Vol 10 Suppl., July 2001
1009-1963/2001/10(Suppl.)/S96-04
Chinese Physics
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and IOP Publishing Ltd

OBSERVATION OF ACTIN POLYMERISATION WITH ATOMIC FORCE MICROSCOPY

Roland M. Gröger†

Forschungszentrum Karlsruhe, Institut für Nanotechnologie, P.O.box 3640, D-76021 *Karlsruhe, Germany*

(Received 5 March 2001; revised manuscript received 16 May 2001)

We used atomic force microscopy (AFM) to image biological filaments deposited on mica substrates. After cutting of an actin fibre with the tip of the AFM, the filament started to grow in length. The growth occured in air under ambient conditions and proceeded over several days with a constant rate.

Keywords: actin, AFM, filament growth
PACC: 8700, 0779

I. INTRODUCTION

Cytoskeletial proteins like actin, intermediate filaments, keratin or tubulin play important roles in the stability and mobility of eucariotic cells. Their structural properties and self-organizing capabilities fulfill special functions in the cell.[1] Purified components of such filaments are commercially available, but to make them perform certain actions, it is required to carefully adjust the surrounding conditions like pH value, salt concentration, temperature and others. In a physiological environment all these conditions are fulfilled and changes of certain external values can trigger reactions of the cellular components. These reactions can also take place in rather unusual environments such as at the surface of a mica substrate as used for imaging of the topography of organic molecules and filaments by atomic force microscopy. Some of these macromolecules might be used outside a cell as building blocks or templates in a bottom-up construction of nanosystems.

II. EXPERIMENTAL

The sample material consisted of mouse tail DNA and other cell components in aqueous solution, originally intended for PCR (polymerase chain reaction) DNA amplification. A two millimeter long tissue sample from a mouse tail is homogenized, suspended in 300μl PBND buffer (PCR buffer with nonionic detergents) and digested by 3μl proteinase K over night at 55°C. The proteinase K is then inactivated by heating the solution to 95°C for 10min. Insoluble remnants are precipitated by spinning for 5min.[2] The remaining solution contains the DNA and other small components of the mouses cells which do not interfere with the PCR DNA amplification process. This solution was diluted 1:200 with ultrapure water. A drop of 10μl was cast on a freshly cleaved muscovite mica single crystal surface (Goodfellow, Cambridge, K 603025) and dried in a desiccator with silica gel (Merck, Darmstadt, Germany) over night. The sample was then imaged with an AFM (Nanoscope IIIa Multimode, Digital Instruments, Santa Barbara, USA) in Tapping Mode with silicon cantilevers (Nanosensors, Wetzlar, Germany) with a force constant of 22–79N/m. Imaging was done in air at 24°C and 40% relative humidity.

Besides other components a special type of filaments was found by AFM imaging, which was identified as actin. We tried to manipulate the filaments with the AFM tip, to find out stuctural properties and the strength of adhesion to the substrate. Manipulation was done by switching the microscope to contact mode and engaging the tip with the desired force with zero scan size. The force was selected empirically by gradually increasing the setpoint until a displacement of the filament was achieved. The movements were performed by manual input of X and Y offset values. Another method was to increase the damping setpoint in Tapping Mode so far, that the cantilever oscillation almost ceased, and then also manually inputting the offset. Before retracting the tip to continue imaging, we moved it some microns away from the filament, because out of the water layer remaining on the mica after the preparation of the sample, a meniscus was formed around the tip. Upon retraction the meniscus would leave behind a drop of water, preventing the

†Corresponding E-mail: roland.groeger@int.fzk.de

imaging of the underlying filament. However, after imaging two or three frames, the drop vanished anyway, the water attracted by the tip spread out again over the surface.

III. RESULTS AND DISCUSSION

The fibres we found were up to 16μm long. Many of them had a loop with a diameter of about 1μm at one end (Fig.1(a)). They appeared to be about three to five nanometer high (Fig.2(a)). In some small areas the mica surface was not completely wetted by the solvent. Here the thickness of the layer covering the surface under ambient conditions was determined to be around three nanometer, which results in an overall diameter of the filaments of six to eight nanometer (Fig.2(b)). The filaments had a superstructure with an average period of 55nm (Fig.1(b)). The only filaments matching these values are actin fibres, with the difference that natural actin has a periodic structure of 38nm, which is the spacing of the troponin complexes where the myosin attaches.[3] During a manipulation experiment one filament was stretched beyond its structural stress limit and broke. One of the newly created loose ends snapped away to an angle of 45° from its original position and the remaining filament was 750 nm shorter than the original length. Either a piece of the filament was lost during the manipulation, or the whole filament adhered to the substrate under strain, which was released by cutting, allowing it to assume its original length.

