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Atomic Force Microscopic Study of Vesicles of Synthetic Surfactant, Vesicles of Thylakoid Membrane, and Whole Cells of Bacteria

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Surface images of vesicles of synthetic surfactant, vesicles of thylakoid membrane isolated from thermophilic cyanobacteria, and whole cells of bacteria, *E. coli* were obtained using atomic force microscopy (AFM). The AFM proved itself to be useful in direct observations for molecular assemblies of biological interest.

Direct observation is an important means of understanding the relationship between the structures and functions of biological materials. Optical microscopy, however, provides only a limited resolution of down to submicrometers, which is sometimes not enough for the purpose. Electron microscopy, having sufficient resolution, requires samples to be placed in vacuum with various kinds of pretreatments such as fixation, staining, or coating with metal. Many samples of biological interest are easily denatured and lose their original structures during the preparation processes, which may lead to artifacts.

In this respect, scanning tunneling microscopy (STM) and atomic force microscopy (AFM) allow us to get a direct image of surface structures of various samples at high resolution of down to an atomic level under various conditions without special pretreatments. AFM has an additional advantage that it can be applied to electrically insulating samples, which is especially suitable for the observation of biological materials. Several applications of AFM to imaging of biological samples have been recently reported.[1-6] In this letter we report the first AFM images of vesicles of synthetic surfactant, vesicles of thylakoid membrane isolated from thermophilic cyanobacteria, and whole cells of bacteria, *E. coli* with flagella.

The vesicle solution of didodecyldimethylammonium bromide (DDAB) was obtained by sonication of 10 mM suspension in pure water at 50 °C. The thermophilic cyanobacterium, *Synechococcus elongatus*, was kindly donated from Dr. S. Katoh of the University of Tokyo and was cultured as described.[7] The vesicles of thylakoid membrane were isolated by lysozyme treatment and sonication of the cells.[8] *E.coli* (K12) was cultured in Luria-Bertani medium. During late log phase the cells were harvested and washed twice with pure water. A small portion of each suspension was applied onto a mica sheet freshly cleaved and subsequently

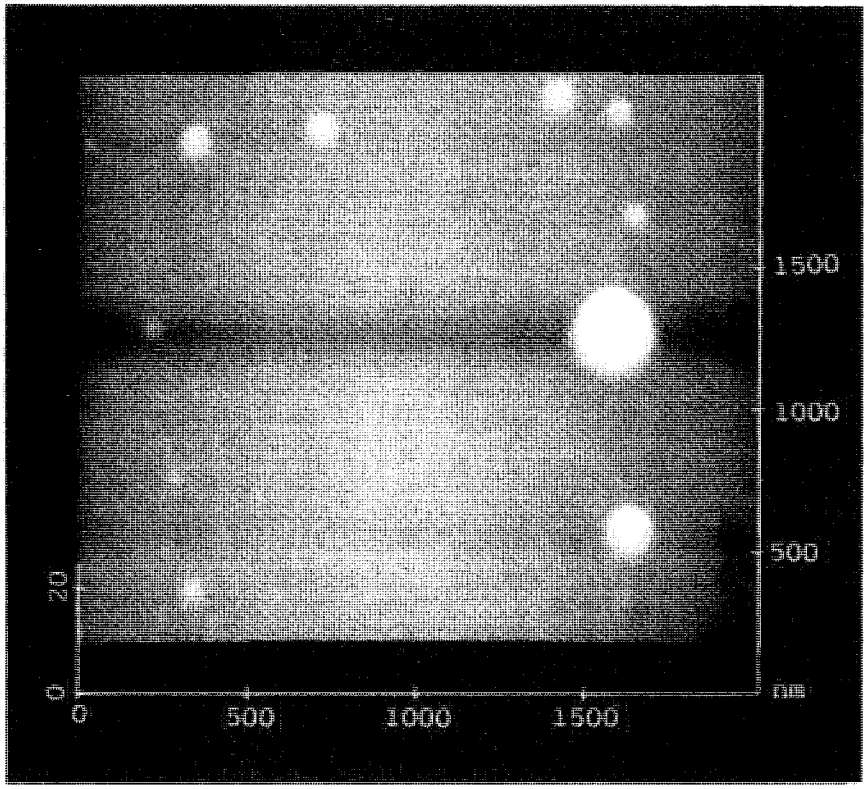

Fig. 1. AFM image of DDAB vesicles on mica (image size: 2.0 μm).

dipped in ethanol for 2 h or a super-cleaned glass substrate of 9 mm x 9 mm. Excess liquid was blotted out and the substrate was air-dried at room temperature.

