PLASTIDS FROM *ZEA MAYS* L.

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1. Introduction

During the greening of etiolated leaves of higher plants exposed to light, chlorophyll appears after a lag phase of about 3 hr. Tracer amounts of chlorophyll, however, are now known to be synthesized from the beginning of illumination by a fast occurring reaction: the phototransformation, within a millisecond, of preexisting protochlorophyllide into chlorophyllide [1]. Nevertheless, the synthesis of new protochlorophyllide is the limiting step of this reaction, and takes place during the first 3 hr of illumination, but it cannot explain by itself all this lag phase of 3 hr.

Thus, little is known of the events which occur during this important phase, and practically nothing upon those which eventually affect the structural proteins of the plastid.

We describe here the disappearance, during the first hours of greening, of an important part of the sugars linked to these proteins.

Moreover, polyacrylamide gel electrophoresis has shown that some of these sugars are linked to the structural protein of the lowest molecular weight. The remaining sugars migrate faster and are not associated with a protein component.

Another protein component previously described [2], with a molecular weight of about 25 000, is present in small amounts in etiolated plastids but increases markedly within 15 hr of illumination, and becomes the main structural protein of the green plastids.

2. Material and methods

2.1. *Preparation of the structural proteins from plastids*

Seeds of *Zea mays* L., variety INRA 260, were germinated on vermiculite impregnated with distilled water in a dark room at 25°. After 10–12 days, a first leaf sample was taken, representing time 0 of the experiment, then the plants were exposed to continuous illumination of 3000 Lux, and samples were taken after 3, 5, 7, 9, 15 and 24 hr of exposure to light.

Structural proteins were extracted from each sample and solubilized as described elsewhere [2].

The chlorophyll/protein nitrogen ratios were determined on aliquots of the protein–pigment solutions by precipitation and extraction with acetone–water (80:20, v/v), until complete decoloration of the residue. A 505 Spectronic Baush and Lomb spectrophotometer was used to record the absorption spectra of the acetone solutions. MacKinney's formulae [3] were used to calculate the amount of chlorophyll. Protein nitrogen was determined by acid titration after wet ashing by the Kjeldhal method.

2.2. *Identification and evaluation of the content of sugars*

Neutral sugars were determined after hydrolysis of the proteins with 2 N HCl at 100° for 2.5 hr. The total content of hexoses was evaluated by a micro method [4] derived from the anthrone method of Shields and Burnett [5]. Determination of the different hexoses was performed by thin-layer chromatography on polycarbonate sheets. After specific color-

ation using TTC (triphenyl tetrazolium chloride), the spots were eluted and their absorbance recorded [6].

Hexosamines were also identified by thin-layer chromatography on polycarbonate sheets, followed by TTC treatment [6]. Here, a preliminary hydrolysis by 4 N HCl at 100° for 4 hr was required. Their total content was determined using a modified Elson–Morgan method [7, 8].

Sialic acids were investigated by the method of Svennerholm [9] and that of Jourdian [10], both on the proteins and on the purified hydrolysate.

Evaluation of galactose from galactolipids was performed as follows: lipids and pigments were extracted by the usual solvents [2]; the extract thus obtained was evaporated to dryness, and then saponified with 0.1 N sodium hydroxide in methanol at 37° for 15 min; the solution was further extracted according to the method of Dawson [11], and hydrolysed by 3 N HCl, for 90 min at 100°, as recommended by Rosenberg [12]. Galactose was then determined as above.

2.3. *Electrophoresis of the protein solutions*

Conditions for electrophoresis were essentially the same as described before [2], but gels were increased to 80 mm in length and 12 mm in diameter, allowing up to 100 μl samples to be deposited.

Gels were stained for protein with Coomassie blue, and for carbohydrates by the Schiff–periodate procedure [13].

3. Results

Structural proteins isolated from the various samples have been investigated for their content of sugars: the longer the plants were illuminated, the lower was the percentage of linked sugars (fig. 1).

The amount of neutral hexoses bound to proteins decreased without a lag phase, and mainly during the first hours of illumination. This reaction occurred before the maximum synthesis of chlorophyll.

The evolution of the linked galactose, which is the prevalent hexose, is also shown in fig. 1. The behaviour of mannose was quite similar to that of galactose, and fits very well the difference between the data for total hexoses and the data for galactose.

