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Biochimica et Biophysica Acta 1712 (2005) 109 – 127

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Review

Watching the components of photosynthetic bacterial membranes and their in situ organisation by atomic force microscopy

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Received 3 February 2005; received in revised form 4 April 2005; accepted 15 April 2005
Available online 5 May 2005

Abstract

The atomic force microscope has developed into a powerful tool in structural biology allowing information to be acquired at submolecular resolution on the protruding structures of membrane proteins. It is now a complementary technique to X-ray crystallography and electron microscopy for structure determination of individual membrane proteins after extraction, purification and reconstitution into lipid bilayers. Moving on from the structures of individual components of biological membranes, atomic force microscopy has recently been demonstrated to be a unique tool to identify in situ the individual components of multi-protein assemblies and to study the supramolecular architecture of these components allowing the efficient performance of a complex biological function.

Here, recent atomic force microscopy studies of native membranes of different photosynthetic bacteria with different polypeptide contents are reviewed. Technology, advantages, feasibilities, restrictions and limits of atomic force microscopy for the acquisition of highly resolved images of up to 10 Å lateral resolution under native conditions are discussed. From a biological point of view, the new insights contributed by the images are analysed and discussed in the context of the strongly debated organisation of the interconnected network of membrane-associated chlorophyll–protein complexes composing the photosynthetic apparatus in different species of purple bacteria.

Keywords: AFM; Photosynthesis; Purple photosynthetic bacteria; Light harvesting complexes; Reaction centre; Core complex; Photosynthetic unit; *Blastochloris viridis*; *Rhodospirillum photometricum*; *Rhodobacter blasticus*; *Rhodobacter sphaeroides*

Contents

Abbreviations: AFM, Atomic Force Microscopy; EM, Electron Microscopy; LH, light-harvesting; RC, reaction centre; 4Hcyt, tetraheme cytochrome; cyt *bc1*, Cytochrome *bc1* complex; PSU, photosynthetic unit; *Rb.*, *Rhodobacter*; *Rsp.*, *Rhodospirillum*; *Blc.*, *Blastochloris*; *Rvi.*, *Rubrivivax*; *Rps.*, *Rhodopseudomonas*

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doi:10.1016/j.bbamem.2005.04.005

1. Introduction

About 25% of the sequenced genomes of different eukaryotic and prokaryotic organisms are predicted to code for membrane proteins and an even larger fraction code for membrane-associated proteins [1]. This large fraction reflects the importance of membrane proteins which cover a wide spectrum of fundamental biological processes. The function, sequence and topology of many of these membrane proteins have been extensively studied. However, despite the important information obtained from biochemical, biophysical and molecular biology strategies, our understanding of membrane phenomena is severely hindered by a dearth of structural information. Indeed, as compared to soluble proteins, only few structures of membrane proteins have been solved by X-ray and electron crystallography (http://www.rcsb.org/pdb/; http://www.mpibp-frankfurt.mpg.de/michel/public/memprotstruct.html).

In this structural context, the atomic force microscopy (AFM) [2] has developed into a powerful tool in membrane protein research and is now a complementary technique to X-ray crystallography and electron microscopy (EM) [3,4]. The AFM is a remarkable instrument allowing high resolution topography of biological samples to be acquired near physiological conditions, i.e., in buffer solution at room temperature and under normal pressure [5–16]. Using the AFM, the heights of membranes and protruding structures can be measured accurately with a vertical resolution of ~1 Å [8,17,18]. The high signal-to-noise ratio of the instrument enables the oligomeric state and sidedness of membrane proteins directly to be assessed in raw data images [19–21] and poorly ordered single particles to be recognized and imaged at high resolution (~10 Å) [4,13,19–26]. The AFM has developed to the point that it permits now to detect flexible and stable extrinsic domains [18,27] and to perform time lapse AFM allowing identification of structural changes of molecules as a function of time [24,28–31]. Further, by applying loading forces to the AFM tip, biological samples can be nanodissected to give insights into protein assembly [21,30,32–36]. Finally, in addition to being used as a microscope, an AFM can measure biomolecular forces with piconewtons sensitivity. Using this method, known as force spectroscopy, a variety of inter- and intramolecular forces have been measured, including protein–protein interaction forces, unfolding forces and molecular recognition with specific antibodies [37–40]. Taking together, although the AFM will never provide an atomic structure due to its restriction to surface contouring, several examples demonstrate the capability of AFM to resolve characteristic substructures of single individual proteins, to study conformational changes, to analyse protein–protein interactions and the assembly of membrane proteins.

Most AFM reports have been related to single membrane proteins reconstituted into lipid bilayers as two-dimensional (2D) crystals or densely packed proteins [6,19–21,31,41,42]. Thus, as for X-ray and electron crystallographic approaches, AFM approaches require the difficult solubilisation, purification and reconstitution steps and are also limited by the high quantity of material required for structure determination. These limitations are even more crucial for the structural analysis of supramolecular complexes, in which different membrane proteins have to interact specifically to fulfil their physiological function. It is obvious that membrane protein structural biology is in need of a technique with a resolution high enough to identify the individual components of a multi-protein complex and to analyse their supramolecular organisation needed for relevant functions.

The last 2 years, high-resolution imaging of non-crystalline native membranes have been reported by AFM [30,43–47]. In particular, the value of this technique has been demonstrated in analysing the assembly of multi-component membrane protein complexes in native membranes of photosynthetic bacteria [30,43–46]. These anaerobic photosynthetic prokaryotes fuel their metabolism with light energy and have developed for this purpose a highly efficient photosynthetic apparatus consisting of a highly organised nanometric assembly of transmembrane protein complexes [48]. Absorption of light and its conversion into chemical energy require only four transmem-