FEBS Letters 480 (2000) 306–310 FEBS 24003

The influence of glycerol and chloroplast lipids on the spectral shifts of pigments associated with NADPH:protochlorophyllide oxidoreductase from *Avena sativa* L.[1]

Harald Klement, Ulrike Oster, Wolfhart Rüdiger*

Botanisches Institut der Ludwig-Maximilians-Universität München, Menzingerstr.67, 80638 Munich, Germany

Received 21 June 2000; accepted 13 July 2000

Edited by Richard Cogdell

Abstract Dark-grown angiosperm seedlings lack chlorophylls, but accumulate protochlorophyllide *a* complexed with the light-dependent enzyme NADPH:protochlorophyllide oxidoreductase. Previous investigators correlated spectral heterogeneity of in vivo protochlorophyllide forms and a shift of chlorophyllide forms from 680 to 672 nm (Shibata shift) occurring after irradiation, with intact membrane structures which are destroyed by solubilization. We demonstrate here that the various protochlorophyllide forms and the Shibata shift which disappear upon solubilization are restored if the reconstituted complex is treated with plastid lipids and 80% (w/v) glycerol. We hypothesize that the lipids can form a cubic phase and that this is the precondition in vitro and in vivo for the observed spectral properties before and after irradiation.

Key words: Chlorophyll biosynthesis; Photoconversion; NADPH:protochlorphyllide oxidoreductase; Prolamellar body; Shibata shift; *Hordeum*

1. Introduction

In angiosperms, the reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide) is catalyzed by the light-dependent enzyme NADPH:protochlorophyllide oxidoreductase (POR; EC 1.3.1.33) [1]. PORA, one of the two involved isoenzymes, is predominant in dark-grown seedlings and is essential at the beginning of greening [2]. Together with its cosubstrates NADPH and Pchlide *a*, it forms a ternary complex [3] which constitutes the main protein complex of the prolamellar bodies (PLBs) of etioplasts [4]. The unusual paracrystalline structure of this membrane system has been studied extensively and is reviewed in detail by Selstam and Wigge [5]. It shows a bicontinuous cubic phase organization with two different branched and interwoven water channels, separated by a continuous bilayer that consists mainly of monogalactosyl diacylglycerol (MGDG) and digalactosyl diacylglycerol (DGDG) in a molar ratio of 1.8. The higher amount of MGDG is considered to be an important factor for building up the cubic phase. The POR enzyme is regarded to be more or less peripherally associated with the membranes, probably by anchoring of tryptophan residues to the lipid polar head groups [6].

In vivo, the endogenous Pchlide *a* shows spectral heterogeneity, detectable by absorption and fluorescence spectroscopy (for reviews see [7,17]). A form absorbing at around 630 nm is regarded to be non-photoconvertible, but can act as precursor for the photoactive forms. Two photoactive forms are detectable, a short-wavelength form absorbing at 638 nm (Pchlide638) and a long-wavelength form absorbing at 650 nm (Pchlide650). Both correspond to the ternary NADPH:Pchlide–POR complex and have high transformation rates. Pchlide650 is believed to represent Pchlide *a* in highly aggregated complexes. Pchlide638 is thought to be present in less aggregated, probably monomeric or dimeric complexes. Spectral heterogeneity is found also for Chlide *a*, the product of the light reaction. The first stable product formed upon irradiation under saturating light intensities has an absorption maximum at 678 nm, which shifts within seconds to 684 nm. After subsequent incubation in the light for several minutes, the absorption maximum is blue shifted to 672 nm (Shibata shift). If the incubation is performed in darkness, the shift is accompanied by a regeneration of photoactive Pchlide absorbing at 650 nm. Several explanations for the Shibata shift, based on indirect evidence, have been offered by some authors, but questioned by other authors (for the Shibata shift), including esterification of newly formed Chlide *a* [18], displacement of Chlide *a* from the POR–product complex [14], and disaggregation of large enzyme–product aggregates into smaller ones [16,19], possibly connected with dephosphorylation of the POR–product complex [15].

