RecA-double stranded DNA complexes studied by atomic force microscopy

Kazuo Umemura, Jun Komatsu, Takayuki Uchihashi, Nami Choi, Shukuko Ikawa, Taro Nishinaka, Takehiko Shibata, Yoshikazu Nakayama, Shinji Katsura, Akira Mizuno, Hiroshi Tokumoto, Mitsuru Ishikawa and Reiko Kuroda

1Joint Research Center for Atom Technology, 1-1-4 Higashi, Tsukuba, Ibaraki 305-0046, Japan, 2Department of Ecological Engineering, Toyohashi University of Technology, Tempaku-cho, Toyohashi, Aichi 441-8580, Japan, 3The Institute of Physical and Chemical Research (RIKEN), Hirosawa, Wako, Saitama 351-0198, Japan, 4Department of Physics and Electronics, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka 599-8531, Japan and 5Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan

ABSTRACT

RecA-double stranded (ds) DNA complexes have been studied by atomic force microscopy (AFM). When the complexes were prepared in the presence of ATPγS, fully covered RecA-dsDNA filaments were observed by AFM. When the concentration of RecA proteins was lower, various lengths of filaments were found. The variation of the observed structures may directly reflect the real distribution of the intermediate complexes in the reaction mixture, as the mixture was simply deposited on a mica surface for AFM observation without special fixation or staining. The use of a carbon nanotube (CNT) AFM tip enabled high resolution to reveal the periodicity of RecA-dsDNA filaments. Our observations demonstrated the potential of the AFM method for the structural studies of the RecA-dsDNA complexes, especially their intermediate states.

INTRODUCTION

One of the unique advantages of atomic force microscopy (AFM) is that it can provide high resolution of three dimensional structures of various samples without reconstruction of obtained images (1). The AFM method has been applied not only for material sciences but also biosciences because the AFM imaging is available not only in vacuum but also in air and in liquid.

RecA protein is a key protein for homologous recombination in *Escherichia coli*. In vitro, purified RecA proteins catalyze the exchange of two DNA strands (2). AFM has been recently applied to structural study of RecA-DNA complexes by several groups, but only a few papers have been so far published (3-5). For example, Seong *et al.* successfully imaged the pairing of a RecA-single stranded (ss) DNA complex with a double stranded (ds) DNA molecule that has a specific complementary sequence (5). The resolution of the reported AFM images was not high enough to reveal the periodicity of the RecA-DNA filaments which was observed by electron microscopy (EM) (2,6).

Previously, we reported AFM imaging of RecA-ssDNA complexes (7). This time, we focused on AFM imaging of RecA-dsDNA complexes, especially intermediate structures of the filaments, to elucidate the formation process of RecA-DNA filaments and compare it with that studied by EM (6). Additionally, a carbon nanotube (CNT) tip was employed to observe the periodicity of RecA-dsDNA filaments.

RESULTS AND DISCUSSION

Figure 1 shows AFM images of RecA-dsDNA complexes deposited on a mica surface pretreated with 3-aminopropyltriethoxysilane (APS). RecA and φX174 RFII dsDNA were mixed in the presence of ATPγS, and incubated for 60 min at 37 °C. When the ratio of RecA and dsDNA was 1:3, fully covered circular filaments were observed as shown in Figure 1A. The typical circumference of
Figure 1 AFM images of RecA-dsDNA filaments observed with a commercially available sharpened tip. RecA and dsDNA were mixed at the molecular ratio of (A) 1:3 and (B) 1:12.

Figure 2 A scanning electron micrograph of a carbon nanotube AFM tip.

Figure 3 An enhanced AFM images of RecA-dsDNA filaments observed with a CNT tip. The arrows indicate periodicity of the filaments.

the circles was 2.5 μm although shorter ones were additionally observed. Since the normal length of a φX174 dsDNA (5386 bps) is known to be 1.8 μm based on 0.332 nm per bp, the length of a RecA-dsDNA complex was extended 1.5 times of a normal φX174 dsDNA.

On the other hand, when RecA and dsDNA were mixed at the molecular ratio of 1:12, partially covered RecA-dsDNA complexes were observed although more than half of DNA molecules were bare (Figure 1B). RecA proteins bound to a DNA molecule obviously formed filamentous domains along the DNA strand in many cases. The varied structures may reflect different stages of the RecA-dsDNA complex formation.

Ten nm periodicity of RecA-dsDNA filaments which is known by electron microscopy was not observed in the above AFM images. Lyubchenko et al. also met the same problem, and pointed out two possible reasons: convolution effect of the AFM tip, and deformation of the RecA-DNA filaments during sample preparation.

We employed a CNT tip to improve the resolution problem. Figure 2 shows a scanning electron micrograph of a CNT tip. A multiwall CNT was attached to the top of an conventional AFM tip by previously described method (8). Although length of the CNT on the tip was estimated by the EM image as several hundreds nm, the curvature of radius of the CNT tip is impossible to estimate.

Figure 3 shows an AFM image of a part of a RecA-dsDNA filament observed using a CNT tip in air. Periodicity of the filament (around ten nm) was clearly visualized after a graphic enhancement of the AFM image (see the arrows). This could not be observed with the conventional tip even if the images were graphically enhanced.

REFERENCES