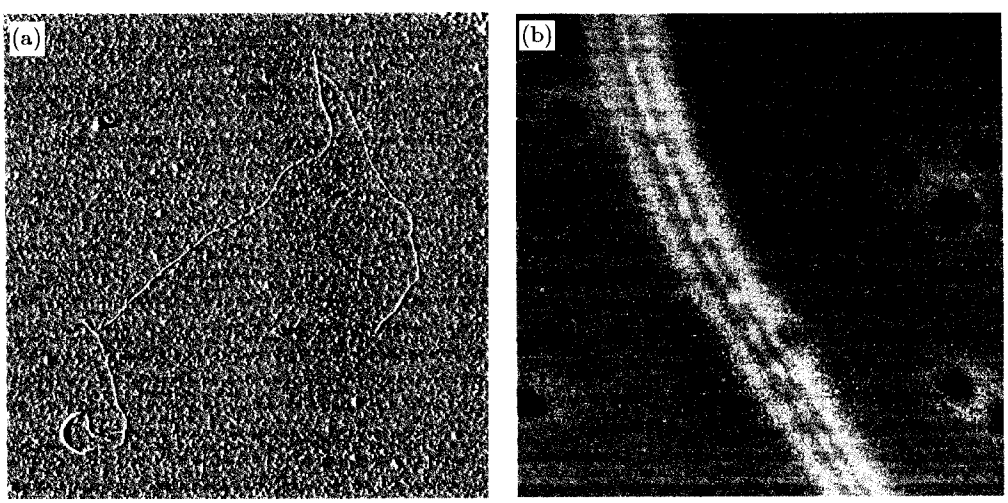


Fig.1. (a) A complete filament with 16μm length and a typical loop at one end. Picture size 16μm×16μm (Tapping Mode Amplitude) (b) Characteristic periodic superstructure on a filament with a spacing of 55nm. Picture size 600×600nm^2. (Tapping Mode Phase).

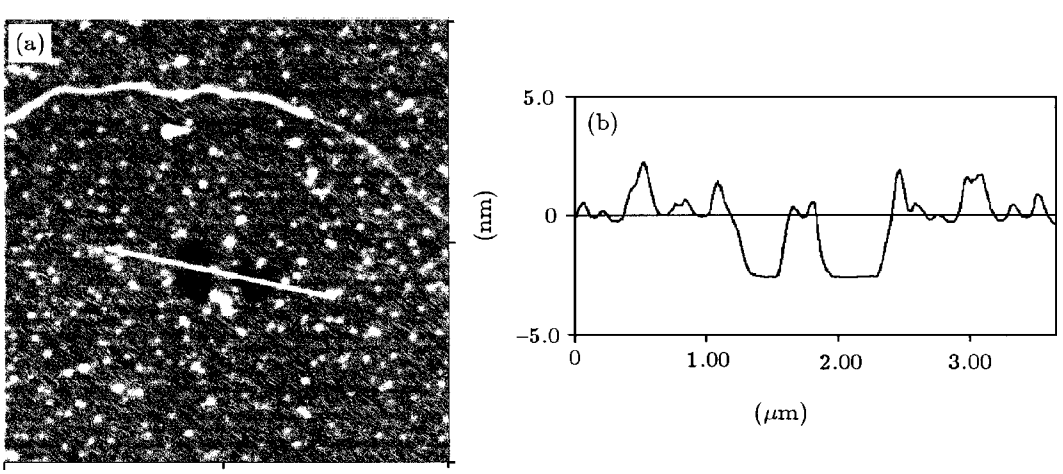


Fig.2. (a) Section of an actin filament on mica and the visualization of small surface areas which were not covered by solvent remnants. Picture size 6μm×6μm. (Tapping Mode Topography) (b) The profile shows a thickness of the solvent layer of 3nm.

After several hours it was found that both newly created open ends had grown in length. One end extended until it touched the other filament part and then ceased further growth. The other one, which snapped away, continued its growth over the next 74 hours, extending over 3.5μm (Fig.3(a)–(d)). The average growth rate was 50 nm per hour (Fig.4). The growth direction could be changed by bending the front end with the AFM tip. The new sections appeared smooth and had no periodic structure. Their height was the same as of the original filaments. On the surrounding substrate surface many dotlike precipitates were distributed, with heights around 5nm, deposited out of the solution. Neither did one of them change its position nor was one consumed by the growing filament. Hence, the source of the material for the growth must have been in the solvent layer on the substrate, undetectable with the AFM. A possible explanation for this observation is, that single globular G-actin proteins were present in abundance in the water layer and polymerized to the filamentous F-actin fibre, however no troponin complexes were attached to the fibres, and so the newly created pieces had no periodic structure. However, from its known size,[3] we should have been able to resolve G-actin proteins with the AFM, but this was not the case. Possibly the conformation of the single actin proteins on the surface is very different from that in a physiological environment, probably no longer globular. In other experiments on the same sample the cut filaments grew only a few nanometers. The external conditions were always the same, but maybe here some components for the growth were not present in the required quantity.