So far, Pchlide650 and the Shibata shift have only been observed in intact leaves, in isolated etioplasts or in membrane fractions containing PLBs or at least PLB fragments. Partial solubilization with low concentrations of dodecylmaltoside yielded inter alia a 1000-kDa photoactive complex of Pchlide650, which still contained carotenoids and membrane components. Higher concentrations of this detergent resulted in a mixture of photoactive and non-photoactive Pchlide absorbing at 643 nm [12]. Complete solubilization of non-irra-

*Corresponding author. Fax: (49)-89-17861 185.
E-mail: ruediger@botanik.biologie.uni-muenchen.de

[1] Dedicated to Professor Otto Kandler on the occasion of his 80th birthday.

Abbreviations: Chlide, chlorophyllide; DGDG, digalactosyl diacylglycerol; MGDG, monogalactosyl diacylglcerol; Pchlide, protochlorophyllide; PLB, prolamellar body; POR, NADPH:protochlorophyllide oxidoreductase; Zn-Pheide *a*, zinc pheophorbide *a*; Zn-Protopheide *a*, zinc protopheide *a*; Chlide684, Chlide672, Pchlide650, Pchlide638, Chlide and Pchlide in the enzyme–substrate complex with absorption maximum at the indicated wavelength

PII: S0014-5793(00)01887-1

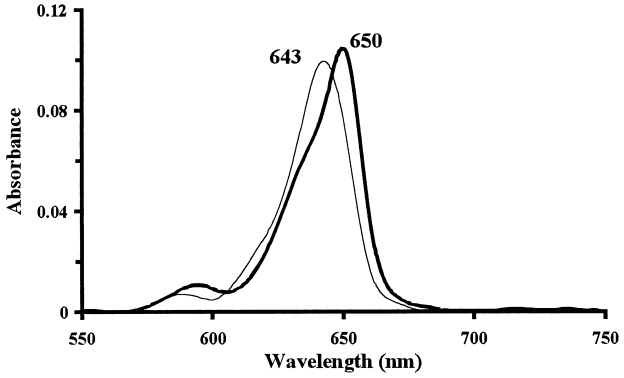

Fig. 1. Absorption maxima of solubilized etioplasts in the presence of 30% (w/v) and 80% (w/v) glycerol. By dialysis of an etioplast preparation in 30% (w/v) glycerol against 80% (w/v) glycerol the absorption maximum of Pchlide *a* (4.15 nmol ml^{-1}) at 643 nm is red-shifted to 650 nm.

diated membranes with octylglucoside yielded only the short-wavelength Pchlide absorbing at 641 nm, and solubilization of both the Chlide684 and the Chlide672 forms obtained by irradiation of the membrane resulted only in the short-wavelength form Chlide672 [11]. The authors stated that the long-wavelength form, "the dominating form in vivo, was not obtainable in solubilized form".

Recently, we reported a simple and rapid method for the preparation of highly active POR apoprotein from oat etioplasts, which was purified to homogeneity and was depleted of Pchlide, carotinoides and lipids [8]. In the present study, we describe conditions for the in vitro transition of soluble Pchlide638 into Pchlide650. Furthermore, we demonstrate the reconstitution of a long-wavelength form of the pigment–POR complex in a solubilized form starting from the individual components. Whereas reconstitution of the POR apoenzyme with NADPH and the zinc analog of Pchlide *a* yielded the photoactive short-wavelength form, the transition to the photoactive long-wavelength form was achieved by addition of plastid lipids and by high concentrations of glycerol. The reconstituted long-wavelength complex shows the typical properties of the in vivo complex including the Shibata shift.

2. Materials and methods

Etioplasts were prepared from 8-day-old etiolated oat seedlings (*Avena sativa* L. cv. Pirol, var. Irlbad) according to [8].

