PRE-EXISTENCE OF CHLOROPLAST LAMELLAR PROTEINS IN WHEAT ETIOPLASTS. FUNCTIONAL AND PROTEIN CHANGES DURING GREENING UNDER CONTINUOUS OR INTERMITTENT LIGHT

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1. Introduction

When etiolated plants are exposed to light, important structural and functional changes are noted in plastids [1–4]. The prolamellar bodies characteristic of etioplasts disappear, whereas lamellae and grana are differentiated.

For some investigators working on etiolated Euglena [5] as well as on higher plants [6, 7], the etiolated material exposed to light leads necessarily to a de novo synthesis of new proteins. For others, etiolated plastids contain all or at least a large part of the proteins necessary for the building of lamellae and grana [8] and consequently, it has been proposed that structural changes might be due to a reorganization of the existing protein molecules rather than to a de novo protein synthesis.

The purpose of this investigation was to determine whether the etioplast lamellar proteins are different from the chloroplast lamellar proteins and to study their evolution in relation with functional changes during the differentiation of etioplast into photosynthetically active chloroplasts.

The experiments were conducted with wheat greened under continuous or intermittent light. Greening under intermittent light was investigated because plastids of such leaves only contained "primary thylakoïds" and were devoid of grana [9]. Moreover it has been reported that these plastids do not exhibit any photosystem II (PS II) activity. During greening, PS II development was followed by dichlorophenol indophenol (DPIP) photoreduction and lamellar proteins were analysed with the acrylamide gel electrophoresis method.

2. Materials and methods

2.1. *Plant material*

Wheat plants (*Triticum sativum* L., var. Florence aurore) were cultivated for eight days in aquiculture on a standard Arnon and Hoagland nutritive solution [10].

Four kinds of plants were investigated:
i) Etiolated plants grown in complete darkness. ii) Plants grown under a 12 hr photoperiod at 4 000 lux. (control chloroplasts). iii) Plants grown 7 days in darkness and then submitted to increasing periods of continuous illumination at 4 000 lux. (from 1 to 24 hr). iv) Plants grown five days in darkness and then submitted to intermittent light: 1 msec flash every 15 min produced by a Braun Hobby EF 300 electronic flash tube delivering approx. 8.3×10^4 ergs cm^{-2} sec^{-1}.

The stage of greening obtained after 300 flashes corresponds to a plastids structural development and a foliar chlorophyll content similar to that observed after 4 hr under continuous light.

2.2. *Subcellular fractionation*

For chloroplast isolation, leaves were cut with scissors into small pieces prior to homogenization in a Waring Blendor at full speed, three times 5 sec. The grinding medium contained 5×10^{-2} M phosphate-buffer, pH 7.5; 10^{-3} M $MgCl_2$; 0.4 M Sorbitol.

The slurry was filtered through six layers of gauze and one layer of nylon cloth (blutex 50). The filtrate was centrifuged for 5 min at 200 *g*, a centrifugation of the supernatant at 1500 *g* for 10 min yielded the chloroplasts employed in our experiments.

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For etioplast isolation, a similar procedure was employed except that it was carried out under dim green light and the last centrifugation was performed at 2 000 *g* for 10 min.

2.3. *Measurement of DPIP photoreduction*

A Cary 14 spectrophotometer equipped with a scattered light transmission accessory was used to follow DPIP photoreduction by measuring the decrease of absorbance at 590 nm. The actinic white light was 80 000 lux. The reaction mixture employed: 50 mM of a Na_2HPO_4–NaH_2PO_4 mixture pH 7.5, 300 mM sucrose, 10 mM $MgCl_2$, 0.1 mM DPIP, plastids equivalent to 50 μg Chl per ml and where indicated 0.5 mM 1,5 diphenylcarbazide (DPC), 0.5 mM semicarbazide (SMC), 1 mM 3-[*p*-chlorophenyl]-1,1-dimethylurea (CMU).