Galactosamine is the only important hexosamine; its amount fell after 3 hr of illumination from an initial value of 0.83% of the structural proteins weight to a final value of 0.22%.

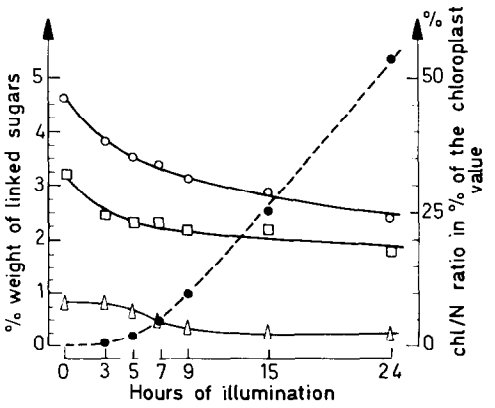

Fig. 1. Content of sugars linked to the structural proteins as a function of illumination time:
(•–•–•) Chlorophyll/protein nitrogen ratio expressed as a percent of the value for chloroplasts.
(○–○–○) Total neutral hexoses as percent of structural protein weight.
(□–□–□) Percent of linked galactose.
(△–△–△) Percent of linked galactosamine.

Colorimetric assays for sialic acids were not conclusive.

The disappearance of linked sugars is not the result of an increase in the amount of structural proteins since, in maize plastids, there is practically no modification in the level of structural proteins after 24 hr of greening [14], while such an explanation would imply a 2-fold increase in the content of these proteins.

It was of interest to know if these sugars were attached to one or more proteins, and what happens to them during greening.

Polyacrylamide gel electrophoresis of structural proteins from etiolated plastids, from plastids after 15 hr of greening and from green plastids, were compared (fig. 2). It can be seen from the results of fig. 1 that after 15 hr of illumination the percentage of linked sugars reached practically the lowest value.

Only two bands were stained by the Schiff-periodate procedure in all cases examined (fig. 2).

Staining with Coomassie blue shows that one of the glucidic bands is associated with the fast moving component (approx. M.W. 10 000 [2]), and that the other appears free of protein and moves faster than all protein species.

