Imaging the RecA-DNA complex by atomic force microscopy

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Abstract
Atomic force microscopy (AFM) was applied to study the RecA protein and its complexes with DNA in air and in aqueous solution. RecA and DNA were reacted under several conditions, and deposited onto a mica substrate pre-treated in various ways. We found that the structure of the RecA and RecA-DNA complexes, especially the height of the molecules, was affected by the sample preparation method such as gel filtration, and environment during imaging.

Introduction
RecA protein from Escherichia coli is a DNA-dependent ATPase that catalyzes homologous pairing of single-stranded DNA with duplex DNA. Visualization of the RecA-DNA complex has to date been carried out mainly by electron microscopy to study the formation of presynaptic filaments and the exchange process between the two DNAs at the molecular level. We have attempted to visualize RecA and RecA-DNA complexes using AFM in air and in aqueous solution. In principle, AFM has several advantages over electron microscopy particularly for biological samples: 1) Sub-molecular resolution is available in air and in liquid without fixation and staining; 2) The height or thickness of molecules can be easily measured from three dimensional images; 3) Not only topographies but also physical and chemical properties of sample surface can be measured or imaged.

To realize the above advantages of the AFM method, molecules should be deposited suitably on a flat substrate. This means that establishment of appropriate sample preparation methods is one of the most important aspects of the work to maximize the potential of AFM. In this paper, we focused on methods to investigate the RecA DNA complex by AFM.

Results and Discussion
When single stranded M13 DNA (7249 nucleotides) which had been incubated with excess RecA (1 RecA molecule per 3 nucleotides) was deposited onto a pre-treated mica surface without removing excess protein, typical RecA-DNA filaments were observed as shown in Fig.1. Prior to imaging, the cleaved mica surface was modified with 3-aminopropyltriethoxysilane (AP-mica). Several other surface modifications of mica were studied to examine their effect on the structure of the RecA-DNA complex. Control of the affinity between the mica substrate and RecA filaments was an important factor in allowing uniform adhesion of the molecules. Although uncomplexed RecA molecules were surrounding the filaments, individual filaments could be clearly distinguished. Thus, the AFM method was effective for simple verification of the sample without a prior purification step. RecA-DNA filaments prepared with shorter single-stranded DNA (5386 nucleotides) or double stranded DNA (1200 bp) have been previously imaged by AFM in air. The filaments we observed appeared to be somewhat more entangled than these and this may be due to different sample preparation procedure, the details of which were not reported in literature.

Fig.2 shows RecA-DNA complexes after removing excess proteins by Sepharose 2B gel column chromatography. From a cross-section of the AFM
When a small amount of RecA molecules was mixed with single-stranded or double stranded DNA, in most cases, RecA molecules just adsorbed onto DNA as aggregates. Only on a few DNAs were partially filamentous structures formed (data not shown).

We tried to image RecA molecules in aqueous solution as well. Many particles were observed as shown in Fig.3(a). Although big aggregates were also observed, RecA polymers were not found. It seems possible that the RecA molecules formed hexamers and not polymers because a diluted RecA solution (10 nM) was employed. Further experiments are in progress to understand the behavior of RecA and DNA molecules on the AP-mica surface.

From the cross-section of the AFM image, the typical height of the particles was measured to be 5-7 nm on AP-mica surface (Fig.3(b)). By contrast, even in aqueous solution, the height of particles was less than 4 nm when RecA was deposited on bare mica. When imaging was carried out in air, the height was 1 nm or less both on bare- and AP-mica. These results suggest that RecA molecules are easily flattened when bare mica is used or samples are dried. Imaging in aqueous solution on suitably modified mica is essential for the investigation of the native structure of RecA molecules.

References
3. Shibata, T., Osber, L. and Radding, C.M.