The Three Dimensional Structure of Chloroplast Membranes. 3D Imaging of CLSM Data

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Keywords: 3D chloroplast structure, confocal laser scanning microscopy, 3D imaging

In higher plants photosynthetic light reactions are mediated by chlorophyll-protein complexes which are embedded in thylakoid membranes inside the chloroplast. Thylakoid membranes are differentiated into cylindrical granum stacks of appressed (stacked) membranes and non-appressed (unstacked) stroma thylakoids. This type of spatial organization corresponds to lateral segregation of main photosyntetic complexes. Dimeric form of photosystem II complex (PSII) occurs in grana while monomeric PSII mostly in stroma thylakoids. Photosystem I complex (PSI) is localized in non-appressed regions: stroma lamella and grana margins [1].

Currently there are two main methods for high resolution analysis of the three dimensional (3D) network of thylakoid membranes: electron microscopy (EM) and electron tomography (ET). Comparison of data obtained from EM and ET experiments lead to creation of a new quasihelical model of thylakoid membranes [2].

In earlier studies we have shown another approach to chloroplast structure analysis by means of fluorescence confocal laser scanning microscopy (CLSM) [3,4]. This work is a continuation of these researches and was extended by 3D imaging of CLSM data.

In situ CLSM measurements were performed using intact chloroplasts which were isolated from leaves of several plant species (e.g. pea and bean) or plants grown in different light or temperature conditions. Collected data stacks were subjected to deconvolution procedure to increase image resolution and signal-to-noise ratio. Deconvolved images were subsequently processed using 3D imaging software to obtain final structure. Example for 3D imaging of CLSM data is shown on figure 1. For detail spectral analysis of chlorophyll fluorescence and localization of PSII complexes inside thylakoid membranes, a confocal microscope equipped in spectral detector was used.

CLSM imaging enables the analysis of intact chloroplasts without any fixing procedures and guarantees native origin of observed structures.

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5. This research was supported by Polish Ministry of Science and Higher Education founds (N303 4185 33).

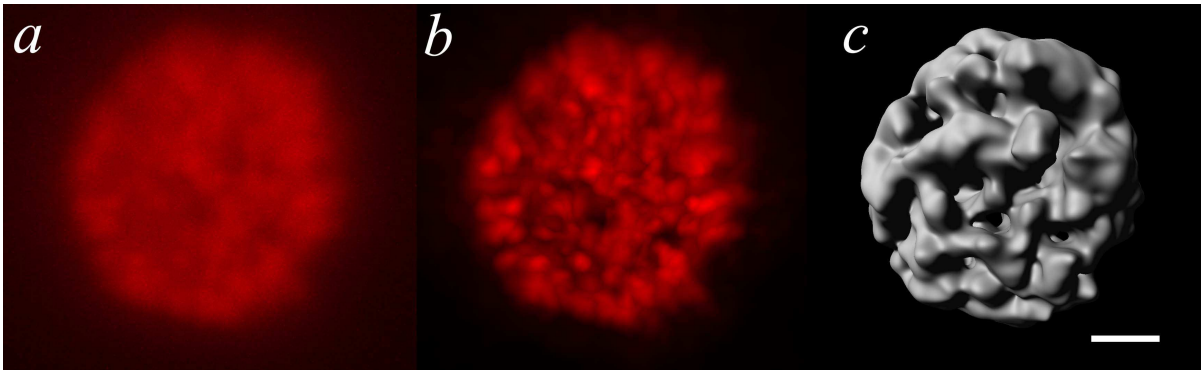

Figure 1. Images of intact chloroplast isolated from bean leaves (plants were grown at low light conditions) performed by CLSM and further 3D imaging. To improve signal-to-noise ratio raw CLSM data (*a*) were deconvolved (*b*) using the AutoQuant X2 software (Media Cybernetic, Bethesda, USA). Three dimensional model of thylakoid surface (*c*) was obtained using the Imaris 6.1.0 software (Bitplane, Zurich, Switzerland). *Bar* - 2 μm.

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-214, © Verlag der TU Graz 2009