The isolation of lipids was based on a method described in [9]. All organic solvents were chilled and saturated with nitrogen. The procedure was carried out under dim green light. 9.5×10^9 etioplasts were resuspended with 30 ml of resuspension buffer (50 mM NaH_2PO_4, 300 mM sucrose, 100 mM KCl, pH 7.2) and incubated on ice for 30 min. The lipids were extracted with 300 ml of CH_2Cl_2/methanol (1:2). The extract was filtrated and the residue was extracted once more with 100 ml of CH_2Cl_2. The combined organic phase was washed with 150 ml of 100 mM NaCl and the solvent was removed by evaporation. The different lipid classes were separated by column chromatography on silicic acid Si60 (Merck, Darmstadt). The column (19 mm o.d.) containing 28 g of silicic acid was equilibrated with CH_2Cl_2 and a step gradient was applied with CH_2Cl_2, CH_2Cl_2/acetone (2:1), CH_2Cl_2/acetone (1:2), acetone, and mixtures, of acetone/methanol (4:1), acetone/methanol (3:1), acetone/methanol (2:1), and acetone/methanol (1:1). The fractions were analyzed by thin layer chromatography for MGDG and DGDG according to [10]. Lipid–pigment mixtures were prepared for the enzyme assay with purified POR obtained as described below as follows: MGDG and DGDG were mixed in a ratio of nearly 1:1. A portion (8 mg) of this mixture was solubilized with 6 μl of DMSO, and 6 nmol of Zn-Protopheide *a* in 3 μl DMSO was added. Three microliters of this mixture was used per nmol of POR in 1 ml of buffer 1 (10 mM Tricine, 10 mM HEPES/KOH pH 7.2, 0.3 mM Na_2-EDTA, 0.3 mM $MgCl_2$, 14.25 mM *n*-octyl-β-D-glucoside, 30% (w/v) glycerol, 1 mM NADPH).

The fractions for enzyme assays were prepared as follows: 2.4×10^9 etioplasts (fraction ET) were resuspended in 6 ml of buffer 2 (10 mM Tricine, 10 mM HEPES/KOH pH 7.2, 1 mM Na_2-EDTA, 1 mM $MgCl_2$, 15 mM *n*-octyl-β-D-glucoside, 30% (w/v) glycerol, 1 mM NADPH). The preparation of partly purified, but solubilized POR in fraction S3 and the isolation of pigment-free pure POR in fraction EL was performed as described in [8], with the modification, that the concentration of NADPH in S3 was increased from 0.3 to 1 mM.

For dialysis, 3 ml aliquots were added to a dialysis cassette (Type 66380, Pierce). The samples were dialyzed against 500 ml of buffer 3 (10 mM Tricine, 10 mM HEPES/KOH pH 7.2, 0.3 mM Na_2-EDTA, 0.3 mM $MgCl_2$, 80% (w/v) glycerol) during a time period of 14 h at 4°C.

UV/Vis spectra were recorded at room temperature on a double beam spectrophotometer (UV 2401 PC, Shimadzu) equipped with an Ulbricht globe for collection of scattered light.

3. Results

Three fractions obtained from the isolation of POR from

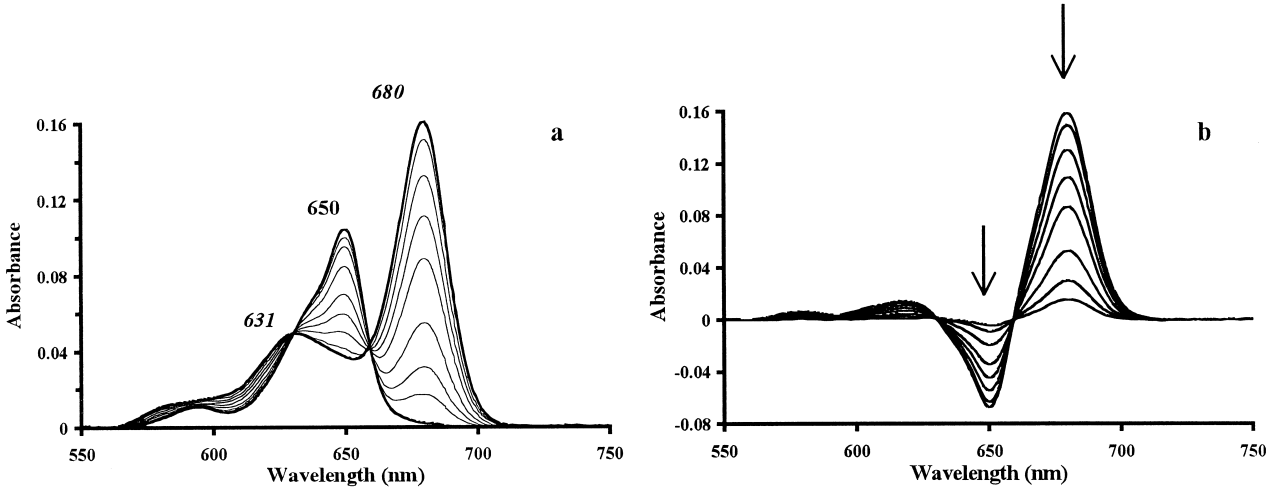

Fig. 2. Monitoring the photoconversion of Pchlide650 in the presence of 80% (w/v) glycerol (the same sample as described in Fig. 1). (a) Absorption spectra recorded after 0, 5, 10, 20, 40, 60, 90, 150 and 270 s irradiation (3.6 μE m^{-2} s^{-1}). (b) Corresponding difference absorption spectra. Dashed arrows indicate the decrease and increase of absorption.