2.4. *Plastid lamellar preparation and protein analysis*

The procedure for purification and solubilization of lamellar proteins and subsequent analysis by disc electrophoresis in acrylamide gels had been described in a previous report [11].

2.5. *Molecular weight estimation of the lamellar protein complexes*

An electrophoretic method according to Shapiro et al. [12] and to Weber and Osborn [13] was employed.

3. Results and discussion

3.1. *Development of photochemical activity*

3.1.1. Under continuous light

It has been previously reported that photosynthetic electron flow in isolated plastids is first detectable after 4 hr of greening under continuous light, after which it increases to a maximum rate at about 16 hr [14].

3.1.2. Under intermittent light

The only detectable photosynthetic activity in plastids isolated from leaves greened under intermittent light is the cyclic photophosphorylation with phenazine methosulfate, suggesting that only photosystem I (PS I) is functional [14]. Recently we have briefly described [15] that induction of PS II in isolated plastids can be obtained by submitting the previously flashed leaves to a short period of continuous illumination. In the same way, the appearance of an oxygen evolution with whole bean leaves has been reported by Strasser et al. [16–18]. We observed that an illumination as short as 5 sec given to seedlings after greening under intermittent light is sufficient to induce PS II activity. It is likely that no detectable protein synthesis occurs during this short post-illumination and that no new lamellae are formed. Therefore, PS II might be present in primary thylakoids but in an inactive form. As shown in table 1, the probable cause of deficiency is located on the water side of PS II. Effectively, electron donors to PS II such as diphenylcarbazide or semicarbazide are able to produce a CMU sensitive DPIP photoreduction with plastids isolated from leaves greened under a regime of 300 flashes. NADP can also be photoreduced by these plastids with DPC as electron donor.

Table 1

Photoreduction of DPIP with plastids isolated from wheat leaves greened under intermittent light (300 F = 300 flashes, 1 flash every 15 min); effect of 1,5-diphenylcarbazide (DPC) or semicarbazide (SMC).

Condition of greening	Electron donor	(μmoles DPIP reduced/hr/mg Chl)
Intermittent light 300 F	H_2O	0
	DPC	46
	SMC	25
	SMC (+ CMU)	0
Continuous light control	H_2O	80

These results suggest that:

i) Intermittent light is able to establish PS II in these plastids, but in an inactive form.

ii) The deficiency occurs between water and photosystem II.

3.2. *Development of lamellar proteins*

3.2.1. Lamellar proteins from light grown and etiolated wheat leaves

In a previous study [11] it has been reported that SDS solubilized lamellar proteins resolved to about ten protein components after polyacrylamide gel electrophoresis. It was suggested that protein components 3 and 8 of mature chloroplasts (see fig. 1d) might be the protein moiety of chlorophyll holo-

