TOPICS

Molecular Scale Imaging of Cytoskeletal Filamentous Actin Supramolecules

Taiji Ikawa

アクチンフィラメント超分子構造の観察

井川泰爾

Progress in atomic force microscopy (AFM) over the past decade has achieved imaging of single biomolecules such as DNA and proteins under native conditions with (sub-) nanometer resolution, and it offers promising prospects on direct investigation of biological self-assemblies, which are essentials of cellular structures. In many AFM sample preparations, however, biomolecules easily detach from the substrate surface during AFM scanning, resulting in poor resolution and imaging artifacts. To visualize precise images, special sample preparation methods must be developed that can reliably preserve the conditions under which the structures are formed.

Here we report a new method for AFM imaging of biological self-assemblies based on a unique biomolecule immobilization technique using a non-ionic polymer containing an azo-dye (azopolymer). Recently, we have demonstrated that the azopolymer is capable of immobilizing biomolecules in an aqueous solution upon exposure to visible light.[1, 2] In principle, because of the photo-isomerization motion of the azo-dye and succeeding photo-plasticization of the azopolymer matrix, the azopolymer surface deforms along the shapes of the biomolecules and thus immobilizes them. The biomolecules immobilized on the azopolymer surface can be clearly imaged by AFM because of a non-reactive, non-ionic and flat surface (roughness < 0.3 nm) of the azopolymer.

Using this method we examined the association of cytoskeletal filamentous actin (F-actin) with a divalent cation. F-actin appears to be a double stranded filament consisting of a small protein, actin, of diameter 8 nm and persistence length 10 μm. It self-assembles into parallel bundles and gel-like networks in the presence of linker proteins and/or polyvalent cations *in vivo/vitro*, which are essential for cell motility, muscle contraction and other cellular events. Recently, a high precision small-angle X-ray scattering (SAXS) study suggested F-actin formed supramolecular structures with divalent cations.[3] With increasing divalent cation concentration, F-actin associates into a newly found layered structure, and then into a bundle structure with hexagonal packing. The structural behavior of the F-actin supramolecules has been a complicated problem.

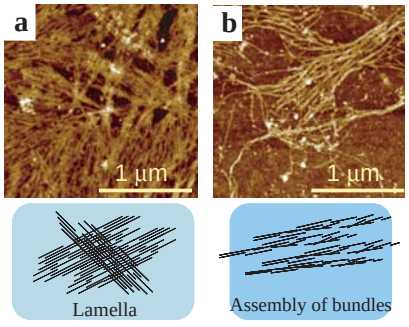

Fig. 1 AFM images of F-actin supramolecules with (a) 10 mM $MgCl_2$ and (b) 80 mM $MgCl_2$.

Figure 1 shows AFM images of F-actin supramolecules on the azopolymer. The aqueous solutions containing F-actin and $MgCl_2$ were spotted on an azopolymer film, and then the film was exposed to visible light. The film was probed by AFM after removing the solvent. At an intermediate concentration of Mg^{2+} (10 mM), F-actin forms the 2D nematic-like raft structure (Fig. 1a). In the raft, the filaments lie in a side-by-side configuration with a lateral repeat distance of about 12 nm, and the rafts are stacked at a large angle from each other (lamella structure). On the other hand, at a high concentration of Mg^{2+} (80 mM), multifilament aggregations are observed (Fig. 1b). Height analysis shows that the observed filaments are bundles consisting of several single F-actin, and the bundles are stacked on each other. The overall structural behavior of the F-actin supramolecules observed by this method is consistent with the result obtained by the previous SAXS study.

This method provides a detailed view of the individual structural elements of F-actin supramolecules at the molecular level. The method has distinct advantages over conventional methods because it allows position-controllable immobilization of a wide variety of biomolecules with less damage to molecular function. The method is, therefore, promising not only for biological chips and sensors but for biomolecule imaging.

References

1) Ikawa, T., et al. : Langmuir, **22**(2006), 2747
2) Watanabe, O., et al. : Appl. Phys. Lett., **88**(2006), 204107
3) Wong, G. C. L., et al. : Phys. Rev. Lett., **91**(2003), 018103

(Report received on Jul. 4, 2006)