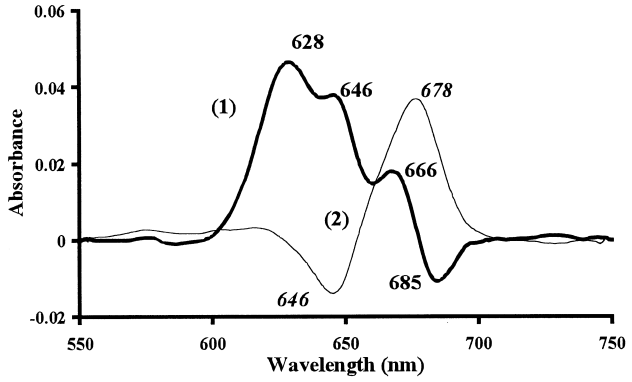

Fig. 3. Spectral shifts after addition of exogenous Zn-Protopheide *a* to the fully photoconverted sample shown in Fig. 1 and subsequent phototransformation. (1) Difference absorption spectrum after addition of Zn-Protopheide *a* (absorption maximum 628 nm) and incubation in darkness for 45 min (positive peaks at 628 and 646 nm). (2) Difference absorption spectrum caused by subsequent irradiation resulting in the formation of Zn-Pheide *a* (678 nm) from Zn-Protopheide *a* (646 nm).

etiolated oat seedlings, by the procedure described in [8], were used to create spectral shifts corresponding to those occurring in vivo. These fractions were the solubilized etioplasts, the partially purified pigment–POR complex (fraction S3), and pigment-free POR (fraction EL).

At first, we examined the spectral shifts in solubilized etioplasts at different glycerol concentrations. In 30% (w/v) glycerol (buffer 2), Pchlide was characterized by an absorption maximum at 643 nm. This confirms the finding of a pigment complex absorbing at 641 nm in similar preparations [11]. Upon dialysis against 80% (w/v) glycerol (buffer 3), this maximum shifted to 650 nm (Fig. 1). If this long wavelength maximum corresponds to the phototransformable form in vivo, then a long wavelength maximum at around 680 nm, due to Chlide, should arise upon irradiation with saturating quantities of white light.

Exposure of the sample for different periods of time to light intensities at 3.6 μE m^{-2} s^{-1} yielded Chlide *a* with an absorption maximum at 680 nm (Fig. 2a). The corresponding difference spectrum (Fig. 2b) of the monitored phototransformation showed two isosbestic points at 630 and 660 nm, indicating a monomolecular reaction type. Predominantly, the Pchlide absorbing at 650 nm was photoconverted, whereas the non-photoconvertible Pchlide631 remained unchanged during the test. The position of the maximum at 680 nm did not change over a long period of observation (15 016 min) if the sample was stored in the dark. Only a minimal blue-shift of about 1 nm was detectable.

Addition of Zn-Protopheide *a* (628 nm) had two effects which were strictly coupled to each other. A shift of Chlide685 to Chlide666, corresponding to the Shibata shift, was initiated immediately after the addition of Zn-Protopheide *a*. At the same time, a long wavelength (646 nm) phototransformable adduct was formed. Both processes were completed in darkness within 45 min (Fig. 3).

The experiments with etioplasts were performed in the presence of 15 mM octylglucoside which should completely solubilize the Pchlide–POR complex [8]. However, we could not exclude the possibility that the described results depend on membrane components which were still present in the preparation. Therefore we repeated the experiments with the Pchlide–POR complex after removal of most of the contaminating proteins and membrane components by ultracentrifugation (fraction S3, see [8]). The completeness of solubilization and the absence of membrane fragments were tested by an additional ultracentrifugation step, at 200 000×*g* for 1 h, of the fraction S3. Even when the concentrations of detergent and glycerol were lowered, no pelleted membranes or spectral shifts in the supernatant were obtained (data not shown).

