Chlorophyll a Formation in the Chlorophyll b Reductase Reaction Requires Reduced Ferredoxin*

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The reduction of chlorophyllide b and its analogue zinc pheophorbide b in etioplasts of barley (Hordeum vulgare L.) was investigated in detail. In intact etioplasts, the reduction proceeds to chlorophyllide a and zinc pheophorbide a or, if incubated together with phytidyphosphate, to chlorophyll a and zinc pheophytin a, respectively. In lysed etioplasts supplied with NADPH, the reduction stops at the intermediate step of 7-1-OH-chlorophyll(ide) and Zn-7-1-OH-pheophorbide or Zn-7-1-OH-pheophytin. However, the final reduction is achieved when reduced ferredoxin is added to the lysed etioplasts, suggesting that ferredoxin is the natural cofactor for reduction of chlorophyll b to chlorophyll a. The reduction to chlorophyll a requires ATP in intact etioplasts but not in lysed etioplasts when reduced ferredoxin is supplied. The role of ATP and the significance of two cofactors for the two steps of reduction are discussed.

Chlorophylls (Chls) are abundant pigments of photosynthesis in marine (oceans), aquatic (freshwater), and terrestrial environments (land). The appearance and disappearance of Chls (their synthesis and degradation), can be detected by remote sensing from satellites (1, 2) and are the only biochemical processes on earth that can currently be observed from outer space. The biosynthesis of Chl a, the most widely occurring Chl, is well known and has been treated in several recent reviews (3–7). The biosynthesis of Chl b, typical in addition to Chl a for green algae, mosses, ferns and seed plants, is not yet known in detail. The only difference in these chlorophyll structures is located at C-7; the substituent is a formyl group in Chl b but a methyl group in Chl a (see Fig. 1). The formyl oxygen is derived from molecular oxygen (8, 9). It is generally assumed that the early steps of biosynthesis of Chl a and b are identical and that oxygen is introduced during one of the final steps of the Chl b biosynthesis; however, the exact location in the biosynthetic chain where the oxygenation occurs, before or after phytynylation, is not precisely known.

The reverse reaction, formation of Chl a from Chl b, has been repeatedly discussed as a process involved in reorganization of the photosynthetic apparatus during acclimation to various light environments (10–12). Acclimation implies redistribution of Chls between the different chlorophyll-protein complexes (12, 13). Reduction of Chl b to Chl a must also play a role in the process of Chl degradation, because during the senescence of higher plants, Chl b disappears together with Chl a, but the degradation products are entirely derived from Chl a (14). Further, the key enzyme of Chl degradation, pheophorbide oxygenase, accepts only Pheide a, whereas Pheide b is a competitive inhibitor (15).

The Tanaka group detected the in vitro reduction of the formyl group to the methyl group of Chl derivatives when they investigated the esterification of chlorophyllide (Chlide) b with etioplasts from cucumber and barley (16–18). The esterified products were Chl b, Chl a, and in barley the intermediate 7-1-OH-Chl a (Ref. 18; see Fig. 1). The yield of products, especially of Chl a, was increased by adding ATP. The reduction of pyro-Chlide b, lacking the methoxycarbonyl substituent at C-13 (see Fig. 1), to pyro-Chl a in greening etioplasts of cucumber extended this finding and showed that reductase activity increased slightly during greening in the light (19). We have demonstrated that the same reduction can be performed with zinc rather than magnesium complexes. Thus, the formyl group of zinc pheophorbide (ZnPheide) b can be chemically reduced with cyanoborohydride to a methyl group producing ZnPheide a (20). Zn-7-1-OH-Pheide a and Zn-7-1-OCH3-Pheide a are intermediates in this chemical reduction. Earlier, Veitzikii and Schcherbakov (21) infiltrated etiolated rye leaves with Chlide b and ZnPheide b and demonstrated the reduction to Chl a and ZnPhe a, respectively. Likewise, infiltration of ZnPheide b or Zn-7-1-OH-Pheide a into primary leaves of etiolated oat seedlings yielded ZnPhe a (20). In cooperation with Tanaka’s group, we showed that the first step, reduction of ZnPheide b to Zn-7-1-OH-Pheide a, proceeds before or after esterification in purified, broken etioplasts of barley (22). This step required NADPH or NADH but no ATP.

In this paper, we describe the second step, the formation of Chl a and ZnPheide a, in more detail; we compare this reduction in both intact and broken etioplasts and describe the effect of ATP. The comparison led to the discovery that the second step of reduction requires reduced ferredoxin.

