

**THE BIOCHEMICAL AND FUNCTIONAL PROPERTIES OF
THYLAKOID PROTEIN COMPLEXES FROM MESOPHYLL AND
BUNDLE SHEATH CHLOROPLASTS OF THREE TYPES OF C₄
PLANTS**

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Leaves of C₄ plants contain two distinct types of photosynthetic cells, mesophyll (M) and bundle sheath (BS) which are quite different organized both structurally and functionally. There are three C₄ subgroups based on differences in decarboxylating mechanisms: NADP-ME, NAD-ME and PEP-CK type [1]. The BS chloroplasts in NADP-ME subtype are agranal with structures like stroma lamellae of C₃ chloroplasts. The BS and M chloroplasts in both NAD-ME and PEP-CK subtypes are granal but is unknown if the structural and functional heterogeneities in PSI and PSII exist. In NADP-ME species, BS thylakoids exhibit only PSI activity and little is known about the amount, composition, and the function of the PSII centers. Because the conflicting data on PSII activity has been reported, the degree of PSII deficiency in bundle-sheath chloroplasts of NADP-ME species has always been a matter of debate. Discrepancy could be due either to different experimental assay conditions or to cross-contamination of the bundle-sheath with the mesophyll chloroplasts. Pfündel et al. [2] using flow cytometry method for sorting the chloroplasts showed, that PSII light absorption in BS chloroplasts contributes significantly to the functional antenna of PSI despite their relatively low concentration. This agree with observation of Bassi et al. [3] who found that LHCII acts as an antenna of PSI in bundle sheath maize chloroplasts. PSII in BS cells of young seedlings of maize is active but it is progressively reduced during leaf differentiation. A loss of activity was caused by reduced rates of synthesis of polipeptides of oxygen evolving complex (OEC); D1, D2, CP47 polypeptides [4]. The appearance of M and BS chloroplasts is very similar in NAD-ME and PEP-CK types of C₄ plants, but PSII concentration in bundle sheath cells is elevated in NAD-ME species [2]. Information about the organization of chlorophyll-protein complexes and photochemical activity of PSI and PSII in both types of chloroplasts are very limited. Dynamic and reversible changes of composition in thylakoids membranes have great importance to sustain of photosynthetic activities of plants under changing conditions. We have investigated the PSI and PSII activity, the relative amounts of individual thylakoid proteins, composition of Chl-P complexes in M and BS chloroplasts of *Zea mays* (NADP-ME), *Panicum*

miliaceum (NAD-ME) and *Panicum maximum* (PEP-CK) C4 species growing in the same conditions.

Chloroplasts of BS cells have been obtained by mechanical isolation or by enzymatic digestion. Chl-P complexes were analyzed by polyacrylamide native gel electrophoresis and fluorescence excitation, and emission spectra at 25°C and 77K. The electron-transport activities were determined using oxygen electrode or DCPIP/DPC assay. Proteins were identified by immunodetection.

Chloroplasts of BS cells, which were obtained by enzymatic digestion, as compared to chloroplasts isolated mechanically, had lower by about 60% chlorophyll *a* fluorescence and exhibited rapid proteolysis of Chl-P complexes in all species. Western blot analysis of BS chloroplasts showed that 33 and 23 kDa proteins from OEC, α CF1 and D1 proteins has been partly lost in enzymatic preparations; during mechanical isolation all analyzed subunits were present. Thus, in further studies only chloroplasts isolated mechanically were used. Immunological work provides evidence that OEC occurs in the BS chloroplasts of maize and substantial amounts of some of PSII polypeptides analyzed were still detectable, whereas PSII activity was not noticeable. The results presented in this paper show that PSII is essentially inactive in maize bundle sheath tissue, whereas PSI activity was 2-fold higher in BS than in M. Bundle sheath chloroplasts contained significant amounts of PSI core complex and PSI-LHCI, and the cyt. b/f complex but lacking a major portion of PSII core complex and the trimeric LHCII subcomplex. As detected by the specific D1 antibody the two forms of the D1 protein were present in the M and BS chloroplasts. Besides the chloroplasts from M and BS cells in all C4 species differ slightly in Chl-P complexes composition as shown by both electrophoresis and fluorescence emission, and excitation spectra. There was close correlation between Chl-P complexes composition and PSI and PSII activity in M and BS chloroplasts. Higher activity of PSI was accompanied with larger amount of PSI-LHCI in M and BS chloroplasts of *P. miliaceum*, and in BS chloroplasts of *Z. mays*. In both *Panicum* species PSII was more active in M than in BS chloroplasts, where higher amount of oligomeric form of light harvesting complex was noted. Our results suggest that M and BS chloroplasts of three subgroups of C4 plants growing under the same conditions differ with respect to organization and activity.

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