We obtained basically the same results with fraction S3 as with the solubilized etioplast fraction. In 30% (w/v) glycerol the Pchlide in fraction S3 absorbs at 641 nm. If the sample was exposed to irradiation (3.6 μE m^{-2} s^{-1}) for 20, 60, 120 and 150 s, Chlide absorption maxima detected by difference spectra at 672, 675, 678 to 680 nm arose from respective Pchlide forms at around 635, 637, 640 and 642 nm (data not shown). In an unirradiated sample, which was dialyzed against 80% (w/v) glycerol, an absorption maximum at 649 nm developed with the loss of absorption at 641 nm. This shift is highly similar to the observed spectral shift in the etioplast fraction (Fig. 1). Exposure to white light resulted in the appearance of an absorption maximum at 680 nm. Since the photoconversion in this case was only about 50%, the peak shifted to 672 nm within 90 min without the necessity of adding exogenous pigment.

Fig. 4a shows this shift during dark incubation periods of 15, 30, 45 and 90 min in a sample after partial phototransfor-

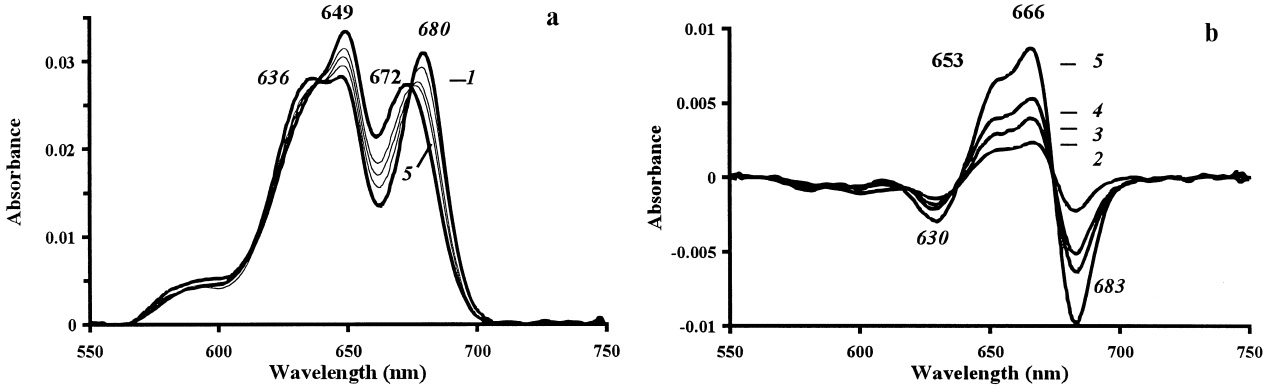

Fig. 4. Monitoring the short wavelength shift (680 nm Ø 672 nm) of Chlide *a* and the simultaneous regeneration of Pchlide *a* absorbing at 649 nm in a partially (50%) photoconverted sample of the fraction S3 (8) in 80% (w/v) glycerol. (a) Absorption spectra obtained by incubation in darkness for 0 min (curve 1), 15, 30, 45 and 90 min (curves 2–5). (b) Corresponding difference spectra to a, showing the regeneration of Pchlide *a* at 653 nm from endogenous Pchlide *a* at 630 nm and the simultaneous shift of Chlide683 to Chlide666.

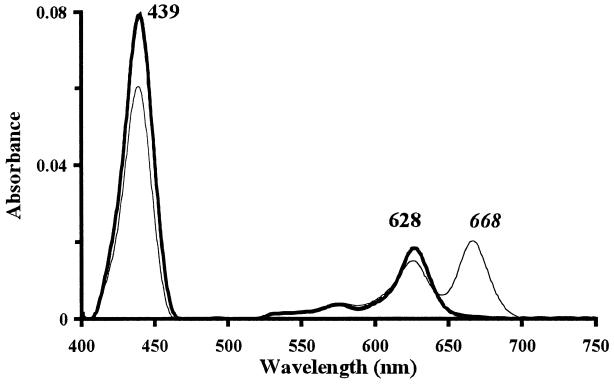

Fig. 5. The phototransformation of Zn-Protopheide *a* with pigment-free purified POR (fraction EL, (8)) in 30% (w/v) glycerol. The irradiation of the sample with 15 μE m^{-2} s^{-1} (60 s) resulted in the formation of short-wavelength Zn-Pheide *a* (668 nm) from short-wavelength educt (628 nm).

mation of Pchlide. The corresponding difference spectra shown in Fig. 4b demonstrate the strict time correlation between the shift and the regeneration of the long wavelength Pchlide from endogenous short wavelength Pchlide absorbing at 630 nm.

