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Force-induced Conformational Change of Bacteriorhodopsin

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The cytoplasmic surface topography of purple membranes imaged by the atomic force microscope depends mainly on the force applied to the stylus. Imaged at forces of 300 pN, individual bacteriorhodopsin molecules reveal two domains. The resulting donut-shaped trimers reversibly transform into structures exhibiting three prominent protrusions when scanned at 100 pN. In parallel, the height of the protein moiety above the lipid layer increases from 4 Å to 6 Å. From the known structure of bacteriorhodopsin it appears that this change may be related to a bending of the most prominent cytoplasmic loop.

Keywords: atomic force microscopy; purple membrane; bacteriorhodopsin; loops connecting helices; force dependence

Bacteriorhodopsin, a light-driven proton pump (Oesterhelt & Stoeckenius, 1973), is packed into highly ordered two-dimensional (2-D) crystals (Blaurock & Stoeckenius, 1971), the purple membranes, which are integrated into the plasma membrane of *Halobacterium salinarium*. The structure of bacteriorhodopsin has been solved by electron crystallography (Henderson *et al*., 1990) revealing seven closely packed α helices that surround the photoactive retinal (Jubb *et al*., 1984). A similar arrangement of seven α-helical membrane spanning segments has been observed for halorhodopsin (Havelka *et al*., 1993) and rhodopsin (Schertler *et al*., 1993). This feature also appears to represent a common motif of the G protein-coupled receptor family (Baldwin, 1993). Besides electron microscopy (Henderson & Unwin, 1975; Neugebauer & Zingsheim, 1978; Studer *et al*., 1981; Henderson *et al*., 1990), X-ray diffraction (Blaurock & Stoeckenius, 1971; Henderson, 1975), neutron diffraction (Jubb *et al*., 1984; Dencher *et al*., 1989), and, more recently, scanning tunneling microscopy (Fisher *et al*., 1990) and atomic force microscopy (AFM: Butt *et al*., 1990, 1991) have been used to study the purple membrane. Despite these extensive structural analyses, the surface topography of bacteriorhodopsin is not well resolved (Henderson *et al*., 1990). We have established conditions to reproducibly acquire topographs of purple membranes at subnanometer resolution using the AFM and have identified the cytoplasmic surface (Müller *et al.*, 1995) by its preferential adsorption to polylysine (Fisher *et al*., 1978). Time-dependent fluctuations of the force sensing AFM cantilever have recently been interpreted as conformational changes of active lysozyme (Radmacher *et al*., 1994). Here, we demonstrate that subnanometer force-induced conformational changes of bacteriorhodopsin can be directly visualized using this microscope.

Figure 1 shows the cytoplasmic surface of a purple membrane that was adsorbed to mica and imaged in buffer solution using the AFM. Provided the force applied to the stylus ranged between 300 and 600 pN the features observed were reproducible under different conditions and remained stable during hours of repeated scanning. The donut-shaped protrusions arranged in a trigonal lattice ($a = b = 62(\pm 2)$ Å) represent the bacteriorhodopsin trimers. As displayed by the average in the inset, each molecule exhibits two domains of comparable height, $4.1(\pm 0.3)$ Å above the lipid surface.

When the force applied to the stylus was reduced from 300 pN (bottom of Figure 2a) to 100 pN (top of Figure 2a) during imaging, the donut-shaped structure of the bacteriorhodopsin trimer exhibiting six protrusions changed into a trimer that had only three, but more pronounced protrusions. This conformational change was fully reversible. It was thus possible to repeat the process over hours of continuous scanning and imaging of the same area at high resolution. The force-dependent surface

Abbreviations used: AFM, atomic force microscope.

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topography of bacteriorhodopsin is more distinct after local averaging by Fourier peak filtration (Figure 2b). To interpret the structural change, correlation averages of the two conformations were merged and displayed as a relief (Figure 3a). The positions of helices A to G from the atomic model (Henderson *et al*., 1990) are indicated in the corresponding topograph (Figure 3b). At 300 pN (right), the outer domains arranged in a circle of 17 Å radius may represent the loop connecting helices A and B, whereas the inner domains arranged in a circle of 15 Å may represent the loop connecting helices C and D. The most prominent loop at the cytoplasmic surface which connects helices E and F, is probably bent by the stylus. In contrast, the topograph recorded at 100 pN (left) exhibits a prominent protrusion at the position of the E–F loop. It appears that at lower forces this loop extends and rises by approximately 2 Å to a height of 6 Å above the bilayer surface.

