Biomolecular Stress-Sensitive Gauges: Surface-Mediated Immobilization of Mechanosensitive Membrane Protein

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Stress-sensitive proteins, which serve as pressure relief valves in the cell membrane, are considered as a promising model of biomolecular gauges.[1] Studies of a mechanosensitive protein of large conductance (MscL) had shown that a dramatic change in the protein conformation is caused by membrane tension rather than the ion concentration gradient. Recent structural studies of such membrane channel proteins suggested an architecture with a large pore opening under mechanical stresses.[1-3] The elastic barrel composed of highly tilted helices acts as a tension sensor and was responsible for a large "iris"-like expansion and flattening of the protein triggered by local stresses in the cell membrane.[4,5]

A variation of the lateral pressure within the lipid bilayer, asymmetric bending, and, to lesser extent, membrane thinning were found to be the main driving forces for pore opening. The transition from a closed state to a closed-expanded state occurs initially as a result of a gradual increase in helical tilt angle. Under further membrane tension, the helices move away and dock to the rest of the open structure, resulting in a flat structure with a large central opening.[6,7] However, to date, no attempts have been undertaken to immobilize these proteins in a controlled open/close state on a microelectronic-related surface as an initial step toward designing biomolecular gauges. An open structure of this membrane protein causes a significant challenge in its immobilization because it is prone to collapse on a solid surface.

Here, we report on the design of protein–organic surface nanostructures containing stress-sensitive membrane protein, MscL, from Salmonella typhymurium. We designed supported organic monolayers (alkylsilane self-assembled (SAM) and lipid Langmuir–Blodgett (LB)) with different packing densities and surface tensions, which can be directly used for membrane protein immobilization (see Supporting Information).[8-10] Previous studies had shown that the density of alkyl SAM affects the conformation of other proteins during adsorption.[11,12] We used high-resolution atomic force microscopy (AFM) as a tool for the direct observation of the shape of MscL proteins embedded in organic monolayers with varying surface tension/adhesion, tuned by the hydrophilic–hydrophobic interfacial balance.[13,14] Light tapping mode scanning in ambient humid conditions was utilized for characterization of MscL proteins. All samples were scanned immediately after adsorption, to avoid drying of the sample. As we observed, protein conformation changes dramatically depending upon the packing density of alkyl chains within the supporting organic layer.

We estimated the effective surface energies (or tensions) ranged from 20 mJ/m^2 for hydrophobic monolayers to 68 mJ/m^2 for loose disordered monolayers using measured contact angles of water.[15] Initial studies showed that proteins could not be embedded in a highly hydrophobic surface with a surface tension below 22 mJ/m^2. On the other hand, surface tension exceeding 68 mJ/m^2 for the

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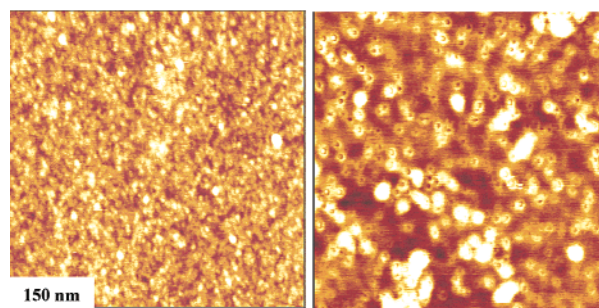
Figure 1. AFM images of immobilized protein molecules in closed (left) and open (right) states; *z* scale is 5 nm.

hydrophilic surface resulted in the complete unfolding of protein molecules. For intermediate interfacial tensions, we observed a spectrum of protein shapes indicative of conformational changes.

AFM images for the protein molecules adsorbed on modestly hydrophobic monolayers (surface tension within 22–30 mJ/m^2) showed the presence of individual MscL molecules embedded in the lipid monolayers as nanoscale "bumps" (Figure 1). The overall vertical dimension of the molecules was 4–5 nm, which was smaller than molecular dimensions in an unperturbed conformation (about 8 nm in closed conformation with stem) (Figure 2a). We suggest that this was due to the compression stress induced by the AFM tip which is usually observed for compliant macromolecules and the partially embedded state of proteins within vertically oriented alkyl chains.[16] No indications of pore structure were found for the protein under these conditions (Figure 2a). Thus, we concluded that under low surface tension, the MscL molecules retained their closed-pore conformation and prolate shape.

A very different structure was observed when MscL molecules were embedded in less-ordered, loosely packed SAMs with fluidlike packing of alkyl chains and surface tensions between 30 and 60 mJ/m^2. Under these conditions, the adsorbed protein molecules consistently showed a doughnut-shape with a large central opening (Figure 1). The height of the protein molecules in this state was within 2.2–2.7 nm, indicating their significant flattening. A large opening was consistently observed at highest magnifications (Figure 2b). The apparent width of this opening, calculated as the distance between edges of the rim, reached 11–15 nm, whereas the overall width of the molecules increased to 20–30 nm. These values were greater than those expected for the open-pore molecular models of the protein in an upright orientation (Figure 2). The upright protein orientation is expected due to the predominantly hydrophobic nature of the bottom part of the molecule, which favors interactions with nonpolar alkyl chains. We also suggest that the proteins can be partially embedded in loosely packed alkyl chains with a high level of free volume.