Pigment-free, highly active and pure POR can be isolated from fraction S3 by DEAE-cellulose chromatography [8]. In 30% (w/v) glycerol (buffer 1) this pigment-free POR shows in 30% glycerol (buffer 1) only a short wavelength form of added Zn-Protopheide *a* absorbing at 628 nm which is rapidly phototransformed into short-wavelength Zn-Pheide *a* absorbing at 668 nm (Fig. 5). No further shift was observed upon incubation in darkness. The enzyme activity was completely destroyed by dialysis against 80% (w/v) glycerol. This observation raised the question of whether the loss of cofactors which were present before the chromatographic step are responsible for the capability to form photoactive long-wavelength complexes. For all the experiments with this fraction, we added Zn-Protopheide *a* instead of Pchlide a for two reasons. (1) It has been shown that a long-wavelength form at 646 nm, comparable to the 650 nm form of Pchlide, can be produced (Fig. 3, spectrum 1). (2) Zn-Protopheide *a* does not form aggregates in solution, whereas Pchlide does. We isolated and analyzed the lipids from etioplasts. After depletion of carotenoids, the separately purified MGDG (monogalactosyl diacylglycerol) and DGDG (digalactosyl diacylglycerol) were mixed in a molar ratio of about 1:1. Zn-Protopheide *a* was added and the mixture was applied to the pigment-free POR.

Immediately after mixing, we detected an absorption maximum for Zn-Protopheide *a* at 627 nm. The resulting spectrum after dialysis against 80% (w/v) glycerol is shown in Fig. 6a. A maximum at 646 nm had arisen which disappeared upon irradiation. The new absorption peak at 678 nm indicated the formation of long-wavelength Zn-Pheide *a*. Within 30 min of incubation in darkness at room temperature, a blue shift to 668 nm corresponding to the Shibata shift took place, resulting in a short-wavelength Zn-Pheide *a* species, which absorbs at 662 nm according to the difference spectrum (Fig. 6b).

4. Discussion

Non-irradiated etioplast preparations treated with 15 mM octylglucoside and 30% (w/v) glycerol show the main absorbance maximum of Pchlide at 643 nm, which indicates that such samples contain a mixture of the photoactive pigments Pchlide638 and Pchlide650. An absorption maximum at 641 nm has been observed earlier by the treatment of a PLB preparation with 10 mM OG without glycerol [11]. The authors considered this to be due to the short-wavelength form of photoactive Pchlide, especially since photoconversion yielded only the short-wavelength Chlide form, and furthermore discussed that such short-wavelength forms which they considered as non-aggregated pigment–protein complexes are always obtained by solubilization with detergents. We have now found that dialysis of our sample against 80% (w/v) glycerol shifted the absorption maximum towards 650 nm (Fig. 1). This shift occurred with the Pchlide–POR complex in a solubilized form, as shown by ultracentrifugation of the partially purified complex (fraction S3 [8], Fig. 4). The shift of Pchlide638 to Pchlide650 included the bulk of Pchlide in the preparation, and hence has to be considered as being basically different from results obtained by fractionation of etioplast membranes with the aim of preserving the naturally occurring long-wavelength Pchlide form during partial solubilization [12]. The photoconversion of the etioplast (Fig. 2) and the S3 fraction (Fig. 3) in 80% (w/v) glycerol led to a Chlide