The conformational change of the bacteriorhodopsin surface depended mainly on the force applied to the stylus, and the pH of the buffer solution. The force, ΔF, of approximately 200 pN required to induce the change is remarkably close to the binding force of avidin to biotin, which is 160 pN (Florin *et al*., 1994), or to the 340 pN of the streptavidin-biotin bond (Lee *et al*., 1994). Furthermore, the deformation of the surface by about 2 Å corresponds to an energy of 6 kcal/mol. This low value is compatible with the observed reversibility of the process. At basic pH, the effect was more readily and reproducibly achieved than at acidic pH, where the characteristic trimeric protrusions were not stable even at minimal force. Since the purple membrane is known to change its thickness by 10% at pH 5.2 (Henderson, 1975; Müller *et al*., 1995), the isoelectric point of bacteriorhodopsin (Ross *et al*., 1989), our data suggest that the loop connecting helices E and F may protrude out of the surface due to electrostatic forces at high pH values. Our observations imply that subnanometer conformational changes of single loops connecting transmembrane helices can be reproducibly imaged by the AFM. This opens new avenues for analyzing the relationship between structure and function of biomolecules. However, imaging at forces $\leqslant 100$ pN is required to minimize distortions of the native conformation by the stylus.

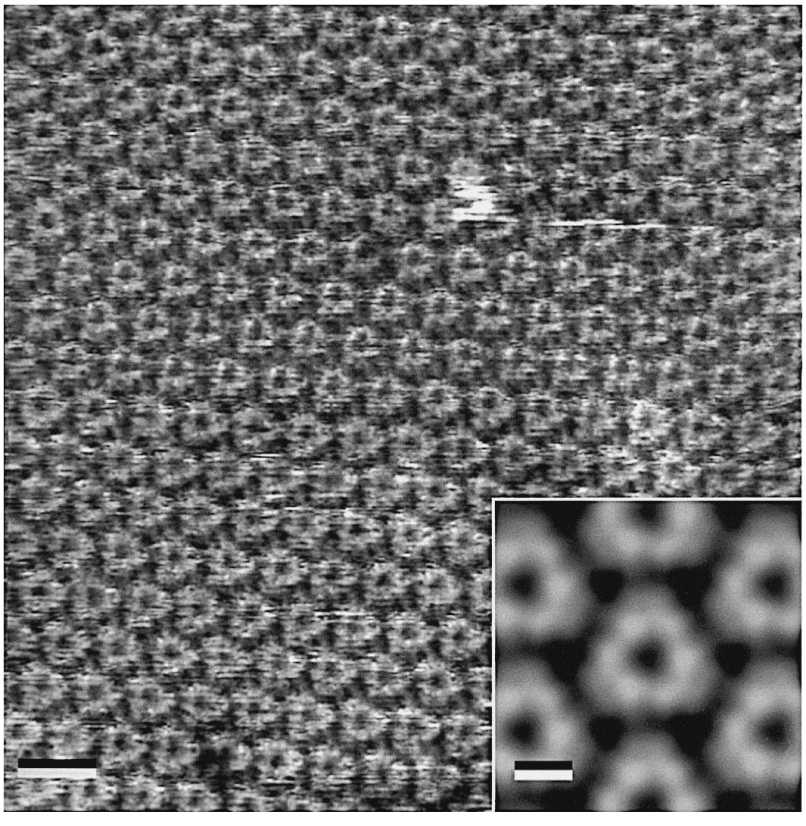

Figure 1. Cytoplasmic surface of purple membrane in aqueous solution. The AFM topograph (scale bar represents 10 nm) shows individual bacteriorhodopsin molecules imaged at subnanometer resolution. As shown in the inset (scale bar represents 2 nm), bacteriorhodopsin molecules exhibit 2 protrusions at their cytoplasmic surface. Purple membranes (50 μg/ml) suspended in 150 KCl, 10 mM Tris (pH 9.2) were adsorbed to freshly cleaved mica for 30 minutes, and subsequently washed to remove membranes that were not firmly adsorbed. High magnification scans were recorded at a force of approximately 300 pN with a scan speed of 2 μm/second. AFM measurements were performed with a Nanoscope III AFM (Digital Instruments, Santa Barbara, CA) equipped with a fluid cell. A 15 μm or a 130 μm piezo scanner and oxide sharpened silicon nitride tips mounted on a 100 μm cantilever (Olympus Ltd., Tokyo, Japan) were used. The averaged and 3-fold symmetrized topograph was calculated from approximately 220 unit cells using the SEMPER image processing system (Saxton *et al*., 1979).

