ELSEVIER

Current Opinion in Colloid & Interface Science 13 (2008) 338–350

Current Opinion in COLLOID and INTERFACE SCIENCE

www.elsevier.com/locate/cocis

Strategies to prepare and characterize native membrane proteins and protein membranes by AFM

Daniel J. Muller [a,*], Andreas Engel [b]

[a] *Center of Biotechnology, Technische Universität Dresden, Tatzberg 47-51, 01307 Dresden, Germany*
[b] *M.E. Müller Institute for Structural Biology, Biozentrum, University of Basel, Klingelbergstrasse 70, 4056 Basel, Switzerland*

Received 13 June 2007; accepted 19 September 2007
Available online 26 September 2007

Abstract

Progress in characterizing native membrane proteins and protein membranes by atomic force microscopy (AFM) opens exciting possibilities. While the structure, oligomeric state and supramolecular assembly of membrane proteins are assessed directly by AFM, single-molecule force spectroscopy (SMFS) identifies interactions that stabilize the fold, and characterize the switching between functional states of membrane proteins. But what is next? How can we approach cell biological, pharmaceutical and medical questions associated with native cellular membranes? How can we probe the functional state of cell membranes and study the dynamic formation of compartments? Such questions have been addressed by immobilizing membranes on solid supports, which ensures the integrity of the native state of membrane proteins but does not necessarily provide a native-like environment. Direct attachment of membranes to solid supports involves non-specific interactions that may change the physical state of supported lipids and proteins possibly hindering the assembly of membrane proteins into native functional compartments. Thus, to observe the dynamic assembly and working of proteins in native membranes by AFM, supports are required that mimic the native environment of the cell membrane as closely as possible. This review reports on recent progress in characterizing native membrane proteins by AFM, and surveys conventional and new approaches of supporting surfaces, which will allow the function, dynamics, and assembly of membrane proteins to be studied by AFM in native cell membranes.

Keywords: Atomic force microscopy; Compartments; Native protein membranes; Single-molecule force spectroscopy; Tethered membranes; Polymer supported membranes; Protein assembly; Structure and function relationship; Molecular interactions

1. Introduction

Membranes are vital components of all living systems, forming the boundaries of cells and their organelles. Biological membranes consist largely of a lipid bilayer and of proteins embedded in or anchored to the bilayer. Lipids and membrane proteins form domains, which can vary and adapt to the functional state of the cell [1,2]. Thus, a cellular membrane can be viewed as a unique and dynamic combination of various domains coupled together spatially and functionally. The dynamic assembly of such multicomponent complexes into compartments ensures a high efficiency of various processes taking place simultaneously and warrants organelle integrity. Such supramolecular assemblies of membrane proteins are involved in essentially all processes of life, i.e., signal transduction, energy conversion, cell–cell communication, cell adhesion, trafficking, and transmembrane transport. In contrast to this importance, little is known about how single components, proteins and lipids, are organized into higher order structures and how this organization determines their biological activity.

Atomic force microscopy (AFM) enables imaging of native membrane protein surfaces at subnanometer resolution [3]. An important advantage over conventional optical microscopes is that AFM does not require fixation or labeling of the sample. Importantly, AFM allows observing the biological membrane in

Abbreviations: AFM, atomic force microscopy; AQP, aquaporin; SMFS, single-molecule force spectroscopy; LH1, light-harvesting1; LH2, light-harvesting2; RC, reaction center.

* Corresponding author. Tel.: +49 351 463 40331.
E-mail address: mueller@biotec.tu-dresden.de (D.J. Muller).

1359-0294/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cocis.2007.09.002

the buffer solution and at the temperature needed to maintain its physiological function. In the past 15 years AFM has been established as an imaging tool to observe the structure of native membrane proteins embedded in the lipid membrane. A lateral spatial resolution of up to 0.5nm and an exceptional signal-to-noise ratio suffice to directly observe the oligomeric assembly of single membrane proteins such as channels, receptors, or transporters, to characterize their structural features, and to visualize single polypeptide loops connecting transmembrane alpha-helices or beta-sheets. Since AFM allows imaging of membrane proteins under physiological conditions, this technique has made it possible to directly observe membrane proteins at work [3–5••], and to track their diffusion in the lipid bilayer at subnanometer resolution [6]. Most of these exciting results have been achieved on reconstituted membrane proteins or on membranes containing only one or a few different membrane protein species [7]. However, within the past decade first attempts have been undertaken to image the supramolecular assembly of reconstituted membrane protein complexes [8••,9] and of proteins in native membrane patches extracted from cellular compartments [10••,11,12••,13•].

