In Situ Conversion of Protochlorophyllide b to Protochlorophyllide a in Barley

EVIDENCE FOR A NOVEL ROLE OF 7-FORMYL REDUCTASE IN THE PROLAMELLAR BODY OF ETIoplasts

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We recently put forth a model of a protochlorophyllide (Pchlide) light-harvesting complex operative during angiosperm seedling de-etiolation (Reinbothe, C., Lebedev, N., and Reinbothe, S. (1999) Nature 397, 80–84). This model, which was based on in vitro reconstitution experiments with zinc analogs of Pchlide a and Pchlide b and the two NADPH:protochlorophyllide oxidoreductases (PORs), PORA and PORB, of barley, predicted a 5-fold excess of Pchlide b, relative to Pchlide a, in the prolamellar body of etioplasts. Recent work (Scheumann, V., Klement, H., Helfrich, M., Oster, U., Schoch, S., and Rüdiger, W. (1999) FEBS Lett. 445, 445–448), however, contradicted this model and reported that Pchlide b would not be present in etiolated plants. Here we demonstrate that Pchlide b is an abundant pigment in barley etioplasts but is rather metabolically unstable. It is rapidly converted to Pchlide a by virtue of 7-formyl reductase activity, an enzyme that had previously been implicated in the chlorophyll (Chl) b to Chl a reaction cycle. Our findings suggest that etiolated plants make use of 7-formyl reductase to fine tune the levels of Pchlide b and Pchlide a and thereby may regulate the steady-state level of light-harvesting POR-Pchlide complex.

Angiosperms have developed sophisticated mechanisms to harvest sunlight and to convert this into various physiological responses (1). They make use of various photoreceptors, such as the red/far red-absorbing phytochromes, the blue light-absorbing cryptochromes, and the blue light-absorbing phototropins, to adapt to different light qualities and quantities and to sense the direction and duration of incident light (for a review, see Ref. 2). All of these photoreceptors are chromoproteins, which undergo characteristic spectral changes upon illumination.

Another blue and red light-absorbing protein-pigment complex is the protochlorophyllide (Pchlide)3 holochrome (3). It is localized in the prolamellar body of etioplasts. These plastids form when angiosperms germinate in darkness. The entire developmental process of seedling germination leading to prolamellar bodies is termed skotomorphogenesis or etiolation (1).

In plants displaying a hypogeous type of germination, it takes place underneath the soil. The newborn seedlings then utilize all nutrient reserves contained in the seed to bring the cotyledons above the soil.

Previous work has shown that the Pchlide holochrome is a higher molecular mass complex of about 600 kDa (3). More recent work suggested that it may be composed of galacto- and sulfolipids (4), Pchlide (5, 6), and an enzyme called the NADPH:Pchlide oxidoreductase (POR; EC 1.3.3.1) (7, 8). It was discovered that two distinct forms of POR exist in barley etioplasts, called PORA and PORB (9, 10). Moreover, two species of Pchlide have been distinguished in isolated prolamellar bodies by low temperature in situ fluorescence measurements: Pchlide 628/632 (the first number indicates the absorption maximum, the second the respective fluorescence emission maximum at the chosen excitation wavelength) and Pchlide 650/657 (for a review, see Ref. 11). Whereas the former remained quantitatively unchanged upon illumination with a single, 1-ms flash of white light, the latter was readily converted to Chlide 684/690. This differential behavior led scientists to name Pchlide 628/632 photoinactive and Pchlide 650/657 photoactive (summarized in Ref. 11). Both before and after flash light illumination, energy transfer was observed, taking place from photoinactive Pchlide to photoactive Pchlide in etiolated plants and from photoinactive Pchlide to Chlide in preflashed plants (12–17).

To resolve all of these puzzling previous observations, we proposed a model of a “light-harvesting POR-Pchlide” complex, named LHPP (18). Based on in vitro reconstitution experiments with synthetic zinc analogs of Pchlide, we put forth the idea that LHPP may be composed of 5 PORA-Pchlide b-NADPH ternary complexes and 1 PORB-Pchlide a-NADPH ternary complex embedded into the lipid bilayers of the prolamellar body of etioplasts (18) (see also Ref. 19 for a summary).