One protein species, of M.W. 25 000 [2], is mark-

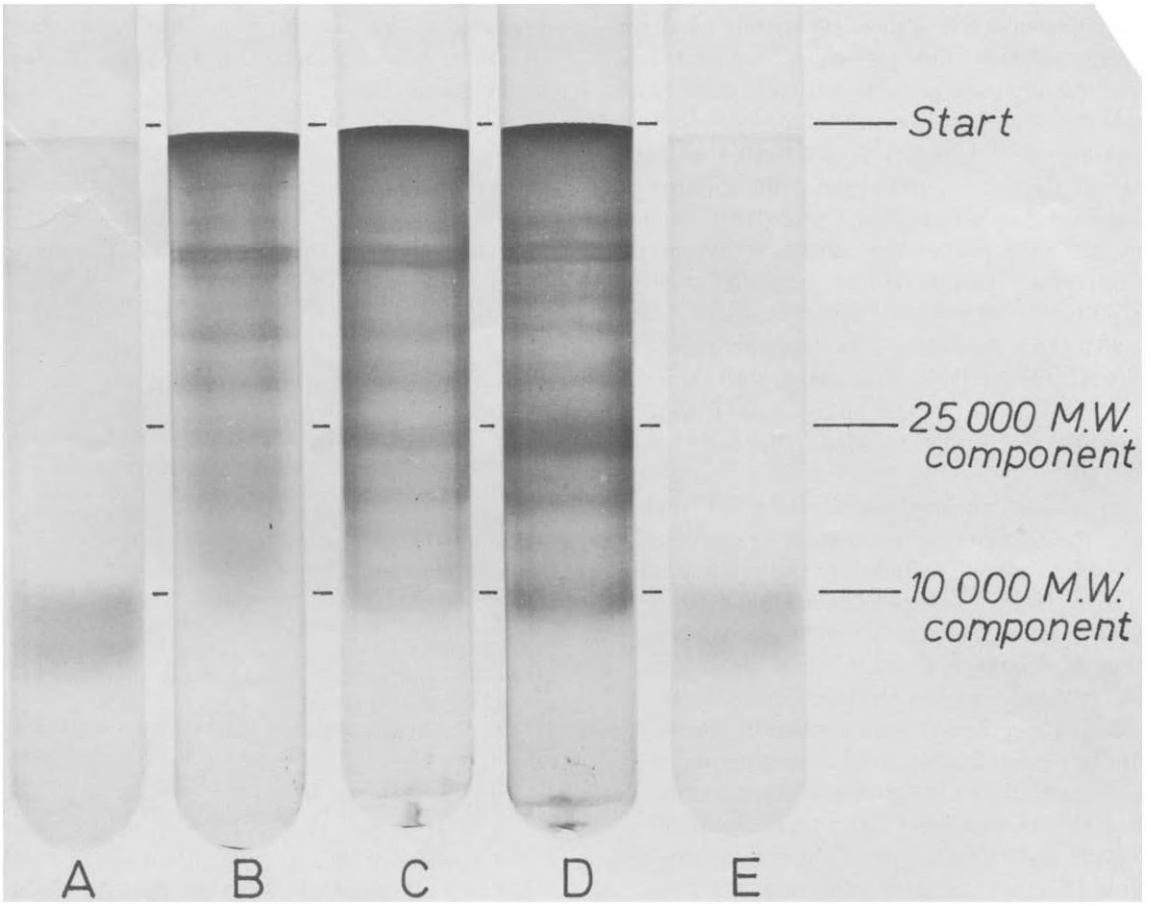

Fig. 2. Electrophoresis of structural proteins solutions in 10% polyacrylamide gels. Gels A and E were stained by the Schiff-periodate procedure; gels B, C and D were stained with Coomassie blue. The samples are: etiolated structural proteins (gels A and B), structural proteins after 15 hr of greening (gel C), structural proteins of chloroplast (gels D and E).

edly increased after 15 hr of greening, and becomes the main protein constituent of green plastids.

Since it is known that, for etiolated higher plants, the content of galactolipids increases after about 10 hr in the light, we also measured the level of lipidic galactose, before and after greening. This level, expressed as percent of structural protein weight, was found to increase from 6.3% in etiolated plastids to 8.0% in green plastids. This increase of 1.7% of the lipidic galactose is of the same order as the decrease in the galactose from structural proteins.

4. Discussion

Transformation of etioplast into chloroplast takes place within 24 to 48 hr, and the first lamellae appear after about 4 hr of illumination. For peas and beans, Roughan and Boardman have shown that, with the exception of chlorophyll, all lipid components needed to build the lamellar structures are present in the prolamellar body of the etioplast [15]. During the greening of etiolated plastids of maize, there is no rise in the amount of structural proteins, and no important specific incorporation of amino acids into these proteins [14]. It is possible to explain this by a reorganisation and perhaps some modification of preexisting proteins.

We observed that, among these structural proteins, a component with a molecular weight of 25 000 is markedly increased during greening. In chloroplasts, after electrophoresis of pigment–protein complexes, a part of the chlorophyll remains associated with this protein (unpublished results). So it is likely that this component plays an important part in the appearance of photosynthesis. Nevertheless, it seems that, qualitatively, almost all protein components are present in etiolated plastid. The protein with the lowest molecular weight is especially interesting; for etiolated plastids as well as for chloroplasts, its electrophoretic mobility is quite similar, it is associated with carbohydrate, probably by covalent linkages, and it also seems to bind specifically the same amount of magnesium [16].

Illumination of etiolated plastids induces a rapid falling of the amount of linked sugars. As a fraction of these sugars seem to be linked to the smallest protein, two explanations are possible for this decrease: the first one is a partial destruction of this component, but its staining by Coomassie blue after electrophoresis increases with greening; the second, more plausible, is a specific release of glucidic fragments.

If the latter explanation is right, we can imagine that such a transformation gives new properties to this protein: either enzymic activity, like thrombin which is obtained after release of sugars from prothrombin [17], or fixation of other structural components by the unmasking of important sites.

Another question is what becomes of the galactose thus liberated during greening. An important use would be the incorporation, in situ, in the newly synthesized galactolipids; indeed, the amounts of galactose involved in these two processes are quite similar, and, moreover, the increase of galactolipids occurs when the content of galactose liberated from the structural proteins is maximal.

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