EXPERIMENTAL PROCEDURES

Etioplast Preparation—Barley seedlings (Hordeum vulgare, L. var. Steffi, obtained from Dr. Ackermann Co.) were grown on moist vermiculite in the dark in a light-tight growth chamber (25–30 °C) for 6 days. To prevent photoversion of protochlorophyllide, the etioplasts were purified under dim green light (<10 µmol·m−2·s−1) according to the method of Eichacker et al. (23). The primary leaves were ground in an isolation medium of 0.4 M sorbitol and 2 mM EDTA in 50 mM HEPES/KOH buffer (pH 8.0). The homogenate was filtered through a nylon mesh (20 µm), and the etioplasts were collected by centrifugation (1 min, 3800 × g). The etioplasts were resuspended in isolation buffer and purified over a Percoll step gradient (65%/35% (v/v) Percoll in isolation medium without EDTA). The intact etioplasts were removed from the 35%/65% Percoll interphase, diluted with isolation medium without EDTA, and centrifuged again (4097 × g, 3 min). The intact plastids...
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were resuspended in reaction buffer containing 0.4 mM sorbitol and 10 mM MgCl₂ in 50 mM HEPE/ROH (pH 7.5) and then counted in a hemacytometer under a phase-contrast microscope. The plastids were either used as intact etioplasts or centrifuged again and resuspended in reaction buffer without sorbitol to break them osmotically. In some experiments, the plastids were additionally frozen in liquid nitrogen and thawed. If only the membranes were used, the broken etioplasts were washed twice with reaction buffer without sorbitol to remove the stroma (3250 × g, 2 min). If not otherwise indicated, 8.5 × 10⁶ etioplasts/sample were used.

**Pigment Preparation**—Chlide b was extracted from leaves of *Ailan-thus altissima* using the chlorophyllase reaction (24, 25). It was purified by preparative high pressure liquid chromatography (HPLC). The HPLC unit consisted of a pump and controller (Watere 600E multisolv-ent delivery system), an in-line degasser (Waters), a diode-array spectrophotometer (Tidas, J & M, Germany), and a spectrofluorometric detector (RF-551, Shimadzu). The pigments were separated on a reverse-phase column (19 × 300 mm), packed with silica gel C-18 (μBondapak® C18, 125 Å, 10 μm). Chlides were eluted isocratically with a flow rate of 6.5 ml/min at 50% acetone, 50% 25 mM NH₄ acetate, and a flow rate of 1.3 ml/min. For separation of the pigmented esters (as in Figs. 3 and 5), the column was maintained at 70% acetone, 30% water for 2 min, followed by a linear gradient to 82% acetone within 2 min, another flat linear increase to 88% acetone within 11 min, and a steep linear gradient to 100% acetone within 4 min. The spectrofluorometric detector was set at 425 nm (excitation) and 665 nm (emission), and was highly sensitive for Chl a and ZnPhe a. Additionally, an absorption spectrum from 350 to 750 nm was recorded every second with the diode-array spectrophotometer. The data were evaluated with the LabControl software Spectra Chrom Version 1.5. The amount of zinc pigments was determined by calculating the areas of the corresponding emission peaks at 665 nm after calibration with a standard.

**Protein Analysis**—The purified intact etioplasts were collected by centrifugation. After freezing and thawing, the stroma was separated from the membranes by centrifugation (6500 × g, 2 min) and the membranes were washed twice with washing buffer (3800 × g, 2 min).

**RESULTS**

At first, we addressed the question of why only the first step of Chl b reduction to 7'-OH-Chl a was catalyzed by purified, lysed etioplasts (22) but varying amounts of Chl a, the product of the second reduction step, were found if the reaction was carried out with freshly prepared, crude etioplast preparations (17, 18). The following two possibilities for this finding were considered. 1) During the lengthy purification procedure, a sensitive compound of the second reductase system could be inactivated by oxidation, but the addition of the antioxidant, diithiothreitol, did not restore this second reductase to form Chl a (data not shown). 2) Crude etioplast preparations always involve partial lysis of etioplasts. The crude etioplast preparation contained intact and lysed etioplasts in variable amounts. For that reason Scheumann et al. (22) used purified etioplasts that were lysed osmotically. To test whether intactness of the etioplasts enhanced the second reduction step, we performed the assay with intact etioplasts in isocratic conditions.

