THE ENERGY MIGRATION IN PIGMENT ASSEMBLY IN RELATION TO THE CHLOROPHYLL BIOSYNTHESIS

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The energetic interactions between pigment molecules are characteristic for functioning the photosynthetic apparatus. Therefore a study of the heterogeneous and homogeneous energy transfer is an important tool to learn more about the localization of different pigment molecules and the development of the pigment-pigment interactions in greening and ontogenesis. This approach allowed us to discover the energy transfer to Chl a even from the very first molecules of Chl b in shortly illuminated and darkened etiolated leaves (1). As Chl b arises from Chl a (2,3), their neighbouring position indicates that primary Chl a molecules were located in groups. In this way the hypothesis of group organization of Chl biosynthesis was forwarded.

In fact, a group localization of pigment molecules was revealed even at a stage of Pchlide accumulation. The active Pchlide is aggregated (4-6) and an energy transfer was observed between its various spectral forms as well as from Pchlide to the products of its phototransformation (7-9).

Pchlide reduction is followed by Shibata hypsochromic spectral shift and the energy transfer from Pchlide to Chl(ide) gradually fades due to Chl(ide) movement in the process of membrane development (8). This may be the cause for the absence of the energy transfer from Pchlide, reaccumulated upon repeated darkening, to the previously synthesized Chl (8). The observation of the Pchlide fluorescence in green leaves (10).

Abbreviations: Chl - chlorophyll; Chlide - chlorophyllide; Pchlide - protochlorophyllide.
was also explained (11) by a spatial uncoupling Pchlide from Chl in chloroplasts.

Nevertheless, no kind of fractionation of the photosynthetic membranes by using various detergents, ultrasonication, French press, and grinding in combination with centrifugation or electrophoresis led to complete separation of Pchlide from Chl (12,13). The examination of pigment-protein complexes of various ranks of organization was especially impressive in this respect (14-17). Such joint localization of Pchlide and Chl in chloroplast membranes, which persisted at any procedure of membrane disintegration implied that in green leaves an energetic interaction between Chl and Pchlide, resynthesized upon darkening, is still possible. The special search really revealed an energy transfer from Pchlide to Chl in green plant (18). In the following this is described alongside with the development of such a transfer in greening etiolated leaves.

The leaves of barley of wild type and its Chl b-less mutants 2807 and 3613 from collection of H. Sagromsky (the authors are deeply thankful for this kind gift) were used. Three-day-old light-grown leaves of 4 to 5 cm length or 6-day-old leaves of 9 to 10 cm length were cut along the central vein and a solution of digitonin in 5 mM phosphate buffer, pH 7.2, was vacuum-infiltrated in one moiety while the other either remained intact or was infiltrated with the buffer only. In 20 minutes their fluorescence (excitation at 440 nm) and excitation (monitored at 730 nm) spectra were recorded.

It was reported earlier (18) that the infiltration of digitonin into green leaves causes a partial rearrangement of the Chl molecules out of its longwave forms into the shorter-wave ones. The treatment with 4% digitonin results in (Fig. 1a) a decrease in the longwave form alongside with its shift from 740 or 735 nm (before or after 5-h darkening) to 725 nm and a growth of emission at 692-695 nm. Such changes could be ascribed not only to Chl rearrangement out of the longerwave forms but also to a failure in the energy transfer to them. By comparing the excitation spectra (curves 3 and 4 in Fig. 1b) one can see the actual decrease of the band attributed to the energy transfer.