A particularly important question that had thus far remained unanswered was whether Pchlide b implicit in the LHPP model would be present in etiolated barley plants. Whereas previous work had indicated that Pchlide b is present in green plants (20), no comparable study had thus far been available reporting the identification of Pchlide b in etiolated plants, where the pigment, according to our in vitro reconstitution experiments (18), should be found in maximum levels. In a recent paper, Scheumann et al. (21) even generally questioned the existence of Pchlide b, but at the same time demonstrated that barley etioplasts rapidly convert exogenously added zinc protopheophorbide b (ZnPPb) to ZnPPa. This prompted us to conclude that Chl(ide) b reductase, presumably responsible for this conversion (22–26), could also metabolize...
the endogenously occurring Pchlide $b$ to Pchlide $a$.

In the present study, we readdressed the experimental design of Scheuermann et al. (21). We demonstrate that Chl(ide) $b$ reductase (which may alternatively be named 7-formyl reductase; see below) is indeed able to convert Pchlide $b$ to Pchlide $a$ in situ. This reaction already occurs upon plastid lysis and subsequent detergent solubilization of isolated prolamellar bodies. Both experimental steps lead to a denaturation of the prolamellar body and the release of the PORA and make Pchlide $b$ readily accessible to 7-formyl reductase. Our results provoke the idea that 7-formyl reductase may be involved in fine tuning the levels of Pchlide $b$ and Pchlide $a$ in etioplasts.

MATERIALS AND METHODS

Pigments—All glassware used throughout this study was pretreated with diethyl pyrocarbonate (DEP). This compound had previously been shown to inhibit bacterial and plant Rieske-type oxygenases (27, 28), to which Chlide $a$ oxygenase and related enzymes belong (29, 30). To block this activity seemed particularly necessary, in order to allow accurate determination of Pchlide $a$ and Pchlide $b$ levels, respectively.

Pigments were extracted from intact barley etioplasts as described herein and in Ref. 31. Separation by HPLC was performed on a C18 reverse phase silica gel column (Macherey-Nagel Co., 250 × 4.6 mm, NurseBond XRs, 5 μm) as described in Ref. 32. Either a step or gradient was used, starting with 34% 25 mM aqueous ammonium acetate, 15% acetone, and 51% methanol (buffer A), increasing to 16% H$_2$O, 60% acetone, and 24% methanol within 20 min (buffer B), and finally to 100% acetone another 4 min later, or linear gradients from buffer A to buffer B. Absorbance measurements were made at 455 nm, which corresponds to the Soret band of Pchlide $a$ and Pchlide $b$ levels. At this wavelength, the extinction coefficients of Pchlide $b$ and Pchlide $a$ are 5-fold different (21). As internal standards, we used synthetic Pchlides $a$ and $b$, which were prepared from Chlides $a$ and $b$ with an excess of 2,3-dichloro-5,6-dicyanobenzoquinone as described in Refs. 21 and 32. At a flow rate of 1 ml/min, Pchlide $a$ has a retention time of ~15 min, and Pchlide $b$ has a retention time of ~12.5 min. For simultaneous separation of Pchlide $a$ and Pchlide $b$ and their reduced products (i.e. Chlides $a$ and $b$, respectively), a C30 reverse phase column (250 × 4.6 mm, 5 μm; YMC Inc., Willmington, NC) (33) was used. HPLC was performed in a Varian ProStar model 410 appara-\natus, a ProStar model 240 pump, and a ProStar 330 photodiode array detector, essentially as described in Ref. 33 (see accompanying paper (31) for details). In some experiments, a combination of octadecyl silica and poly(ethylene) powder media was used, likewise allowing separation of both porphyrins and chlorins in the same HPLC run (34).