As is known, overestimation of lateral dimensions by AFM is due to the dilation effect induced by the AFM tip. "Apparent"
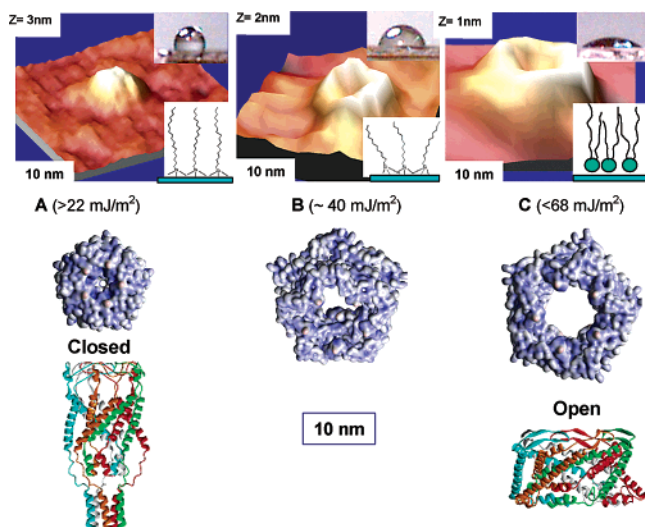
Figure 2. Top: AFM images of individual MscL protein molecules embedded in different organic monolayers in closed (a) and two open states (b, c); insets show microstructure (right, bottom corner) of supporting alkylsilane SAMs and lipid monolayers; corresponding water drop shape (right, top corner) illustrates increasing hydrophilicity of monolayers with different packing density from left to right (dense SAM → loose SAM → loose lipid LB layer). Bottom: molecular models of MscL proteins in different states; schematic side-views and top-views of van der Waals models are shown for closed and open states (top-view of open-pore models includes slightly tilted model in the middle); a common scale bar of 10 nm for all models is displayed.

molecular dimensions are close to those estimated from molecular models by considering a semispherical approximation.[17] On the other hand, the "apparent" internal pore diameter reached $8-9$ nm, which is larger than the expected pore diameter of about $3-4$ nm. We attribute this to the local lateral (shear) stress produced by the tip piercing into the open pore and pushing out a flexible protein ring. The limits of "lateral stretching" estimated from the measured depth of tip penetration ($0.5-1$ nm) and the contact area are, indeed, close to the molecular model values.

AFM images of the MscL molecules embedded in the loosely packed organic monolayer showed even a larger opening in the center (Figure 2c). Consistently observed single protein molecules embedded in a lipid monolayer appeared flattened and, frequently, slightly tilted. The total height of the protein molecules was estimated to be close to $1.8-2.0$ nm, and the overall width of the molecule increased to $14-19$ nm that is statistically different from the previous state (see Supporting Information). This shape of the molecule reflects even wider opening of the central pore.

Our observations of distinctive shapes of the MscL molecules immobilized in different organic monolayers can be understood considering different conformational states proposed from molecular modeling (Figure 2).[18] We suggest that both open-pore structures observed under different surface tensions can be associated with different open-pore states of the protein molecules. The geometrical dimensions of the MscL molecules in open states show significant height decrease accompanied by a correlated, dramatic width increase and pore opening. "Apparent" dimensional parameters obtained directly from AFM images underestimate height and overestimate lateral dimensions as was mentioned above. However, the general trend in the variation of the parameters corresponds to

MscL molecules in different conformational states and confirms the transition from a prolate to an oblate, open-pore molecular shape. It is worth noting that the surface tension of the monolayers, which initiate open protein structures, is several times higher than that obtained electrophysiologically for the lateral pressure of cell membranes.[19] Although the trends for pore opening under higher tension are similar, a direct comparison of these parameters cannot be done because in our monolayer systems, the surface tension affects embedded proteins differently than does the lateral pressure within bilayer membranes. In our design of surface structures, protein molecules are embedded in the monolayer instead of the bilayer; this creates additional tension analogous of that developing in thinning membranes upon stretching. However, if not assisted by higher surface tension, this factor alone does not produce stress sufficient for protein opening to occur.

In conclusion, we designed protein−organic−inorganic surface nanostructures and directly observed structural reorganization of individual MscL proteins comparable to conformational changes associated with gating under external stresses. We demonstrated that the variable surface tension of organic monolayers used for immobilization of MscL molecules is instrumental in mediating protein conformation. AFM confirmed transition from a prolate shape of MscL molecules at low surface tension to a flattened, oblate shape with a wide central opening at higher surface tension.

Supporting Information Available: Experimental routines for surface modification, protein immobilization, and comparison molecular model dimensions and AFM data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA037686Q