AFM images were obtained with a Nanoscope II (Digital Instruments Inc.). Repulsive force between the tip and the sample was kept constant during the measurements and the vertical travel distance of the substrate placed on the piezoelectric scanner was recorded. All images were taken in air at room temperature.

Figure 1 shows the AFM image of DDAB vesicles on mica. Circular structures formed by the adsorption of the vesicles to the substrates are clearly seen. The diameter of the vesicles is 70 nm to 200 nm, which is coincident with the electron microscopic observation reported by Kunitake et al.[9] The thickness of the vesicles is 3 nm to 7 nm, which is rather small for a two fold bilayer structure. There is a possibility that the smaller thickness than is expected is due to the compression of the membrane by the AFM tip during the measurements. The other structures such as tubules seen in dark-field micrographs[10] were not observed. This is the first AFM image reported so far of the vesicles of quarternary ammonium surfactants.

The AFM can be used to investigate directly the fine structures of the surface of biological membranes. Figure 2a shows the first AFM image of thylakoid membrane of thermophilic cyanobacteria. The vesicles are 200 nm to 800 nm in diameter and 20 nm to 40 nm in thickness. These results show that the thylakoid membrane, which is responsible for photosynthetic reaction, forms exclusively vesicular structures when it is isolated from the bacteria. Figure 2b shows the image of the one having been dried for over 10 h in a vacuum