Chemical synthesis of ZnPP was carried out using a photodiode array detector. As shown previously (21, 32), this allowed simultaneous separation and identification of both porphyrins and chlorins in the same HPLC run (34).

Preparation and Solubilization of Prolamellar Bodies—Etioplasts were prepared from etiolated barley plants by Percoll density gradient centrifugation as described previously (35, 36). For low temperature analyses at 77 K (see below) and pigment measurements (see above), the etioplast suspension was directly used. Plastid to be lysed and its content was diluted with the buffer described by Li et al. (32). When etiolated leaf material was extracted with 100% acetone containing 0.1% DEP, which was used in order to block the potential generation of Pchlide $b$ by virtue of the previously identified Chlide $a$ oxygenase and related enzymes (27–30). This solution is referred to as 100% acetone throughout the rest of the paper. In the second case, an aqueous, non-DEP-supple-\nmented solution containing only 80% acetone was used, as reported in Ref. 21. Pigment analyses were made by HPLC, using a photodiode array detector. As shown previously (21, 32), this allowed simultaneous separation and identification of pigments during the actual HPLC run.

Two different pigment extraction procedures were used. In the first case, we used an almost pure, nonaqueous solution of acetone containing 0.1% DEP, which was used in order to block the potential generation of Pchlide $b$ by virtue of the previously identified Chlide $a$ oxygenase and related enzymes (27–30).

RESULTS

Previous studies had shown that barley and cucumber etio-\nplasts contain enzyme activity that converts Chl $b$ to Chl $a$ (22). By analogy, this enzyme activity was found to also convert the nonesterified precursor of Chl $b$, Chlide $b$, as well as pyrochlo-\nphyllide $b$ and the magnesium-free phosphorbidide $b$ into the respective Chl $a$ and 7-hydroxy compounds (23–26).

To test whether this enzyme activity, which we tentatively named 7-formyl reductase to indicate this broad substrate specificity, would also be able to convert Pchlide $b$ to Pchlide $a$ in situ, we followed the experimental design of Scheuermann et al. (21). Briefly, intact barley etioplasts were isolated on a Percoll gradient, sedimented by centrifugation, lysed, and incu-\nbated with ZnPP6, the zinc analog of Pchlide $b$ (32). As a control, barley etioplasts were left intact (26) and incubated identically. Mock incubations lacking ZnPP6 were conducted in parallel.

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![Fig. 1. Detection of pigments in etiolated barley plants. Pigments were extracted from dark-grown barley plants either with 100% acetone containing 0.1% DEP (A) or an aqueous, non-DEP-supple-\mented 80% (v/v) solution of acetone (B). The extracts were separated by HPLC, and porphyrins were identified by absorbance measurements at 455 nm (see "Materials and Methods").](http://www.jbc.org/)

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Chlide pigments by virtue of the chlorophyllase reaction, giving rise to the Q_y band, which is a typical feature of all investigated pigments recovered from peak 1 (A), peak 2 (B), and peak 3 (C), respectively. Main absorption maxima are indicated.

Absorption spectra were recorded between 400 and 750 nm for pigments were extracted and separated by HPLC as described in Fig. 1. Absorption maxima at 578 and 622 nm, respectively (Fig. 2A). These maxima are characteristics of Pchlide a, Chlide a, and 7-hydroxy-Chlide a. These maxima are characteristics of Pchlide a, Chlide a, and 7-hydroxy-Chlide a. Absorption spectra in 100% acetone of the double bond in ring D of the macrocycle was reestablished by chemical dehydrogenation with 2,3-dichloro-5,6-dicyanobenzoquinone. After hydrolysis of the plastoquinone (21, 32). At 622 nm (21). The Q_x band had a higher absorbance than the Q_y band, which is a typical feature of all investigated pigments of the protochlorophyllide series (21, 32).

