

Summary

The internal membranes of photosynthetic organisms (the thylakoids in plants and the chromatophores in purple bacteria) provide location for the light reactions of photosynthesis. The protein complexes comprising the photosynthetic machinery are different for plants and purple bacteria; In plants, the Photosystem I and II (PSI, PSII) and their light harvesting antennas (LHCI and II), ATP synthase and cytochrome b_6f complexes are spatially distributed between stacked grana membranes and unstacked single lamellas of the thylakoid system whereas the bacterial photosynthetic reaction centre RC with its core antenna LH1 (RC-LH1) and peripheral antenna LH2 together with bacterial ATP synthase and cytochrome bc_1 are housed within the same intracytoplasmic membrane of phototropic bacteria. This machinery is highly organized and flexible for effective use of light energy.

Atomic Force Microscopy is a unique and powerful tool for acquiring high resolution topographic images of membrane proteins directly in native membranes. Applied to stacked grana membranes from spinach, the AFM revealed high-resolution images of PSII complexes and their organization within these membranes. Four different packing lattices were observed: co-linear rows of close laying PSII that span the entire membrane, nanometric domains of linear or skewed rows, and disordered domains. Although the membranes appear to be densely packed with protein at lower resolution, the highest resolution images reveal large domains without visible protrusions, which are speculated to be filled with the LHCII antenna complexes. Interestingly it transpired that it is possible for PSII complexes to rearrange from random to row-like organizations when temporarily stored at 4 °C despite grana membranes being adhered to mica surface.

Three different working modes of the AFM microscope were exercised in grana membranes imaging: tapping, jumping and contact. All three have the ability to deliver high resolution images with the jumping mode being the least and contact mode the most intrusive. The height of grana membrane measured in jumping and tapping modes are similar at about 19-20 nm while in the contact mode that figure is slightly lower at 18 nm; similarly the height of PSII core particles is the same, about 5 nm, measured by two first methods, and 4 nm as measured by the latter.

The organization of bacterial photosynthetic membrane has been studied by means of AFM and polarized spectroscopy. The AFM tapping mode has been only employed to probe the flat intracytoplasmic membranes of the purple bacterium *Rhodospseudomonas (Rps.) palustris*, whereas the linear dichroism (LD) spectroscopy technique was used to probe the spherical chromatophores of *Rhodobacter (Rba.) sphaeroides* and *capsulatus*, as well as the membranes of *Rps. palustris*.

Rba. sphaeroides and *capsulatus* contain a special protein within the RC-LH1 complex, protein PufX that is essential for the bacterium photosynthetic growth. In *Rba. sphaeroides* the RC-LH1 complex can have either a monomeric or a dimeric form in which two adjoined RC-LH1 are related by an axis of two-fold symmetry. Dimeric RC-LH1 complexes can form ordered arrays within the membranes with the RC locked in a unique orientation within the complex. The creation of those dimers and fixation of RC in a unique position is also facilitated by PufX protein. In absence of this protein the RC can adopt multiple orientations with respect to the LH1 and there are no ordered arrays in the membrane. The RC-LH1 complexes of *Rba. capsulatus* are only monomeric, despite presence of PufX and the chromatophores of *Rba. capsulatus* lack any long range ordering. Even small alterations of PufX can have severe ramifications for the supramolecular

organization. The single point mutations in the *Rba. sphaeroides* PufX and replacement of the *Rba. sphaeroides* PufX by its *Rba. capsulatus* counterpart produced only monomeric RC-LH1 complexes. The chromatophores however were not completely unordered, corroborating earlier observation that presence or absence of order in the bacterial membrane depends not only on presence of PufX. Interestingly, there were ordered and unordered domains of RC-LH1 complexes also present in native chromatophores even when the PufX was missing from the RC-LH1 complex. The degree of organization of RC-LH1 complexes in the membrane was influenced by a type of carotenoid contained in the RC and antennas. The results support the idea that the type of carotenoid has impact on the structural conformation of the RC-LH1 complex that is independent on PufX protein.

Neither the AFM images nor LD spectra of native membranes of *Rps. palustris*, which instead of PufX has a protein W, show evidence of specific long range ordering of the RC-LH1 complexes. The AFM data suggests that cytochrome bc_1 complex is present within the domains of RC-LH1 and it might have additional subunit attached while the ATP synthase located in vicinity of cytochrome bc_1 shows c-ring stoichiometry of 11 although the dimerization of that complex is inconclusive.