A comparative study of protochlorophyllide spectral forms during development and greening of four varieties of dark-grown plants

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Abstract: Protochlorophyllide (Pchlide) is an intermediate in the chlorophyll biosynthetic pathway of photosynthetic organisms. Different spectral forms of Pchlide characterized by their absorption, fluorescence excitation and emission spectra accumulate in dark-grown angiosperms. In a comparative study the Pchlide spectral forms of four varieties of dark-grown plants were followed during development and greening. Similar forms were present in all varieties although the exact peak position varied slightly. The emission spectra of maize (*Zea mays*) and wheat (*Triticum aestivum*) leaves had a minor short-wavelength band at 631 nm and a main peak present at 656 nm. For pea (*Pisum sativum*) and especially its *lip1* mutant, however, the 631 nm band surmounted the 656 nm peak. Incubation with 5-aminolevulinic acid changed the ratio in favor of the short-wavelength Pchlide forms for all the varieties. The fluorescence excitation band at 632 nm was especially increased and dominated the spectrum. An energy transfer from short- to long-wavelength Pchlide forms could occur. Sometimes the energy transfer is reduced as a consequence of a separate localization of the different Pchlide spectral forms. The presence of several far-red Pchlide forms in dark-grown leaves is suggested indicating additional aggregation states of Pchlide under native conditions.

The Pchlide fluorescence lifetime values varied depending on plant variety. They were composed of a fast component in the range 0.3 – 0.8 ns and a slow component in the range 5.1 – 7.1 ns for both the short- and long- wavelength Pchlide forms. Although the short-wavelength Pchlide forms have been regarded to be free pigment, their lifetime values represent a probable protein-bound pigment.

The fluorescence emission spectrum of Pchlide regenerated in darkened green and greening leaves showed mainly the presence of a short-wavelength form. The excitation spectra, however, revealed the presence of an evident amount of long-wavelength Pchlide, from which energy transfer to chlorophyll. The excitation spectra had properties, which reflected those of the dark-grown leaves and were specific for the plant variety. The results support the idea that the chlorophyll biosynthesis in both dark- and light-grown leaves occurs in a similar way, i.e. through the main photoactive long-wavelength form of Pchlide.

The presence of different spectral forms of Pchlide was age dependent and varied with the plant variety. In general at an early stage of development the short-wavelength Pchlide forms were dominating but already in 3-day-old seedlings of wheat and maize the long-wavelength forms was dominant. PORA encoding mRNA observed during day one for *lip1* , but for pea, maize and wheat it could not be observed before the second day. The POR protein was detectable for all plants on the third day and reached a maximum on day six.

In conclusion the characteristics of Pchlide forms, levels of POR protein and its mRNA became stable at a leaf age of 5-6 days and thus differences among the different plant materials tested at this age can be regarded as plant variety differences and not differences depending on age. The specific variability found in dark-grown materials can also be found in plants during greening and reflects also under such conditions a variety dependent mode of chlorophyll formation.

Key words: chlorophyll, chlorophyllide, etiolated leaves, etioplast, fluorescence excitation, fluorescence emission, fluorescence lifetime, Gaussian components, green leaves, *lip1*, NADPH-protochlorophyllide oxidoreductase, *Pisum sativum*, plant development, prolamellar body, prothylakoid, protochlorophyllide, spectral forms, *Triticum aestvium*, *Zea mays*

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