For the pigments contained in peak 3, a main absorption maximum at 438 nm and a second, lower band at 628 nm were observed (Fig. 2C). These maxima are characteristics of Pchlide b, Chlide b, and Chl a were used as educts in a combined enzymatic and chemical procedure (21, 32). In the first step, the phytoyl chain was removed by the chlorophyllase reaction (40). In the second step, the double bond in ring D of the macrocycle was reestablished by chemical dehydrogenation with 2,3-dichloro-5,6-dicyanobenzoquinone (21, 32).

Fig. 3A shows absorption spectra of Chl a, Chl b, and 7-hydroxy-Chl a. It became apparent that the absorption maxima and the shapes of the curves were identical to those known from the literature (e.g. Refs. 24 and 26). After hydrolysis of the pigments by virtue of the chlorophyllase reaction, giving rise to Chlide a, Chlide b, and 7-hydroxy-Chlide a, respectively, basically the same spectra were obtained (Fig. 3B), which is in agreement with previous findings that the phytoyl chain in the esterified pigments does not affect their absorption properties as compared with the nonesterified pigments (e.g. Refs. 21 and 32). Upon oxidation with 2,3-dichloro-5,6-dicyanobenzoquinone, striking changes occurred in the absorption properties of all three compounds, however. For Chlide b and Chlide a, spectra were obtained (Fig. 3C) that were indistinguishable from those shown in Fig. 2, B and C, respectively, indicating the production of Chlide b and Chlide a. The spectrum of 7-hydroxy-Pchlide a was very similar to that of Pchlide b but was slightly blue-shifted in the red region of the spectrum (Fig. 3C versus Fig. 2A).

Mass spectrometry was used to further characterize the chemically prepared and natural compounds. Table I shows that the molecular ion peaks at m/z 612.4 ± 0.2, 626.9 ± 0.3, and 628.4 ± 0.2 were indistinguishable for the natural and synthetic pigments. According to previous work, they correspond to 7-hydroxy-Pchlide a (m/z 628.4), Pchlide b (m/z 626.9), and Pchlide a (m/z 612.4) (21, 32). These results thus ultimately confirmed the presence of 7-OH-Pchlide a, Pchlide b, and Pchlide a in etiolated barley leaves.

Also with isolated, intact barley etioplasts, 7-OH-Pchlide a, Pchlide b, and Pchlide a were readily detectable (Fig. 4A). Careful quantitative pigment measurements showed that Pchlide b (Fig. 4A, peak 2) was ~4–5-fold more abundant in concentration than Pchlide a (Fig. 4A, peak 3). Again, substantial amounts of 7-hydroxy-Pchlide a accumulated (Fig. 4A, peak 1).

The results presented thus far tempted us to conclude that a major part of Pchlide b originally present in barley etioplasts may be converted to Pchlide a via 7-hydroxy-Pchlide a. We hypothesized that this conversion could be due to 7-formyl reductase activity. To test this idea, conversion of the exogenously administered model substrate ZnPPb was studied in subsequent experiments. Percoll-purified intact etioplasts were allowed to break during the incubation with ZnPPb. Before testing the conversion of the exogenously added ZnPPb, we
TABLE I
Identification of natural and synthetic pigments by liquid secondary ion mass spectrometry

| Pigment          | Natural m/z | Synthetic m/z |
|------------------|-------------|---------------|
| 7-OH-Pchlide a   | 628.4       | 628.2         |
| Pchlide b        | 626.9       | 626.6         |
| Pchlide a        | 612.4       | 612.4         |

Fig. 4. In situ conversion of Pchlide b to Pchlide a. Etioplasts were isolated from dark-grown barley plants, lysed, and fractionated into prolamellar bodies and stroma. The prolamellar body fraction in turn was solubilized with n-octyl-β-D-glucoside. Pigments were extracted with 100% acetone containing 0.1% (v/v) DEP and analyzed by HPLC (see Fig. 1). The different chromatograms show absorbance readings of porphyrin pigments in intact etioplasts (A), lysed etioplasts (B), and solubilized prolamellar bodies (C). Peaks 1–3 correspond to those shown in Fig. 1. The insets show respective quantitative data. Percentages hereby refer to the sum of Pchlide a (gray columns, lanes 1, 3, and 5) and Pchlide b (white columns, lanes 2, 4, and 6) in the different samples, set as 100.