The method of preparation was essentially that of Eichacker et al. (23). The crude etioplast preparation was further purified by density gradient centrifugation (Fig. 2A). The fraction on top of the 35% Percoll, “lysed etioplasts” (Fig. 2A) consisted mainly of broken etioplasts. The fraction at the boundary layer 35%/65% Percoll contained intact etioplasts according to Eichacker
et al. (23). We confirmed the intactness by the presence of stromal proteins in this fraction, e.g. the large subunit of ribulose 1,5-diphosphate carboxylase (LSU, Fig. 2B, lane 4) and ferredoxin (see Fig. 4A). Stromal proteins were absent in the fraction of lysed etioplasts as expected (Fig. 2B, lane 3). Membrane fractions of both intact and lysed etioplasts contain the NADPH-protocorhirylide oxidoreductase (POR) as the main protein band (Fig. 2B, lanes 2 and 5).

These intact etioplasts were incubated in isotonic medium at 24 °C for 90 min with phytyl-POP and ZnPheide b. It is necessary to perform etioplast preparation and the assay in darkness or under very dim green safe light, because light causes phototransformation of endogenous protocorhirylide a to Chlide a, which will be esterified with phytyl-POP to Chl a. We used ZnPheide b rather than Chlide b as a substrate for reduction in most experiments. Thus, we were able to distinguish between Chl a produced by the undesired light effect upon endogenous substrate and ZnPhe a, the product of reduction of the exogenous substrate. Furthermore, the zinc compounds are more stable against demetallation than the magnesium compounds.

The etioplasts contain protocorhirylide a and traces of esterified protorhodin a (Fig. 3a). We obtained significant amounts of ZnPhe a and Zn-7′-OH-Phe a from ZnPheide b (Fig. 3b). ZnPhe a had never been obtained before with lysed etioplasts. Because some etioplasts may be lysed in the course of the assay, we reisolated the intact etioplasts after the assay and analyzed their pigment content. The yield of ZnPhe a relative to Zn-7′-OH-Phe a was significantly higher in the reisolated organelles (Fig. 3, compare c with b), clearly showing that the second step of reduction is restricted to intact etioplasts under our experimental conditions. Only the first reduction occurs in lysed etioplasts when NADPH is supplied. Thus, lysis leads to the loss of a factor different from NADPH that is necessary for the second but not for the first reduction step. The most probable explanation is loss of a soluble factor by lysis or under very dim green safe light, because light causes phototransformation of endogenous protorhodin a to Chl b, which will be esterified with phytyl-POP to Chl a. Ferredoxin is not only involved in electron transfer to NADP + but is also an essential cofactor of plastid-located fatty acid desaturases (36), of protorhodin oxidoreductase (15), and of plant heme oxygenase (37). It is notable that animal heme oxygenase uses NADPH rather than ferredoxin. Ferredoxin and the enzyme ferredoxin-NADP + oxidoreductase are present in etioplasts as shown by immunoblotting (Fig. 4, A and B); we loaded a gel with the stroma and membranes of barley etioplasts and barley chloroplasts together with authentic ferredoxin and ferredoxin-NADP + oxidoreductase from spinach. The proteins were blot-

**Fig. 2. Demonstration of the intactness of the purified etioplasts.** Etioplasts were isolated from primary leaves of barley as described under "Experimental Procedures." After centrifugation, lysed etioplasts (m) and intact etioplasts (e) were removed from the Percoll gradient (A), washed with isotonic buffer, and lysed osmotically and by freezing and thawing. Both fractions were adjusted to equal protorhodin concentrations. After centrifugation, the supernatants of m and e were removed, and the membranes were washed twice and collected by centrifugation. The proteins were separated on a 17% polyacrylamide gel and silver-stained (B). The lanes correspond to the following fractions: molecular marker (lane 1), membranes of m (lane 2), supernatant of m (lane 3), supernatant of e (lane 4), membranes of e (lane 5). The main protein bands of the fractions are marked by arrows: large subunit of ribulose 1,5-diphosphate carboxylase (LSU) and NADPH-protocorhirylide oxidoreductase (POR).
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To compare different substrates and cofactors, we standardized the reaction conditions. Fig. 6 shows the time course of the second reduction step, namely of $\text{Zn-7'}$-OH-Pheide to ZnPheide with reduced ferredoxin. We used the $\text{7'}$-OH intermediate of the Chlb reductase reaction and omitted the esterification reaction so that the second reduction step alone was measured. The amount of product was determined by HPLC (not shown). Linear reaction kinetics were observed up to 90 min. This reaction period was used in all further experiments. Without the addition of ferredoxin, no ZnPheide was formed.

