Probing the Nature of Cholera Toxin B Oligomer by the Atomic Force Microscopy

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The nature of cholera toxin B oligomer was examined via the atomic force microscopy on the nanometer scale at the fluid tapping mode. The specific binding force between the B oligomer and the ganglioside GM1 receptor was quantified.

Ligand–receptor interactions provide the molecular basis for biological activities and molecular communications within cells. As the typical sizes of proteins range from several nanometers to several tens of nanometers, a challenging task in bioscience is to explore the protein structures and functions on the nanometer scale. Recent advancements in atomic force microscopy (AFM) enable biomolecules to be imaged at molecular resolution under physiological conditions.[1,2] In addition to topographical measurements, AFM is also capable of probing the interaction between a pair of biomolecules[3–5] because the typical specific binding force falls in the range of 10^{-11} N to 10^{-9} N whereas the theoretical force sensitivity of AFM is on the order of 10^{-14} N.

In this work, cholera toxin has been chosen for studying the protein structure and the binding features. Cholera toxin is a member of the AB_5 family of bacterial toxins containing a catalytic A subunit and a pentameric B oligomer (CTB) with a central pore formed by the long α-helices on each monomer.[6,7] The B-oligomer participates in specific binding to the ganglioside GM1 receptor present in the outer leaflets of a wide range of cell membrane. In a previous report,[8] the protein association upon the CTB–GM1 specific binding was thoroughly investigated by the AFM. Here the structure of CTB has been examined down to the submolecular level, and the specific binding force between CTB and GM1 has been quantified.

The study was carried out by a multimode Nanoscope IIIa (Digital Instruments, Santa Barbara, CA). The protein images were acquired in the fluid tapping mode using oxide sharpened Si_3N_4 tips, operating at a thermal resonance frequency of 8–10 kHz. Height and phase images were collected simultaneously. The sample was prepared by incubating 5 μL of 10^{-7} M CTB solution on a piece of silicon wafer for 45 min, allowing the adsorption of CTB on the silicon wafer via electrostatic interaction. The specific binding force was measured between a GM1 modified AFM tip and a CTB modified substrate. The gold coated AFM tips and the gold coated silicon wafers were similarly functionalized via the thiol–gold chemistry, where the succinimidyl 3-(2-pyridyldithio)propionate served as a cross linker. A spring constant of 0.17 nN/nm was measured for the GM1 modified AFM tip. All the AFM measurements were performed in a commercially available fluid cell in phosphate buffered saline (PBS) solution, mimicking the physiological condition.

Figure 1 shows the raw data of 600 × 600 nm height (Figure 1a) and phase (Figure 1b) images of the CTBs on a silicon wafer. The round bright spots in Figure 1a are randomly distributed across the surface, exhibiting a uniform size of 5.6 ± 0.4 nm in diameter which is in good consistence with the size of a CTB as reported in the other AFM research[7,9] and in the X-ray crystal structure study.[10] The large bright grains are correlated to the clusters of CTBs that were occasionally formed and adsorbed on top of the CTB layer. The inset of Figure 1a (low-pass filtered) illustrates the details of an individual CTB molecule as acquired at high resolution. The characteristic pentameric structure of the cholera toxin B-oligomer (arrow pointed) as well as the central pore (1.5 ± 0.3 nm) is well resolved.[7–10] Note that the bright patch on top of the upper subunit of the pentamer was not reproducibly observed on the other oligomers, thus is considered as an irrelevant artificial effect. Comparing Figure 1a with Figure 1b, the individual protein shows dark contrast in the phase image. As reported by Hansma et al.,[11] hydrophilic regions of a sample surface are darker in phase images. This is especially true for biomolecules as the examples they raised in the literature. Our observation thus suggests that the exposed surface of a CTB molecule is highly hydrophilic. Strikingly, the high resolution phase image (inset of Figure 1b) shows the relatively bright contour of an individual CTB however the relatively dark core region. It is consistent with the structure determined by X-ray[10] that the inner surface of the central pore is highly hydrophilic whereas the interdigital region at the subunit interface consists of hydrophobic groups. This, for the first time to our knowledge, provides the information of local hydrophilicity of a protein at the nanometer level.

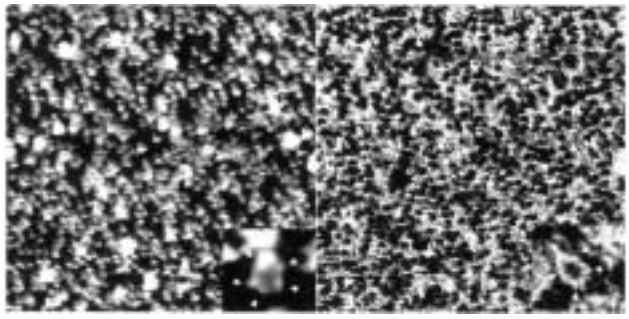

Figure 1. Height (a) and phase (b) images of CTB molecules on a silicon wafer (scale: 600 nm × 600 nm). The inset of (a) shows the pentameric feature of a single CTB oligomer (low-pass filtered), as indicated by the arrows. The inset of (b) illustrates the local hydrophilicity on the exposed surface of a CTB oligomer.