quantified endogenous pigments extractable with practically pure, nonaqueous acetone (see above) in order to plastid lysis. HPLC analyses revealed that already during plastid breakage, a massive pigment conversion occurred (Fig. 4B). Both the relative decrease in Pchlide b and 7-hydroxy-Pchlide a levels and the simultaneous increase in the amount of Pchlide a are clearly indicative of such a pigment conversion. This conversion was further pronounced when reisolated prolamellar bodies obtained from lysed etioplasts were solubilized with n-octyl-β-D-glucoside, a detergent that had frequently been used in previous studies (e.g. Refs. 21 and 34) (Fig. 4C).

Low temperature in situ fluorescence measurements were performed at 77 K (18, 39) in order to analyze the functional state of the different porphyrin pigments. Fig. 5 shows that the intensity of Pchlide F650/657, the predominant fluorescence peak of intact prolamellar bodies of etioplasts (see Introduction), was drastically reduced upon etioplast lysis and subsequent membrane solubilization (Fig. 5, dashed and dotted lines, respectively, versus solid line). With n-octyl-β-D-glucoside-solubilized membranes, in fact, no Pchlide F650/657 could be traced. Instead, Pchlide F628/632 became the prevalent spectral pigment species (Fig. 5, dotted line).

We next analyzed the effect of externally added ZnPPb. We assumed that the pigment, if applied in excess, should be able to compete out Pchlide b to Pchlide a conversion. Percoll-purified intact etioplasts were supplemented with ZnPPb and then lysed hypotonically, and the membranes were sedimented and solubilized with n-octyl-β-D-glucoside. Binding of ZnPPb, as well as that of ZnPPa used as a control, to the solubilized membranes was tested in two different ways. We first performed low temperature spectroscopic measurements at 77 K. These showed that almost indistinguishable levels of ZnPPa and ZnPPb were bound to the membranes (Fig. 6). Interestingly, in neither case was a long wavelength pigment species restored which emitted at 657 nm (Pchlide F650/657) (Fig. 6).

As a second method, we quantified ZnPPa and ZnPPb binding by HPLC (26). However, also by these pigment measurements, no difference in ZnPPa and ZnPPb binding could be seen (Fig. 7, Binding, compare columns 1 and 2).

Conversion of bound pigments was tested in a subsequent experiment. Detergent-treated membranes were subjected to a prolonged dark incubation, and pigment conversion was analyzed by fluorescence and HPLC measurements. Again, no change could be seen (Fig. 7, Conversion, columns 7 and 8 versus columns 3–6), demonstrating that either pigment was stable and not converted into other compounds. The addition of stromal extract to the ZnPPb-containing assays (Fig. 7, Conversion, columns 7 and 8 versus columns 3–6) and/or NADPH, glucose 6-phosphate, glucose-6-phosphate-dehydrogenase, ferredoxin-NADPH oxidoreductase plus ferredoxin, which had collectively been used to restore Chl(ide) b reductase activity in previous studies (23, 26), proved unsuccessful in our experiments (Fig. 7, columns 9 and 10).
The finding that the n-octyl-β-D-glucoside-solubilized membranes bound approximately the same levels of ZnPPb and ZnPPa (Figs. 5 and 6) at first glance seemed to contradict the LHPP model (18). According to this model, at least a 50-fold difference in ZnPPb binding and a 2-fold difference in ZnPPa binding should have been seen, reflecting the 5-fold higher abundance of the PORA as compared with that of the PORB and their ~10-fold different substrate specificities (18). Whereas PORA expressed in vitro has been shown to bind 10-fold higher levels of ZnPPb as compared with ZnPPa, PORB displayed a 10-fold greater specificity for ZnPPa and bound only little ZnPPb (18, 31).