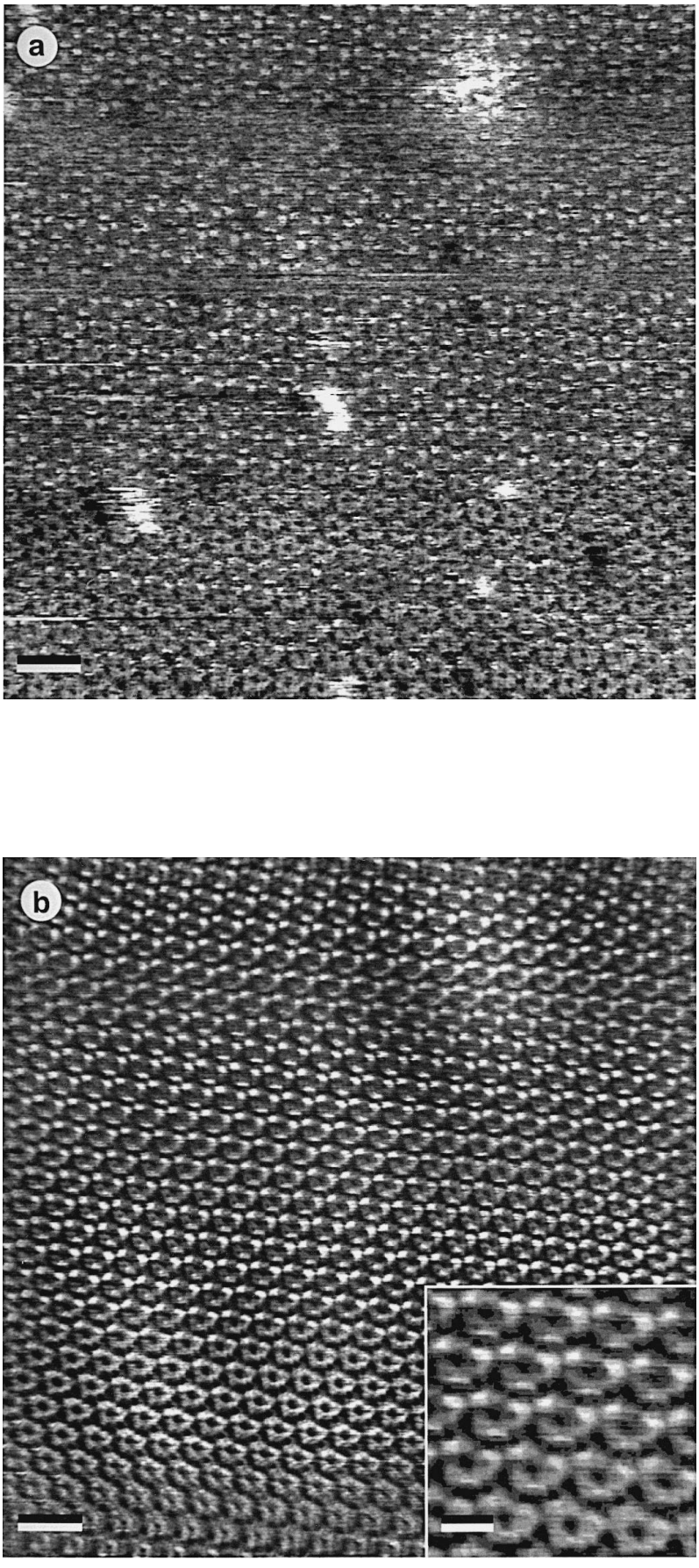

Figure 2. Force dependent surface topography of bacteriorhodopsin. a, AFM image (scale bar represents 10 nm) demonstrating the effect of force variations on the topography of the cytoplasmic purple membrane surface. The initial force of 300 pN (bottom of image) was decreased during the scan to 100 pN (top of image). A conformational change is distinct: donut-shaped bacteriorhodopsin trimers transform into units with three pronounced protrusions at their periphery. b, To improve the visibility of this conformational change, the image was locally averaged and displayed at higher magnification in the inset (scale bar represents 4 nm). Purple membranes suspended in 150 mM KCl, 100 mM Tris (pH 9) were adsorbed to freshly cleaved mica for 30 minutes, washed and imaged. The force applied to the stylus was changed manually. The force constant of the cantilevers used to calibrate the force curve was calculated to be 0.088 N/m as described by Butt *et al*. (1993). To this end, the dimensions of the cantilever used were measured in a scanning electron microscope. Local averaging was achieved by Fourier peak filtration.