For high-resolution imaging by AFM membranes should be immobilized on supporting surfaces. In most cases, this attachment is facilitated by the adsorption of the protein membrane to a relatively chemically inert and hydrophilic solid support. Such so-called solid-supported membranes have allowed, and will still allow us, to gain important insights into the structure and function relationship of native membrane proteins. However, solid-supported membranes may be disadvantageous depending on the question addressed. For example, membrane proteins in membranes directly attached to the support often show impaired mobility [14••,15••]. This is because the gap between membrane and support is only 0.5–2nm, which induces steric constraints that impede the displacement of the protein. Additionally, the adsorption energy may have a feedback on the structural assembly of membrane proteins, as small as it is for a chemically inert and hydrophilic support. For example, it is well known that lipids of a solid-supported lipid bilayer can show very different structural assemblies and thus functional states compared to the lipids of a vesicle or a free-standing lipid bilayer [15••,16]. Since both lipids and proteins are mutually involved in the formation of cellular compartments [1,2], we conclude that the compartments of a supported cellular membrane behave differently from that of the native, intact cell membrane. From a biological point of view it is clear that the cell membrane and compartments react sensitively to their environment. Therefore, to be able to study by AFM how membrane proteins assemble into native compartments requires the development of alternative sample preparation strategies that do not potentially influence the process of compartmentalization.

In this review we provide a brief overview of recent achievements in high-resolution AFM imaging of native membrane proteins and protein membranes. Then, we summarize preparation methods to observe native membrane proteins. Finally, alternative techniques that have been developed to support native biological membranes and possibilities to transfer these strategies for high-resolution AFM imaging of native cell membranes and their compartments are described.

2. High-resolution AFM imaging of membrane proteins and protein membranes

2.1. Imaging purple membrane

The most intensively studied membrane protein by AFM is the light-driven proton pump bacteriorhodopsin (BR) (Fig. 1). BR and lipids naturally assemble into a two-dimensional trigonal lattice, the purple membrane of *Halobacterium halobium*. Since purple membrane is relatively easy to extract from the archaebacterium, chemically and physically stable, commercially available, and its structure has been solved by electron microscopy and X-ray crystallography, purple membrane is often used as a reference for high-resolution AFM. A lateral resolution of ≈0.5 nm has been achieved on purple membrane by AFM in buffer solution concomitant with a vertical resolution of ≈0.1 nm [17]. The exceptionally high signal-to-noise ratio of the AFM allowed structural details of single BR molecules to be observed and these structures to be correlated individual polypeptide loops connecting transmembrane α helices [17]. Calculating topographic averages of the BR trimer allows identifying structural details that appear similar among individual BR trimers and structural regions that exhibit an enhanced flexibility [3,18].

Reconstituted porin OmpF membranes and native purple membrane were the first protein membranes that could be imaged at subnanometer resolution by AFM [3]. Since most of the high-resolution topographs were initially achieved on membrane proteins that were two-dimensionally crystallized [3,19,20], it was thought that a crystalline assembly would be a prerequisite for observing membrane proteins at subnanometer resolution by AFM. In the meantime it could frequently be demonstrated that a comparable spatial resolution can be achieved on non-crystalline assemblies of membrane proteins as well [6,11,12••,21,22••].

Several factors may limit the spatial resolution achieved by AFM. To be imaged at subnanometer resolution the protein membrane should be immobilized onto a very flat supporting surface. A soft membrane adsorbed onto a support follows the modulation of the supporting surface. Thus, if the surface roughness of the support is much higher than the corrugations of the membrane protein the finest structural features will not be resolved. Muscovite mica is the most frequently used support for AFM. Fresh cleavage of the layered mica crystal provides highly reproducible atomically flat surfaces that allow adsorption of membranes to be tuned by ionic strength and pH [23]. High-resolution AFM imaging of fragile biological surfaces requires precise control of the force applied and the feedback parameters guiding the AFM stylus to precisely contour the object. So far all subnanometer resolution topographs have been recorded using contact mode AFM. In this imaging mode the force applied to the AFM stylus must be kept constant at a value smaller than 100pN. We have shown that for reproducible acquisition of high-resolution topographs the electrostatic interactions