An explanation for this apparent paradox could be that the PORA was denatured, was partially degraded, or had become soluble upon etioplast lysis and/or membrane solubilization, including respective centrifugation steps. To follow the fate of the PORA and PORB in the different fractions, we consequently performed Western blot analyses. For comparison, we preflashed isolated, intact etioplasts before analysis and subfractionation with a saturating 1-ms flash of white light, which previously had been used to induce the disintegration of the prolamellar body (13).

Fig. 4A shows that with the dark-incubated, nonflashed samples, both the PORA and PORB proteins were detectable in the intact etioplasts and in the respective sediment fraction obtained after plastid lysis and centrifugation. Upon solubilization of the sedimented membranes, the picture then changed. Only the PORB was retained in the sediment fraction, whereas the PORA was almost quantitatively released into the respective supernatant (Fig. 8A). With the preflashed sample, this release was already detectable upon plastid lysis. Then almost all PORA was found in the supernatant fraction and only traces remained bound to the resedimented membranes (Fig. 8A). Upon detergent treatment, this remainder was released into the supernatant obtained after centrifugation of the assays.

**FIG. 8.** Protochlorophyllide b to Chlide a conversion in relation to the light-induced disintegration of the prolamellar body. Etioplasts were prepared from dark-grown barley plants and either kept in complete darkness (Darkness, lanes 1–5) or exposed to a saturating 1-ms flash of white light (Flashed, lanes 6–10). Protein and pigments were then extracted from the plastids or respective subfractions as described in Fig. 4A, high resolution Western blot analysis of POR-related proteins. Protein was resolved electrophoretically and blotted onto a nitrocellulose membrane, and POR-related proteins were detected with a POR-specific antiserum. The blots show the PORA (36 kDa) and PORB (38 kDa) in the flashed and nonflashed etioplasts before lysis (Intact, lanes 1 and 6), after lysis (Lysed, lanes 2 and 3 versus lanes 7 and 8), and in solubilized etioplast inner membranes (Solubilized, lanes 4 and 5 versus lanes 9 and 10). Both the respective membrane pellet (P, lanes 2, 4, 7, and 9) and supernatant fractions (S, lanes 3, 5, 8, and 10) were analyzed. In all cases, protein equivalent to 25 μg of bovine serum albumin was probed per lane. B, HPLC chromatogram of pigments found in lysed, flashed etioplasts. Intact etioplasts were prepared as described before and flashed. Pigments were extracted from the total sample, corresponding to lanes 7 plus 8 in A, with 100% acetone containing 0.1% (v/v) DEP and analyzed on a C18 column. Peaks 1–3 correspond to those shown in Fig. 1. Peak 4 is due to Chlide a, as shown in a accompanying paper (31).

**TABLE II**

Relative pigment levels in intact barley etioplasts and different plastid subfractions

Pigments were extracted from the various plastid fractions given in Fig. 8 with 100% acetone containing 0.1% (v/v) DEP and separated by HPLC (see “Materials and Methods”). Quantification of HPLC-separated porphyrins was achieved by absorbance measurements at 455 nm, using chemically synthesized Pchlide a and b as standards. Chlorins were quantified as described in Refs. 39 and 41. Numbers refer to the total pigment levels in each fraction, set as 100. ND, nondetectable pigment levels.