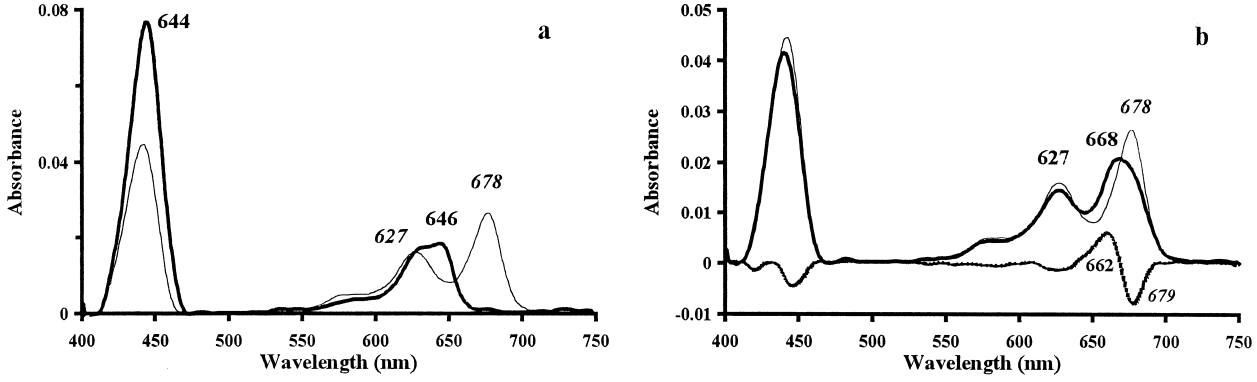

Fig. 6. The phototransformation of Zn-Protopheide *a* with pigment-free purified POR (fraction EL, (8)), supplied with lipids from etioplasts, in 80% (w/v) glycerol and the subsequent short-wavelength shift of Zn-Pheide *a*. (a) The irradiation of the sample with 7.5 μE m^{-2} s^{-1} (120 s) resulted in the formation of long-wavelength Zn-Pheide *a* absorbing at 678 nm from long-wavelength educt absorbing at 646 nm. The short-wavelength absorbing part of the educt was not photoconverted. (b) the short-wavelength shift of Zn-Pheide *a* (678 nm Ø 668 nm) after incubation in darkness for 30 min. In the corresponding difference spectrum, the shift is observed from 679 to 662 nm (dotted line).

maximum at 680 nm. Upon complete photoconversion, this maximum remained stable during a following long dark incubation, a result which agrees with an earlier observed inhibitory influence of glycerol on the Shibata shift [13]. The addition of exogenous pigment (Zn-Protopheide *a*) caused not only an immediate blue shift, but also a simultaneous red shift of the substrate–enzyme adduct (Fig. 3). The replacement of POR-bound endogenous Chlide *a* in etioplast membranes by exogenous Pchlide *a* had been shown before [14]. The occurrence of the Shibata shift became obvious in the S3 fraction after partial photoconversion (Fig. 4). In contrast to the case with the etioplast fraction, it was not necessary to add exogenous pigment to induce the shift, most probably because of the presence of non-photoconvertible Pchlide630, which was restored to Pchlide650 simultaneously to the shift of Chlide680 to Chlide672.

In 30% (w/v) glycerol, our preparation of pigment-free, active and pure POR protein showed only the short-wavelength form of Pchlide and after photoconversion only the short-wavelength form of Chlide. In contrast, no long-wavelength substrate–enzyme adducts nor any photoreduction of added substrates were observed in 80% (w/v) glycerol. The situation changed dramatically, however, if MGDG and DGDG were applied before dialysis against 80% (w/v) glycerol. After dialysis, the sample behaved like the etioplast sample or fraction S3. We found not only the formation of a long-wavelength absorbing substrate–enzyme adduct, but also a blue-shift of the product after irradiation and incubation in darkness (Fig. 6). This result provides strong evidence for the participation of the two lipids in the formation of phototransformable, long-wavelength absorbing Pchlide in vitro and probably also in vivo.

