CONFORMATION STUDY OF THE LARGE FRAGMENT OF E. COLI DNA POLYMERASE I BY STM

Yao Xiaowei (要小未), Li Minqian (李民乾), Xu Yaoliang (徐耀良),
Gu Minming (顾敏明), Hu Jun (胡 钧), Zhang Lanping (张兰平)
Zhang Jiahua (张家骅)

(Institute of Nuclear Research, Shanghai 201800, China)

Lu Changde (陆长德) and Qiu Minyan (裘敏燕)

(Institute of Biochemistry Research, Shanghai 200031, China)

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ABSTRACT

The large fragment of E.coli DNA polymerase I is imaged by scanning tunneling microscope. The specimen is deposited on highly oriented pyrolytic graphite surface, and then covered with pure paraffin oil in order to maintain hydration of the molecules. Images of the enzyme reveal an ellipsoid shape of 5.5—6.0 nm wide and 7.0 —7.5 nm long. The conformation of the enzyme is in agreement with the model derived from X-ray crystallography studies.

Keywords: STM E. coli DNA polymerase I

1 INTRODUCTION

The scanning tunneling microscope (STM) has been proven to be a powerful tool to produce topographic images of crystal surface with atomic resolution. Comparing with electron microscopy and X-ray crystallography, the major advantage of STM is that it can provide direct image of isolated molecule with a high resolution in close to native surroundings. In this paper, the first STM study of the large fragment of E. coli DNA polymerase I is presented.

The large fragment of E. coli DNA polymerase I has two domains corresponding to two enzymatic activities[1]: a DNA polymerase and a 3′ to 5′ exonuclease to edit out mismatched terminal nucleotides. We choose the enzyme as our experimental system because of its simplicity and significance in understanding of DNA replication.

2 EXPERIMENTAL

The 0.02 unit/ μl solution of the enzyme (purchased from USB company) was prepared with 10 mmol/l Tris-HCl buffer at pH 7.8. About 2 μl of the solution was deposited onto freshly cleft highly oriented pyrolytic graphite (HOPG) surface, and was allowed to be evaporated in air until the droplet became a thin aqueous layer.

Then the specimen was covered by 5 μl pure paraffin oil to keep the hydration. The paraffin oil was confirmed having no influence to the STM image in several control experiments.

A self-made SINR-1 STM[2] was used and the room temperature was 0 °C in our experiments. The constant feed-back tunneling current was 0.2 nA and the tip (Pt/Ir) bias was -100 mV. A lateral resolution of 0.1—0.2 nm and a vertical resolution of 0.01 nm were routinely obtained from the atomic structure of the graphite support.

3 RESULT AND DISCUSSION

Fig.1a is a typical STM image of the large fragment of E. coli DNA polymerase I, which reveals the ellipsoid feature of the enzyme. Measurements from several images indicate that the enzyme is 5.5—6.0 nm wide and 7.0—7.5 nm long. The observed shape and measured dimension are in agreement with the model determined by X-ray diffraction[3,4].

Unlike the easily entangled DNA molecules, the enzymes usually distribute scarely on the substrate. We believe that the sample aggregation is prevented by the electrostatic dispersion forces between

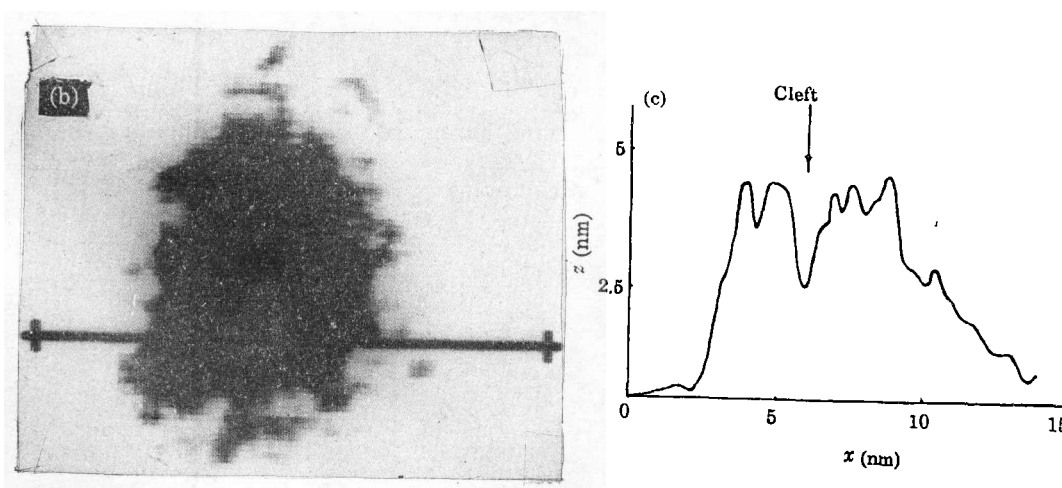

Fig.1 STM images (14.9 nm in x and 12.2 nm in y) of the large fragment of DNA polymerase I

(a) Showing a typical ellipsoid shape of the enzyme (b) Revealing the different aspect of the enzyme

(c) The profile across the enzyme in Fig.1b showing the cleft structure

neighbouring enzymes due to their negative surface charge potential[5].

Fig.1b is another ellipsoid-shaped image of the enzyme revealing some local

structure on its surface. Comparing with Fig.1c, which is a profile drawing across the enzyme in figure 1b from left to right, we can find that the image appears to have a hollow cleft in the middle of the enzyme. The apparent height of the image is 4.3 nm. The width of the cleft is 2.0 nm and the apparent depth is 2.4 nm. Structural information obtained from X-ray crystallography[6] suggest that the large domain of the enzyme has a cleft of 2.0—2.4 nm wide and 2.5—3.5 nm deep. This size and shape are suitable for the enzyme to bind double-stranded B-form DNA.

Some shape variations can be found between Fig.1a and 1b. They are attributed to different profiles of the enzyme. At present stage, the sample preparation and deposition are uncontrollable, so that enzymes are deposited on the graphite surface with random orientations. Since STM provides a direct image of the enzyme, it is reasonable to expect that the images will reveal different aspects of the enzyme.

In order to distinguish the enzyme from the possible artifacts on HOPG surface[7], we have carefully checked the HOPG substrates before and after the experiments. Before sample deposition, about ten percent of the examined areas (about 100 nm × 100 nm each time) show two kinds of defects on the HOPG surface: a. most of the defects (99 %) showing steps or DNA like features; b. in about seven hundred examinations, only one image shows the blob-like feature. When the sample is deposited on the HOPG surface, the ellipsoid image could be found on twenty percent of the scanned area (25 nm × 25 nm each time). So we believe that the observed images represent the conformation of the enzyme on the HOPG surface.

4 CONCLUSION

In this initial study, the large fragment of E. coli DNA polymerase I was imaged by STM in air. The images reveal the ellipsoid shape of the enzyme and its general appearace is in agreement with the X-ray crystallography. The results demonstrate the potential application of STM in studies of DNA replication.

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