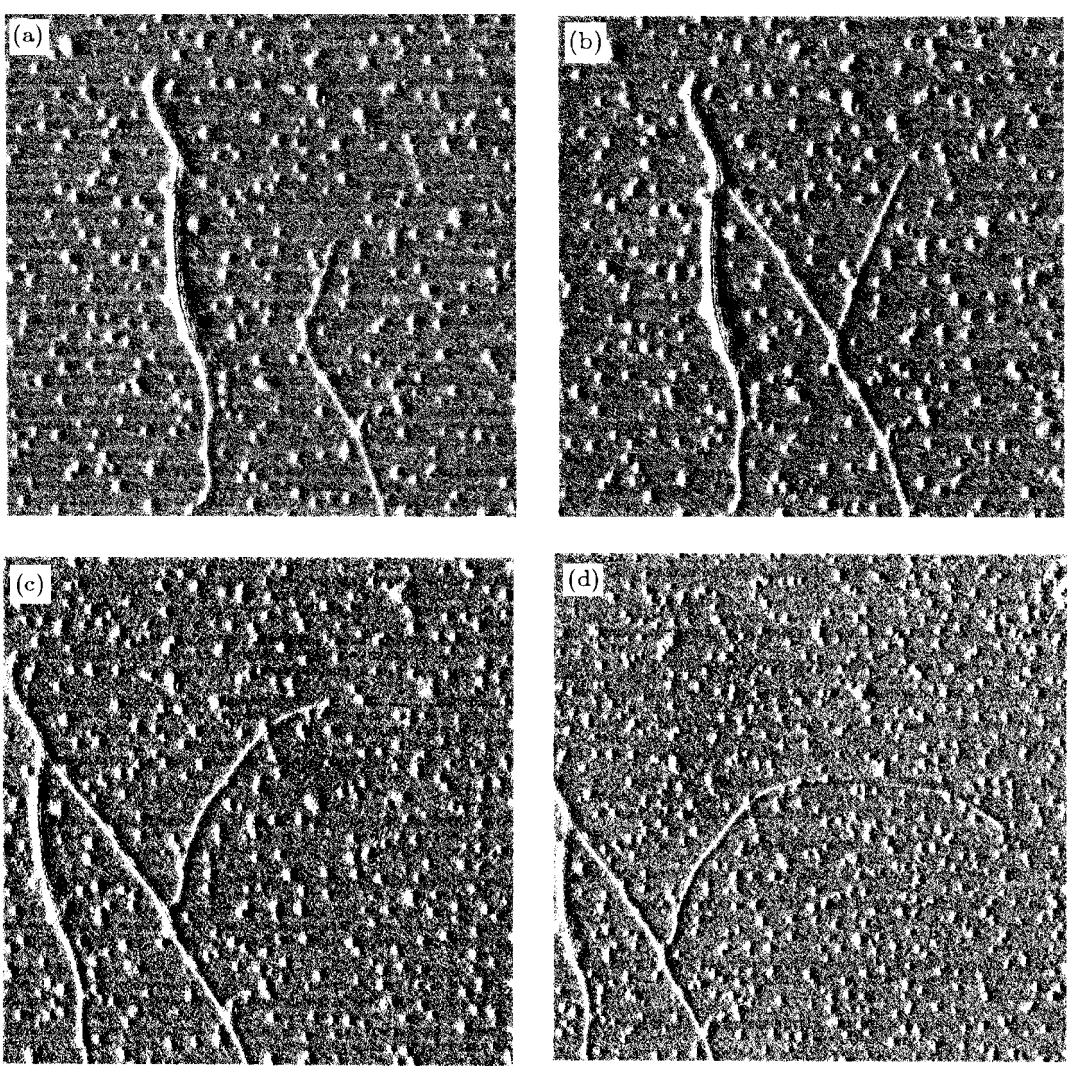


Fig.3. Filament growth sequence. The growth takes place at the upper right section of the filament in Fig.1(a): (a) immediately after cutting; (b) after 25h; (c) 48h; (d) 74h. The total length increase was 3.5μm.

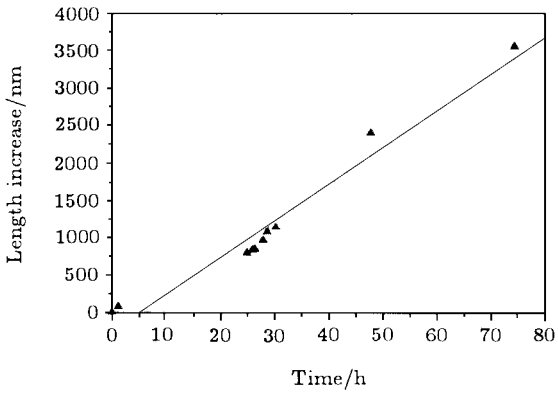


Fig.4. The length gain of the filament of Figs.1 and 3 versus time. The average growth rate was 50nm per hour.

IV. SUMMARY

The observations show that biological proteins and cell components can perform functions like growth, under external conditions far different from those in a living cell, like in the rather two-dimensional environment of a water layer on mica, even under the influence of an AFM tip tapping on it. Further investigations will have to clarify the conditions which promote the growth and and locate and identify the components. Methods to initiate and control the growth have to be developed for a useful application of such fibres as building blocks for nanosystems.

ACKNOWLEDGEMENTS

The author would like to thank Peter von Blanckenhagen, Fangqing Xie (Inst. of Nanotechnology, FZK), Debra Barton and Hubert Schorle (Inst. of Toxicology and Genetics, FZK) for valuable discussions and support in sample preparation.

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