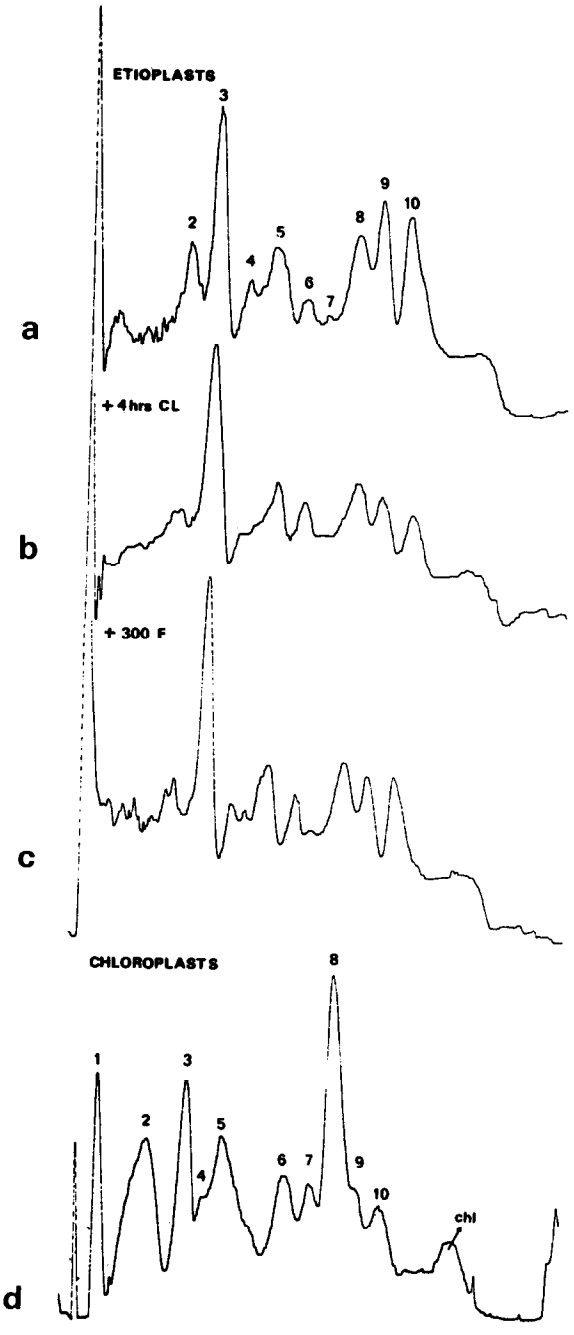

Fig. 1. Densitometric profiles of the protein pattern of gel electrophoresis from wheat lamellar proteins of: a) Etioplasts. b) Plastids isolated from seedlings greened under 4 hr continuous illumination. c) Plastids isolated from seedlings greened under intermittent light (300 flashes, one flash every 15 min). d) Mature chloroplasts.

chromes. Moreover protein complex 3 corresponded to lamellar fragments enriched on chlorophyll *a*, while complex 8 was rather characteristic of chlorophyll *b* enriched lamellar fragments.

These findings are in agreement with the recent reports of Machold et al. [19] and Herrmann and Meister [20] working respectively on Vicia and on Antirrhinum.

Fig. 1a shows a typical densitometric tracing of an etioplast lamellar protein gel electrophoresis. Fig. 1d shows the tracing obtained with lamellar proteins isolated from mature green chloroplasts. A comparison of these two tracings clearly indicates that no qualitative difference is observed between etioplast and chloroplasts, but only quantitative differences. For instance in mature chloroplasts, the strong development of proteins 1, 2 and especially 8 is observed. On the other hand, proteins 9 and 10 are represented in lower quantities in mature chloroplasts than in etioplasts.

3.2.2. Lamellar proteins from greening plastids

Fig. 1b shows the electrophoretic pattern of lamellar proteins isolated after 4 hr of greening. It is surprising to note that no significant change is observed compared with etioplasts, although there is a marked development of the lamellar system correlated with the onset of photochemical activity. It is only after 10 hr of illumination that a net increase of the protein 8 content in lamellar is observed. This protein (M.W. 23 500) is a complex with chlorophylls and in particular chlorophyll *b*. During greening protein 8 increases while proteins 9 and 10 decrease. The marked development of protein 8 in mature barley chloroplast has previously been mentioned by us [14, 21] and recently has been confirmed by Lagoutte and Duranton in maize [22]. In general a good correlation exists between the increase of this protein and the development of grana. Unpublished results with tobacco mutants devoid of grana, but possessing normal PS II activity, show that protein 8 is less developed in agranal than in granal chloroplasts.