The binding nature of CTB to its receptor GM1 was also explored. As shown in Figure 2b, GM1 molecules and CTB molecules were cross-linked to gold-coated AFM tip and gold-coated silicon wafer, respectively. By using a normal tip, similar image as Figure 1 was also acquired on a CTB-modified substrate. As a GM1-modified tip approached to a CTB-modi-

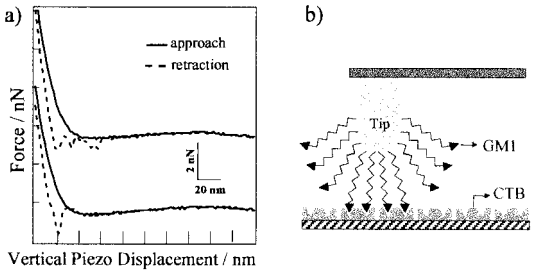

Figure 2. Force curves (a) measured between a GM1-modified tip and a CTB-modified substrate as shown in (b).

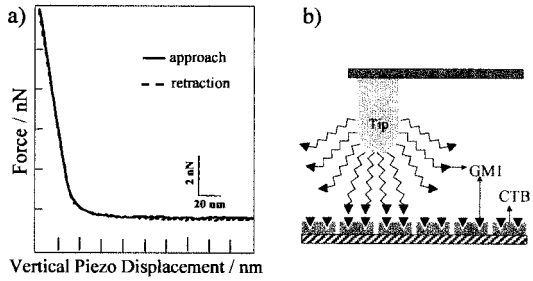

Figure 3. Force curves (a) measured between a GM1-modified tip and a CTB-modified substrate, where the CTB-modified substrate was pre-treated by the GM1 solution as illustrated in (b).

fied substrate closely, the CTB–GM1 specific bond formed; when the tip retracted from the substrate, it was held by the substrate at the beginning and the bond was eventually ruptured as detected as the adhesion force. The force curves of two typical approach–retraction cycles are shown in Figure 2a. The adhesion peaks appeared in the retraction process is quantitatively correlated to the CTB–GM1 specific binding force. The force curve in the upper panel exhibits multiple adhesion peaks, indicating that multiple CTB–GM1 binding occurred and the bonds were ruptured subsequently as the GM1-modified tip retracted from the CTB surface. The forces are of 0.94, 0.65, 0.46 and 0.53 nN, respectively, corresponding to the four peaks. The lower panel of Figure 2a shows a single adhesion peak of 1.28 nN, which indicates the specific bonds were instantly ruptured during the tip retraction. The measured force largely varies in distinct approach–retraction cycles, however the lowest force obtained is of 0.16 nN. We tentatively correlate the 0.16 nN to the single CTB–GM1 specific binding force since the measured forces in distinct cycles are roughly the multiples of this value. Due to the remarkable size difference between GM1 (less than 1nm in diameter of the cross section) and CTB, the local concentration of GM1 is much higher than that of CTB. Because the GM1 molecules were tethered to the tip via flexible long chains, the high affinity between GM1 and CTB may allow the GM1 molecules to easily and equally reach the five binding sites of each CTB pentamer. Therefore the 0.16 nN force more likely corresponds to the interaction between a CTB and five GM1 molecules. The measured forces are at the same order as measured by Luckham et al.,[7] who conducted the force measurements between the CTB modified AFM tip and the GM1 receptors inserted in a phospholipid layer.

Figure 3a shows the force curves of a control experiment, in which sufficient GM1 solution was applied to the CTB-modified substrate prior to the force measurement. As a result, no adhesion peak is detectable above the noise level, suggesting that the binding sites of CTB were completely blocked by the GM1 molecules in the solution phase, so that no reactive site was available for binding to the GM1 molecules on the tip (see Figure 3b). This provides strong evidence that the adhesion peak that appeared in Figure 2a was generated from the CTB–GM1 specific binding. The lack of adhesion peak between the GM1 modified tip and the bovine serum albumin (BSA) modified surface furnishes an additional evidence of the measured CTB–GM1 specific binding force.

In conclusion, the pentameric nature of the CTB oligomer has been resolved at the submolecular level on the nanometer scale. The phase image revealed the local hydrophilicity on the exposed CTB surface. The force measurement may provide a remarkable way to quantify the specific binding force of biomolecular recognition.

References and Notes

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