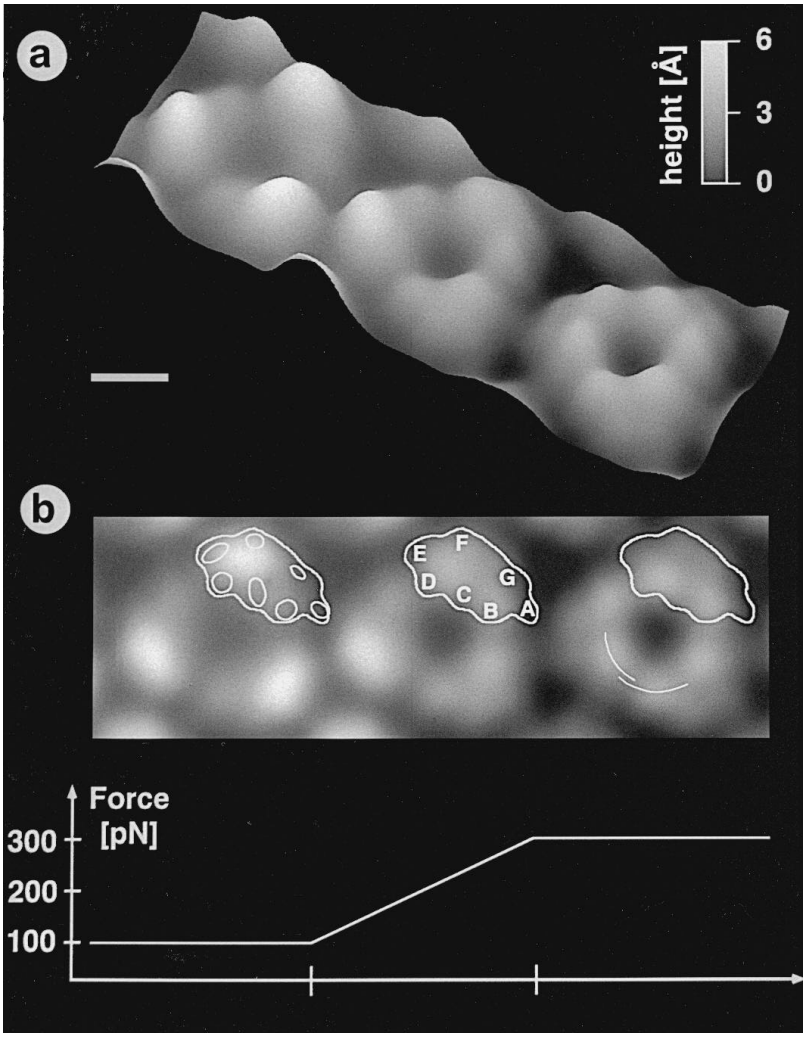

Figure 3. Interpretation of the force dependent conformational change of bacteriorhodopsin. a, Perspective view (scale bar represents 2 nm) of the transition from native (left) to donut-shaped bacteriorhodopsin trimers (right). The central trimer is a composition of the left trimer recorded at 100 pN and the right trimer recorded at 300 pN. b, In the corresponding topograph the helices derived from the atomic model of bacteriorhodopsin (Henderson *et al*., 1990) are superimposed. On the left side, the domains are identified as the loop connecting helices E and F. On the right side, domains arranged in a circle of 17 Å radius represent the loop connecting helices A and B, while domains at 15 Å radius may be the loop between C and D. Approximately 700 unit cells from three images recorded at 50 to 150 pN (left) and 800 unit cells from four images recorded at 300 to 600 pN loading force (right) were averaged, 3-fold symmetrized, and scaled according to the microscope parameters. To adjust the heights of these independent averages, the loops between A and B served as landmarks rather than the lipid bilayer moieties to minimize the influence of the tip geometry. To calculate the composite trimer (center), the left trimer was multiplied with a linear ramp from 1 to 0 and added to the right trimer multiplied with a ramp from 0 to 1.

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