| Pigment | Intact | Lyzed | Solubilized |
|---------|--------|-------|-------------|
|         | Pellet | Supernatant | Pellet | Supernatant |
| In darkness | % | % | % | % |
| Pchlide a | 20 | 70 | ND | 20 | 80 |
| Pchlide b | 80 | 30 | ND | ND | ND |
| Chlide a | ND | ND | ND | ND | ND |
| Chlide b | ND | ND | ND | ND | ND |
| After flash light illumination | | | | |
| Pchlide a | ND | 15 | 30 | ND | ND |
| Pchlide b | 80 | 5 | 30 | ND | ND |
| Chlide a | 20 | 20 | ND | ND | ND |
| Chlide b | ND | ND | ND | ND | ND |
The various findings reported thus far implied that Pchlide b to Pchlide a conversion may be related to the release of the PORA from the prolamellar body, either artificially as a result of detergent solubilization of the isolated membranes or, more naturally, as part of the light-induced disintegration of these structures. Because the latter process should allow PORA, PORA from the prolamellar body, either artificially as a result of hypotonic plastid lysis and membrane solubilization and converted practically all of the preexisting Pchlide b to Pchlide a. In the preflushed sample, 7-formyl reductase was active as well, but it did not seem to gain its full activity. In the supernatant of preflushed, lysedoplasts, only one-third of the total pigment was accounted for by Pchlide a. The remainder was present as Pchlide b. In the respective pellet fraction, we recovered ~3-fold lower levels of Pchlide b relative to Pchlide a and found significant levels of Chlide a (Table II).

Upon membrane solubilization, only Pchlide a could be detected in the resedimented membranes of the preflushed etioplasts (Table II). Remarkably, this fraction did not contain either Chlide b or Chlide a (Table II); nor were we able to trace any Pchlide b. In the respective supernatant, the only detectable pigment was Chlide a (Table II). This suggested that residual Pchlide b present in the pellet of the lysed etioplasts had been converted to Pchlide a.

DISCUSSION

In the present study, we readressed the previously published plastid work-up and pigment extraction procedure of Scheumann et al. (21), which involves hypotonic plastid lysis, the sedimentation of the prolamellar body, and subsequent membrane solubilization. Finally, the solubilized membranes were sedimented, and the obtained pellet and supernatant fractions, respectively, were characterized further.

Our results demonstrate that plastid lysis and membrane solubilization collectively lead to the denaturation of the prolamellar body, the release of the PORA, and the conversion of most, if not all, of the total Pchlide b to Pchlide a. The latter reaction was presumably catalyzed by 7-formyl reductase. This enzyme had originally been implicated in the Chl b to Chl a reaction cycle of chloroplasts during photosynthetic acclimation and leaf senescence (26). But it is also highly active in etioplasts, as shown in this and previous studies (22–26).

We assume that 7-formyl reductase may be involved in fine tuning the amounts of Pchlide b and Pchlide a and thereby could regulate the steady-state level of LHPP in etioplasts.

Flash light illumination of intact etioplasts, which has for a long time been known to induce the disintegration of the prolamellar body (13), caused the simultaneous release of the PORA and Pchlide b from the inner plastid membranes. But it did not induce the immediate enzymatic reduction of Pchlide b to Chlide b or the quantitative transformation of Pchlide b to Pchlide a. These important results lend more, although indirect, support to our previous conclusions that the PORA remains, in the first place, catalytically inactive as a Pchlide b-reducing enzyme (18). Moreover, they demonstrate that 7-formyl reductase activity is partially suppressed during the light-induced transformation of etioplasts into chloroplasts. Although we do not yet know the reasons for this effect, we hypothesize that 7-formyl reductase may be involved in controlling the rate of PORA-driven Chlide b synthesis in illuminated plants. It is tempting to speculate that PORA-derived Chlide b could play regulatory roles for the establishment of the light-harvesting structures in etiolated plants at the beginning of illumination, whereas Chlide a synthesized by virtue of Chlide a oxygenase (29, 30, 41) in light-adapted plants could serve housekeeping functions during photosynthesis (for reviews, see Refs. 42 and 43). According to recent work (44), Chlide a oxygenase is well able to accept both Pchlide a and Chlide a as substrate, although with different apparent affinities. If its expression in etiolated, illuminated, and light-adapted plants and its localization have not yet been examined.

In addition to these aspects, the results presented in this study answer the long-lasting question of whether or not Pchlide b is occurring in etiolated plants (18–21, 45). Our findings show that Pchlide b is present in barley etioplasts and indeed accounts to amounts well compatible with the LHPP model (18, 19).

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