We then studied the effect of ferredoxin for both substrates, ZnPheide and Zn-7'-OH-Pheide, with or without esterification using lyzed etioplasts (Table I). Formation of ZnPheide in incubations with phytol-POP or of ZnPheide incubated without phytol-POP was detected only when reduced ferredoxin was present. This series of experiments confirmed the reduction of ZnPheide to Zn-7'-OH-Pheide with NADPH alone. However, the second reduction step required ferredoxin irrespective of the esterification status of the substrate pigment. It is notable that incubation with Zn-7'-OH-Pheide never resulted in formation of ZnPheide or ZnPhe, indicating that the first step of the Chlb to Chla conversion is not reversible under our experimental conditions.

Because ferredoxin was detected in etioplasts, the observed results might be explained as follows. The concentration of reduced ferredoxin could be high enough for Chla production in intact plastids, but lysis of the plastids would dilute ferredoxin so that Chla was no longer formed. To test this hypothesis, ferredoxin was added together with ferredoxin-NADP$^+$ oxidoreductase, NADPH, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase to the sample with lysed etioplasts. A peak at the position of ZnPheide (peak 3, Fig. 5A) was observed with this reduced ferredoxin (Fig. 5A). A similar reduction was achieved with Chl$\beta$ b was used instead of ZnPheide; a peak at the position of Chla appeared under the same conditions (Fig. 5B). Besides the retention times, the absorption spectra of the peaks proved that peak 5 ($\text{peak 5B}$) was 7'-OH-Chl$\alpha$ (Fig. 5C), peak 6 was the esterified substrate Chl$\beta$ (Fig. 5D), and peak 7 was Chla (Fig. 5E). However, ferredoxin alone without its reducing system caused no reduction (data not shown). Whereas the product 7'-OH-Chl$\alpha$ also appeared with NADPH alone or together with glucose-6-phosphate and glucose-6-phosphate dehydrogenase, Chla was not formed under these conditions (Fig. 5B). This agrees with the previous finding (18, 20) that 7'-OH-Chla is the intermediate in the reduction of the formyl group to the methyl group. Although the fluorescence excitation and emission wavelengths were adjusted for Zn-7'-OH-Pheide and 7'-OH-Chla, respectively, the amount of the esterified b-type pigments was high enough to observe them as a small peak (peaks 2 and 6) in this experiment.

The results in Fig. 5 show that either Chl$\beta$ b or ZnPheide b can be used as substrate to study the reduction of the formyl group. In standard assays, we always used the zinc compounds to distinguish between exogenous and endogenous pigments.

DISCUSSION

We previously showed that barley etioplasts, purified via a Percoll gradient and lyzed before the enzyme assay, catalyzed only the first step of the Chlb reductase reaction, namely the reduction of ZnPheide to Zn-7'-OH-Pheide or (with phytol-POP) to Zn-7'-OH-Phe (22). By way of contrast, crude etioplasts, prepared by a rapid centrifugation procedure, also catalyzed (at least in part) the second step to ZnPheide. We have demonstrated in the present paper that this difference was caused by the presence of intact etioplasts in the crude preparation. When we purified intact etioplasts after conducting the reduction reaction, the ratio ZnPheide to Zn-7'-OH-Phe was even higher than observed with the crude preparation, which consisted of a mixture of intact and lyzed etioplasts. Reactions occurring inside intact organelles require substrate penetration through the envelope when these compounds are added.
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FIG. 5. Effect of reduced ferredoxin upon the Chl(ide) b reductase reaction in lysed etioplasts. Lysed etioplasts (5 × 10^7/sample) were incubated with 1.2 nmol of ZnPheide b (A) or 1.6 nmol of Chlide b (B), respectively, and phytyldiphosphate, ATP (a) together with NADPH, glucose 6-phosphate and glucose-6-phosphate-dehydrogenase (b) and additionally ferredoxin-NADP\(^+\) oxidoreductase and ferredoxin (c). A and B, HPLC of the esterified pigments after incubation at 28 °C for 90 min. Fluorescence was specific for a-type pigments with excitation at 425 nm and emission at 665 nm. The detected reaction products are Zn-7\(^{-}\)-OH-Phe a (1), ZnPhe b (2), ZnPhe a (3), protochlorophyll a (4) 7\(^{-}\)-OH-Chl a (5), Chl b (6), Chl a (7), and Phe b (8). All peaks are followed by their 132-S epimers as smaller peaks or shoulders. The complete reduction to ZnPhe a and Chl a could only be achieved with ferredoxin including the ferredoxin-reducing system (c). Ferredoxin alone had no effect; in this case, the HPLC chromatogram resembled curve a in panels A and B. The total amount of the pigments was calculated by peak integration. A, a, 561 pmol of ZnPhe b; b, 369 pmol of Zn-7\(^{-}\)-OH-Phe a, 177 pmol of ZnPhe b; c, 315 pmol of Zn-7\(^{-}\)-OH-Phe a, 93 pmol of ZnPhe b, and 75 pmol of ZnPhe a. B, a, 1110 pmol of Chl b; b, 264 pmol of 7\(^{-}\)-OH-Chl a, 660 pmol of Chl b; c, 186 pmol of 7\(^{-}\)-OH-Chl a, 630 pmol of Chl b, and 75 pmol of Chl a. C, absorption spectrum of the pigment of peak 5. Retention time (14.4 min) and absorption spectrum are identical with that of 7\(^{-}\)-OH-Chl a. D, absorption spectrum of the pigment of peak 6. Retention time (18.0 min) and absorption spectrum are identical with that of Chl b. E, absorption spectrum of the pigment of peak 7. Retention time (18.2 min) and absorption spectrum are identical with that of Chl a.