Similar to what is observed after 4 hr of greening under continous light, greening under intermittent light (after 300 flashes) induces no qualitative detectable changes of lamellar proteins (fig. 1c). However, component 3 increases slightly due probably to the development of chlorophyll *a* holochromes. Since it is possible to produce the onset of Hill activity with an electron

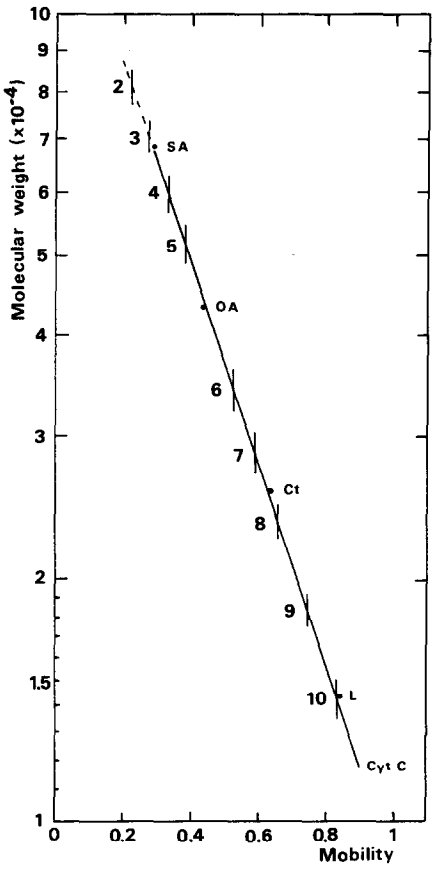

Fig. 2. Graph of electrophoretic mobility as a function of molecular weight. Migration of standard proteins is measured relative to that of bromophenol blue. Standard proteins used are: bovine serum albumin (SA) cryst. Mann, MW = 68 000; ovalbumine (OA) 2 × cryst., Sigma, MW = 43 000; chymotrypsinogen (ct) 6 × cryst., Sigma, MW = 25 700; lysozyme (L) 3 × cryst, Sigma, MW = 14 300; cytochrome *c* (cyt C) Calbiochem, MW = 11 700. Lamellar proteins are numbered from 1 to 10 as in fig. 1d. The molecular weight of component 1 has not been determined accurately enough to be retained.

donor to PS II, PS II is present in primary thylakoïds and it is likely that most of the lamellar proteins preexist in etioplasts.

3.3. *Molecular weight estimation of thylakoïd and prolamellar membrane protein components*

The linear correlation between molecular weights and electrophoretic mobilities is determined with five protein markers (fig. 2). The unknown molecular weights are easily extrapolated on the graph by plotting the calculated electrophoretic mobilities of the membrane polypeptides.

The averaged mobilities are calculated from 5 different densitometric tracings.

The thylakoïd polypeptides numbered from 1 (starting point) to 10 (fig. 1d for example) have molecular weights which spread between 81 000 for component 2 and 14 200 for component 10. Protein 8 has a molecular weight of 23 500.

The above listed values for wheat lamellar proteins are slightly lower than the values recently published for Antirrhinum [22] and maize [24].

In conclusion it seems at present well established that thylakoïd membranes are composed of heterogeneous protein complexes. Some are holochromes, others may be components of the electron transport chain.

It is noteworthy that no fundamental difference is detectable between lamellar proteins of etioplasts and lamellar proteins of photosynthetically functional plastids (greened after 4 hr of continuous illumination). Similarly, no difference between lamellar proteins of etioplasts and plastids greened under intermittent light is observed. This suggests firstly that protein components of PS I and PS II may be already present in etioplasts and secondly that membranes of "primary thylakoïds" are formed from a reorganization of preexisting proteins rather than from a de novo synthesis of new proteins.

But development of proteins 1, 2 and especially protein 8 observed only in later stage of greening (after 10 hr of continuous illumination) correlates well with the development of numerous granal formations.

The fact that plastids developed under intermittent light are able to photoreduce NADP or DPIP when an electron donor to PS II is supplemented, agrees with the hypothesis of a simultaneous formation of the two photosystems in thylakoïds in the course of greening. That PS II activity appears generally later than that of PS I may be due simply to a delay in the water splitting system activation.

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