The molecular basis for the long-wavelength shift of Pchlide and Chlide is still under discussion. Most authors favor aggregation of the pigment–POR complex as the reason for the long-wavelength absorption and consequently disaggregation as the reason for the hypsochromic shift (Shibata shift) [16]. Indirect evidence was obtained for the connection of bathochromic/hypsochromic shifts, interpreted as aggregation/disaggregation, with protein phosphorylation/dephosphorylation in vivo [15]. Even if these reactions are coupled in vivo, our results demonstrate that such biochemical reactions are not an obligatory prerequisite for the spectral shifts, but rather that the shifts are inherent properties of the pigment–POR complexes in the presence of lipids from the plastid membranes. Lipids had been present in the preparations of previous authors, but their essential significance for the spectral shifts was only detected now with our lipid- and pigment-free preparation of the POR apoprotein [8]. One can hypothesize that the lipids allow aggregation of the Pchlide–POR complex but not of the Chlide–POR complex, so that the photoconversion leads to spontaneous disaggregation. Besides the possibility of influencing the aggregation state of pigment–POR complexes, one should consider that the lipid mixture of about equal amounts of MGDG and DGDG is a well-known candidate for phase transitions. In such a mixture, a lamellar liquid phase is formed at high water content and a liquid bicontinuous cubic phase is found at low water content in vitro [5]. The paracrystalline structure of PLBs has been identified as such a cubic phase containing two independent and interwoven branched water channels [5]. We propose now that reduction of the water potential by high glycerol concentrations leads to formation of cubic phases similar to the PLB structure. Furthermore, we propose that the Pchlide–POR complex has a preference for the lipid surface towards the 'inner' water channel, thus coming close to neighboring Pchlide–POR complexes, which causes the long-wavelength shift of the absorption, and that the Chlide–POR complex has either a preference for the opposite lipid surface towards the 'outer' water channel or induces a phase transition from the cubic to a lamellar phase. In either case, the relocation of the Chlide–POR complex would result in the Shibata shift. Although these proposals are speculative, they are able to explain all observations described so far. Furthermore, they allow new predictions which can be tested experimentally and thus open up new perspectives for research on molecular changes occurring during the early stages of greening in higher plants, in particular on the transition of etioplasts to chloroplasts.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft, Bonn (SFB 533) and the Fond der Chemischen Industrie, Frankfurt.

References

[1] Fujita, Y. (1996) Plant Cell Physiol. 37, 411–421.
[2] Runge, S., Sperling, U., Frick, G., Apel, K. and Armstrong, G.A. (1996) Plant J. 9, 513–522.
[3] Griffiths, W.T. (1978) Biochem. J. 174, 681–692.
[4] Ryberg, M. and Dehesh, K. (1986) Physiol. Plant. 66, 616–624.
[5] Selstam, E. and Wigge, A.W. (1993) in Pigment–Protein Complexes in Plastids, (Sundqvist, C. and Ryberg, M., Eds.), pp. 241–277, Academic Press, San Diego.
[6] Birve, S.J., Selstam, E. and Johansson, L.B.-A. (1996) Biochem. J. 317, 549–555.
[7] Griffiths, W.T. (1991) in: Chlorophylls, (Scheer, H., Ed.), pp. 433–449, CRC Press, Boca Raton.
[8] Klement, H., Helfrich, M., Oster, U., Schoch, S. and Rüdiger, W. (1999) Eur. J. Biochem. 265, 862–874.
[9] Sprague, S.G. and Staehelin, L.A. (1987) Methods Enzymol. 148, 319–327.
[10] De Santis-Maciossek, G., Kofer, W., Bock, A., Schoch, S., Maier, R.M., Wanner, G., Rüdiger, W., Koop, H.-U. and Herrmann, R.G. (1999) Plant J. 18, 477–489.
[11] Wiktorsson, B., Ryberg, M., Gough, S. and Sundqvist, C. (1992) Physiol. Plant. 85, 659–669.
[12] Chahdi, M.A.O., Schoefs, B. and Franck, F. (1998) Planta 260, 673–680.
[13] Brodersen, P. (1976) Photosynthetica 10, 33–39.
[14] Oliver, R.P. and Griffiths, W.T. (1982) Plant Physiol. 70, 1019–1025.
[15] Wiktorsson, B., Ryberg, M. and Sundqvist, C. (1996) Plant Physiol. Biochem. 34, 23–34.
[16] Böddi, B., Lindsten, A., Ryberg, M. and Sundqvist, C. (1989) Physiol. Plant. 76, 135–143.
[17] Rüdiger, W. and Schoch, S. (1988) in: Plant Pigments (Goodwin, W.S., Ed.), pp. 1–59, Academic Press, San Diego.
[18] Henningsen, K.W. and Thorne, S.W. (1974) Physiol. Plant. 30, 82–89.
[19] Böddi, B., Lindsten, A., Ryberg, M. and Sundqvist, C. (1990) Photochem. Photobiol. 52, 83–87.