Exogenously, Penetration of phytol-POP and geranylgeranyl-POP through the envelope of etioplasts and chloroplasts has been demonstrated (38, 39), and penetration of Chlide a and Pheide a through the etioplast envelope into prothylakoids and prolamellar bodies that contain the chlorophyll synthase activity (40, 41) has also been shown (42). Penetration of ZnPheide b into intact etioplasts, previously assumed to be due to stabilization of Chl a-binding apoproteins (43), can be deduced by its esterification when it was added together with phytol-POP; the phytylated pigment was detected after extraction from reisolated intact etioplasts (Fig. 3c). Furthermore, the results of reduction discussed below can only be understood with the assumption that these substrates penetrate into intact etioplasts. The penetration of several tetrapyroles through the plastid envelope has been reported; formation of esterified Chl after infiltration of etiolated leaves with Chlide (20, 21) requires penetration of the substrates not only through the plasmalemma but also through the etioplast envelope, since esterification occurs inside the etioplast. Exogenous protoporphyrin passes into plastids for the magnesium chelatase reaction (42), and endogenous protoporphyrinogen, produced in plastids, passes through the plastid membrane for further metabolism in mitochondria or to diffuse throughout the cell after overproduction in plastids (for a review, see Ref. 6). Further, phytoxanthobilin, produced in plastids, passes through the plastid membrane for incorporation into phytochrome apoprotein in the cytoplasm (44), and magnesium protoporphyrin, produced in plastids, also passes into the cytosol, forming a signal for transcription of nuclear genes (45). We do not yet know whether the tetrapyrroles penetrate the plastid membrane by passive diffusion or whether a specific transport system is present. The possibility of a light-activated transport of magnesium protoporphyrin from chloroplasts to the cytosol in *Chlamydomonas* cells has been discussed by Kropat et al. (45).

Ito et al. (17) have shown that the reduction of Chlide b to Chl a required both the membrane and stroma fractions of etioplasts. We found that the stroma fraction can be replaced by reduced ferredoxin. It is likely, therefore, that the stroma factor postulated by Ito et al. (17) is ferredoxin, which is present in the etioplast. We used ferredoxin-NADP\(^+\) oxidoreductase and a NADPH-regenerating system to reduce the ferredoxin added to lysed etioplasts or to the washed membranes. We assume that endogenous ferredoxin is reduced in intact etioplasts in the same way with NADPH, possibly formed, for example, by the pentosephosphate pathway; however, we cannot exclude the possibility that a different pathway of ferredoxin reduction exists in etioplasts that is possibly ATP-dependent (see below).
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FIG. 6. Time course of the reduction of zinc 7′-OH-pheophorbide a to zinc pheophorbide a by reduced ferredoxin in lysed etioplasts (8.5 × 10^7/sample). The percentage of formed reduced pigment added Zn-7′-OH-Pheide a (about 450 pmol) was calculated on the basis of NADPH, glucose 6-phosphate and glucose-6-phosphate dehydrogenase were added without (A) or with ferredoxin-NADP+ oxidoreductase and ferredoxin (B). The reaction proceeded linearly for 90 min under the conditions used. Because all solutions are stored on ice, they require a short time to reach the reaction temperature of 28 °C. This explains the delay occurring in the first 15 min.

