Visualizing the Photosynthetic Membrane Proteins in Situ: Atomic Force Microscopy

The photosynthetic membrane is the microscopic powerhouse of our biosphere. It transforms the sun’s energy into the energy of universal biological fuel, ATP and NADPH (light reactions) that is used for the synthesis of Glc from CO₂ (dark reactions). Oxygenic photosynthesis, the process that enabled the explosive diversity of heterotrophic organisms on our planet, oxidizes water in order to drive the light reactions. The photosynthetic membrane contains various types of proteins assembled into multisubunit complexes and often laterally separated heterogenous domains. One such domain is the formation of a three-dimensional, multilayer membrane structure, the granum, with the major membrane protein complex PSII, an enzyme that removes electrons from water and donates them into the energy-transforming photosynthetic electron transport chain. A membrane plane of a granum carries around 100 dimeric PSII complexes (Kouril et al., 2011). In addition, this membrane contains several hundred PSII light-harvesting antenna complexes that greatly enhance light energy input into the reaction center (Johnson et al., 2011), the cytochrome b₆f complex (Johnson et al., 2014), and some PSI complexes of grana margins. In recent decades, excellent atomic resolution structures of all these complexes have been successfully produced (Ben-Shem et al., 2003; Stroebel et al., 2003; Nield and Barber, 2006; Yamashita et al., 2007; Umena et al., 2011; Qin et al., 2015). However, their structural state in the native membrane environment and their collective dynamic rearrangements reflecting the physiologically crucial processes of acclimation and adaptation of the photosynthetic apparatus to environmental factors have only recently started to emerge.

Atomic force microscopy (AFM) of spinach (Spinacia oleracea) membranes enriched in PSII, reported here by Phuthong et al. (2015), is currently one of only a few studies that aims at visualizing the photosynthetic membrane proteins at high resolution (a few nanometers) in an environment close to the native one: unfrozen and aquatic (Sznee et al., 2011; Johnson et al., 2014). Unlike freeze-fracture, negative staining, or cryoelectron microscopy, AFM possesses an enormous potential for the visualization of selected membrane components using the principles of natural protein-cofactor or protein-protein interactions (Johnson et al., 2014). Phuthong et al. (2015) report a high-resolution topography of the lumen-protruding domains of key components of the PSII reaction center: inner antenna chlorophyll proteins, CP43 and CP47, and extrinsic subunits, PsbO/PsbP/PsbQ, involved in oxygen evolution, offering a great potential for monitoring the conformational changes associated with oxygen evolution and related adaptive changes in the complex. In addition, the authors identified a new type of particle protruding on the stromal site of the membrane that is likely to be granal PSI. The report is another milestone on the path to the development of AFM techniques aiming at real-time, in situ resolution and monitoring of the functions of the photosynthetic membrane proteins and their ensembles, an ultimate goal and the future of research in the whole field of cell biology.

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