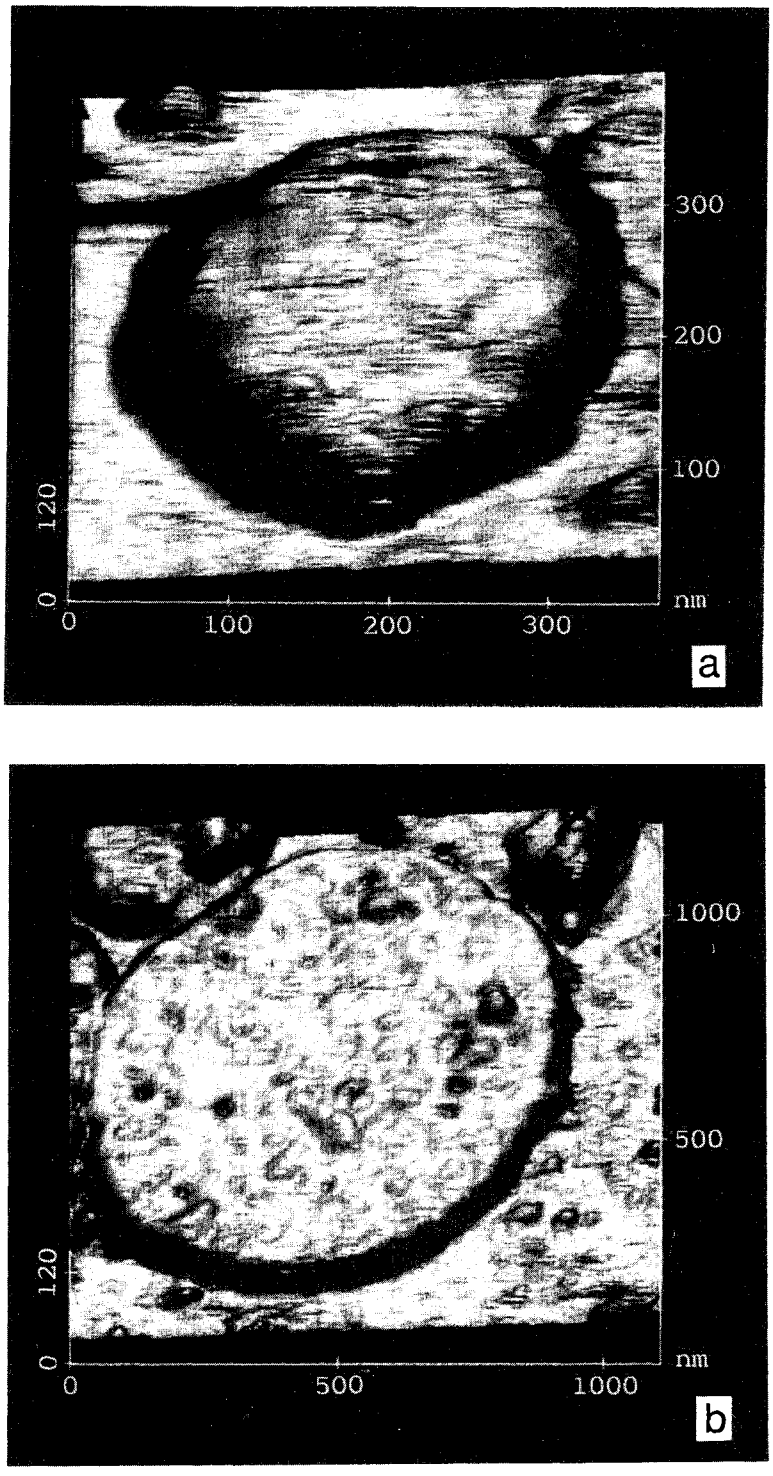

Fig. 2. AFM images of the vesicles of thylakoid membrane of *Synechococcus elongatus* on glass, a) before evacuation (image size: 0.37 μm), b) after evacuation for over 10 h (image size: 1.1 μm).

desiccator. Globular structures on the surface of the membrane are seen in each vesicle. These globular structures may be derived from the assembly of proteins.

The AFM also allows us to observe the surface of whole cells of bacteria. Figure 3 shows the image of bacteria, *E.coli*. Two cells at the last stage of cell division are visualized. The obtained image shows a wrinkled surface of the cell body. Short filaments of flagella elongated from side wall are also observed (indicated by arrows). This AFM image is the first one that clearly shows the shape of *E. coli* with flagella.

This work has shown that AFM can be successfully applied to the observation of biological materials such as vesicles, part of organella, and whole cells which are electrically insulating. AFM provides reliable topographical information of the surfaces of materials in contrast to the cases of STM when it is used to observe the surfaces of electrically insulating materials but with unknown mechanisms. Further improvement on resolution will make it possible to investigate the details of biological phenomena by visualizing, for instance, the interaction between proteins or between small molecules.

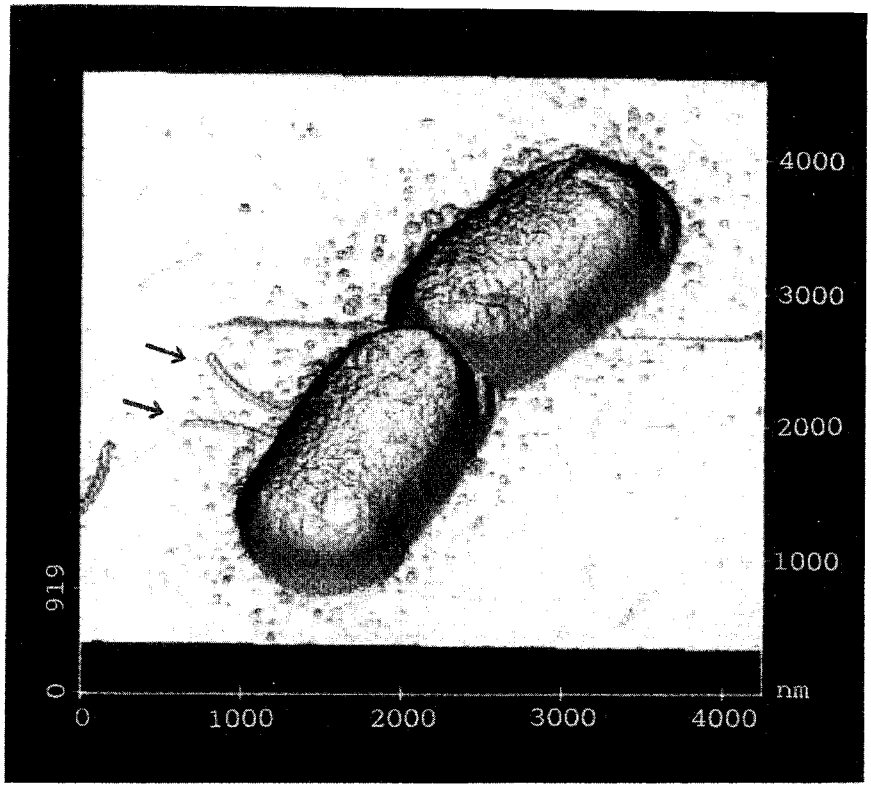

Fig. 3. AFM image of bacteria, *E. coli* on glass (image size: 4.2 μm).

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