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Dynamic interconversion and phototransformation processes of protochlorophyllide complexes during greening

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ABSTRACT Photoreduction and interconversion processes of different protochlorophyllide complexes were studied in epicotyl segments of dark-germinated pea (*Pisum sativum* L.) seedlings illuminated with low-intensity white or 632.8 nm laser light. Analyses of 77 K fluorescence emission spectra showed the direct phototransformation of the monomer, 636 nm emitting complex. The 629 nm emitting complex containing also monomer pigment, regenerated all longer wavelength, flash-photoactive complexes. A dynamic transformation of the protochlorophyllide complexes was also detected during the illumination and/or the subsequent dark-incubation. On the basis of these results, the protochlorophyllide phototransformation schemes can be completed with new pathways.
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KEY WORDS

epicotyl
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In higher plants, the protochlorophyllide (Pchlide) → chlorophyllide (Chlide) photoconversion catalyzed by the light-dependent NADPH:protochlorophyllide oxidoreductase (POR, EC1.3.1.33) is a key step in the chlorophyll biosynthesis (Masuda and Takamiya 2004). In dark-grown angiosperm seedlings Pchlide is accumulated in different pigment-protein complexes in the etioplast inner membranes (prolamellar bodies and prothylakoids), therefore it shows spectral multiplicity. In 77 K fluorescence emission spectra of etiolated leaves four bands were identified with maxima at 631-633, 644-645, 655-657 and 668-670 nm (Böddi et al. 1992). In addition, a minor band at 628 nm was found in the fluorescence spectra of wheat leaves, measured at 10 K (Kis-Petik et al. 1999). The relative ratios of these bands depend on age and growing conditions and vary at species, but usually the 655 nm band is dominant. However, the fluorescence spectra of etiolated stems are different. The main emission peak at 631 nm is composed of two bands with maxima at 629 and 636 nm, the bands at 644, 655 and 670 nm are only minor components in the spectra (Böddi et al. 1998). The different emission bands correspond to various Pchlide and/or Pchlide ester complexes, which are designated in this work as Pxxx, where xxx indicates the fluorescence emission maximum of the given complex. The majority of Pchlide forms ternary complexes with POR protein units and NADPH; these ternary complexes occur in monomer, dimer and oligomer states. P644 and P655 are supposed to be a dimer and oligomer, respectively, of these ternary complexes (Böddi et al. 1992). Both localized in the prolamellar bodies: P655 is an integral component of the interior membranes, whereas P644 is localized mainly on the surface regions (Ryberg and Sundqvist 1982). Pchlide molecules in P629, P633 and P636 are not thought to be bound to functioning POR enzyme. Both P629 and P636 in pea epicotyl contain monomer Pchlide (Böddi et al. 1998). P633 was found in the PT membranes (Ryberg and Sundqvist 1982), but the localization of P629 is unknown.

Various Pchlide forms have different roles in Chl biosynthesis. The flash-photoactivity of P644 and P655 are well known, they transform directly into C684 and C688 (Cxxx where xxx indicates the fluorescence emission maximum of the given chlorophyllide complex). During this process dynamic equilibrium is hypothesized (Kahn and Nielsen 1974) between P644 and P655. P629, P633 and P636 are suggested only to regenerate the previous flash-photoactive complexes, earlier no direct photoactivity of these complexes was detected (Schoefs 2001).

Materials and Methods

Five-eighteen day-old dark-germinated pea (*Pisum sativum* L. cv. Zsuzsi) seedlings were used in the experiments. The middle segments were longitudinally sectioned into 2 or 3 parts. One part was used as a dark control, the others were illuminated or dark-incubated in different ways. The light sources were tungsten lamp and 632.8 nm He-Ne laser light (S101 Globios MOM Budapest). 77 K fluorescence emission spectra were measured with Jobin Yvon - Horiba Fluoromax 3 spectrofluorometer. Spectrum correction and analyses were done with software SPSERV (copyright Bagyinka Cs. BRC, Szeged).

Results and Discussion

To study the phototransformation of monomer Pchlide, pea epicotyls were illuminated with low intensity white or 632.8 nm laser light at room temperature or at -15°C. The difference spectra of illuminated and non-illuminated samples showed the flash-photoactivity of P636 into a 678 nm-emitting Chlide form. Short illumination with laser light provoked the

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phototransformation of P636 more effectively, than white light. At -15°C, all molecular movements are slowed down or inhibited, which would allow aggregation of P636 into longer wavelength forms, and this way the indirect phototransformation of P636 has small probability. The flash-photoactivity of P636 proves that this form is a ternary complex of POR, Pchlide and NADPH. Considering this reaction, the protochlorophyllide flash-phototransformation has three parallel pathways as follows:

P636 → C678
P644 → C684
P655 → C688

However, longer illumination with laser light caused parallel phototransformation of the aggregated Pchlide forms. The following aggregation processes can explain this:

P636→P644→P655

Consequently, direct and indirect phototransformation of P636 took place. The details of this work have been published in Kósa et al. (2005). No direct photoreduction of P629 was observed, however 6 hours continuous illumination with white light of low intensity caused complete phototransformation of this form, too. This shows that the P629 takes part in the greening process. This is in good agreement with earlier models showing that the P629 (P633 in leaves) regenerates the longer wavelength flash-photoactive Pchlide forms (Schoefs et al. 2000). These regeneration processes were studied in illuminated and subsequently dark-incubated (30 minutes) pea epicotyls. The comparison of the spectra of illuminated and dark-incubated samples showed the following regeneration processes:

P629→P636
P629→P644
P629→P655

On the basis of our results, the Pchlide → Chlide reaction schemes known from the literature can be completed with new transformation pathways.

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