FIG. 7. Effect of ATP upon the reduction of zinc pheophorbide b (A) and zinc 7′-OH-pheophorbide a (B) in intact and lysed etioplasts. Intact etioplasts (8.5×10^7/sample) were incubated for 90 min at 28 °C with NADPH and phytidylphosphate. Lysed etioplasts were incubated, in addition, with the ferredoxin-reducing system as in Fig. 5. The values are the means of 4–10 experiments. The amount of the total esterified pigment (sum of ZnPhe b, Zn-7′-OH-Pheide a, and ZnPhe a) varied between 400 and 600 pmol.

FIG. 8. Activity of chlorophyll b reductase in washed etioplast membranes. Aliquots of 8.5 × 10^7 lysed etioplasts were used directly (1), or aliquots of twice-washed membranes prepared from identical aliquots (2) or washed membranes prepared from etioplasts after a freeze-thaw cycle (3) were used. Incubation with ZnPheide b and the ferredoxin-reducing system was as in Fig. 5. The values of Zn-7′-OH-Pheide a and ZnPhe a are expressed as the percentage of the total esterified pigment (about 350 pmol) and are the means of two experiments.

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Ito et al. (17, 18) reported that ATP was required for formation of Chl a in crude etioplast preparations. We confirmed that ATP is required when intact etioplasts were used but not when lysed etioplasts or isolated membranes were used together with reduced ferredoxin. This suggests that the reduction of Chl(id)e b per se does not need ATP and that ATP has some indirect effect with intact plastids. It is not an effect upon import of Chlde b, since we found that there is no effect of ATP upon esterification of Chlde b in intact plastids. Matile et al. (46) reported that ATP was required for production of a Chl catabolite in intact plastids; the same reaction did not need ATP in vitro (15). These observations are interesting in the present context, because reduced ferredoxin is a cofactor for both the key reaction of Chl breakdown and for reduction of Chl(id)e b. If ATP leads to a more negative redox potential in plastids and thus to an increased level of reduced ferredoxin, it would explain the observed effect upon both Chl b reduction and Chl a catabolism. Such an effect of ATP cannot be seen with lysed plastids if reduced ferredoxin is added in excess.

The finding that the two reduction steps in the conversion of Chl b to Chl a are achieved with the two different cofactors NADPH and ferredoxin raises the question of whether the two reactions are catalyzed by one or by two different enzymes; however, it is not clear yet whether NADPH is the proper cofactor for the first step. That NADH supports the first reduction step (22) argues against the strict specificity for NADPH. We cannot yet exclude the possibility that the first step could also use ferredoxin, because our ferredoxin-reducing system contained NADPH. If both steps normally used reduced ferredoxin, there would be no need to assume the presence of two different enzymes. On the other hand, the intermediate product, 7′-OH-Chl a, does not bind to chlorophyll-binding proteins (12), in contrast to the substrate, Chl b, and the final product, Chl a. Thus, the hydroxy intermediate could be a transport form of the pigment. A spatial separation of the two reduction steps would support the notion of two different reductase enzymes. This question will be addressed in future investigations.

TABLE I
Reduction of zinc pheophorbide b and zinc 7′-OH-pheophorbide a, respectively, in lysed etioplasts of barley with or without esterification and with or without reduced ferredoxin

| Substrate (ZnPheide) | Product | Fd | phytyl-POP | Zn-7′-OH-Pheide a | Zn-7′-OH-Pheide b |
|---------------------|---------|-----|------------|-----------------|-----------------|
| b                   | %       | +  | +          | 82              | 0               |
| b                   | %       | +  | +          | 74              | 11              |
| 7′-OH               | %       | +  | +          | 77              | 7               |
| 7′-OH               | %       | +  | +          | 20              | 15              |

ZnPheide b (700 pmol) or Zn-7′-OH-Pheide a (400 pmol) was added to 8.5 × 10^7 purified, lysed etioplasts with or without phytyl-POP and with or without ferredoxin-NADP+ oxidoreductase and ferredoxin (Fd). The NADPH-regenerating system consisting of NADPH, glucose 6-phosphate and glucose-6-phosphate dehydrogenase was added to all samples. The incubation time was 90 min at 28 °C. The values of the products are expressed as percentages of the total extracted (nonesterified) and of the total esterified pigment, respectively. The reduction of Zn-7′-OH-Pheide a to ZnPhe a or ZnPheide a in lysed etioplasts is only possible if reduced ferredoxin is present.

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