Stretch and align virus in nanometer scale on an atomically flat surface

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Manipulation of macromolecules in nanometer scale is becoming an interesting research field. An approach to manipulate supramolecular assemblies is reported in this article. Linear phage viruses were aligned in one direction on atomically flat surfaces by a special method called ''molecular combing.'' Atomic force microscopy was used to check the results. Most of the phage strands were found to be stretch straight from one end to another. A related mechanism is also discussed. © *1998 American Vacuum Society*. [S0734-211X(98)04005-0]

I. INTRODUCTION

Recently, with the rising interest in nanotechnology, manipulation of atoms and molecules in nanometer scale has become a hot scientific issue. Based on the method of scanning probe microscopy (SPM), single atoms and molecules have been arranged to form artificial patterns.[1–7] Even nanodroplets of liquid water can be moved around on a solid surface.[8,9] For more complicated molecular systems, i.e., macrobiomolecules, other kinds of methods such as laser tweezers[10,11] and ''molecular combing''[12] have been developed. Manipulation of biomolecules has a wider interest in science. For example, manipulation of DNA based on those methods has created great interest for both physicists and biologists.[13–22]

In the trend of ''from simple to complicated,'' new approaches will be focused on more complicated life systems. One of the candidates is virus. Viruses are supramolecular assemblies. They have confined structures and most of them are in nanometer size. Manipulation of life in this scale may help us to know more about the rules of nature. However, the challenge is very great. In our earlier work, we have successfully aligned DNA into a two-dimensional (2D) pattern on an atomically flat surface.[23] Based on a similar method, we are pursuing an approach to manipulate virus. In this article, we will report our first results of stretching and aligning a phage virus on an atomically flat surface.

II. EXPERIMENT

The sample of phage virus (wild type phage, fd-tet-GOD1) came from the Shanghai Institute of Biochemistry Research, Chinese Academy of Sciences. It had a linear structure and was about 1.2 μm in length.[24,25] The reason that we chose this kind of phage virus for our first attempt is based on the idea that the liner phage might also be straightened like DNA by a modified ''molecular combing'' method.[23,26] The phage sample was first diluted with a buffer (10 mM tris-HCl, 100 mM KCl, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, *p*H 7.6) to a concentration of 0.1–0.2 A_{260} unit/ml. Then, it was diluted with doubly distilled water (ddwater) by 1000 times in volume. For normal samples, 2 μl of phage solution was deposited onto the surface of APS-mica[26] and washed thoroughly with ddwater. The sample was then dried with a flow of clean nitrogen. For ''manipulation samples,'' a modified molecular combing[23,26] method was used. Briefly, the sample was prepared by first depositing a small drop (typically 2 μl) of virus solution onto a corner of a clean glass cover slip. The glass cover slip was then carefully placed onto the top of the APS-mica. The weight of the thin cover slip forced the solution to spread immediately into a thin layer. The cover slip was made into a narrow long rectangle (8 mm$\times$22 mm) in order to control spreading in one confined direction. During spreading, phage viruses were stretched[15,26] and the mechanism will be discussed in detail next. After a few minutes, to be exact, no more than five minutes, the glass cover slip was removed and the APS-mica surface was rinsed with ddwater and dried with clean nitrogen. All the samples were imaged with an atomic force microscope (AFM). AFM has been widely used in the studies of biomolecules.[27–34] The AFM used here was a Nano IIIa system from Digital Instruments (Santa Barbara, CA). All the images were taken in air in tapping mode.

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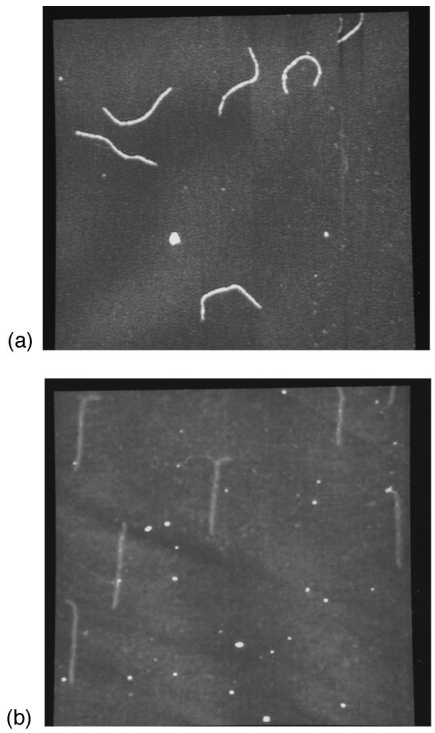

FIG. 1. (a) AFM image of phage virus in an area 4.85 μm×4.85 μm. The sample was prepared by depositing a small drop of 2 μl phage solution onto APS-mica and rinsing it with ddwater. The viruses appeared as soft strands. (b) AFM image of the phage virus in an area 4.85 μm×4.85 μm. The sample was prepared by a modified molecular combing method. All the phage strands in this image were stretched straight from one to another end and in the same direction.

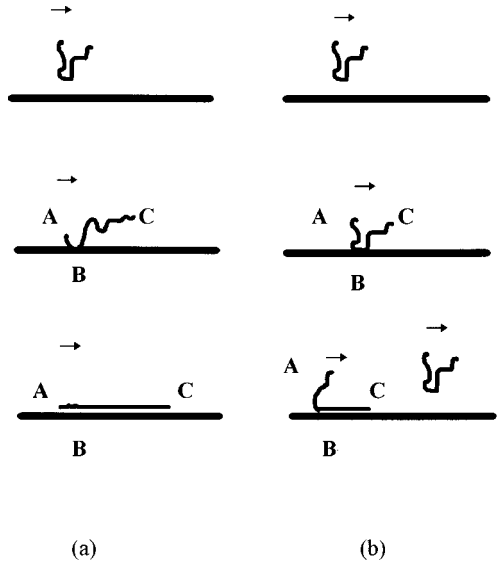

FIG. 2. One possible interpretation of stretching. (a) One end of the phage strand touched the surface first and was adsorbed, resulting in the whole strand being stretched straight. (b) If the first adsorption occurred at the middle, the whole strand would not be fixed on the surface.

III. RESULTS AND DISCUSSIONS

Figure 1(a) shows an AFM image of phage virus normally prepared on the surface of APS-mica. APS-mica was prepared atomically flat and this was very important for the AFM study.[26] We found that the phage molecules could be tightly adsorbed on APS-mica and could not be washed away by water. We also tried to deposit phage molecules on the surface of bare mica but they were easily washed away during rinsing. Strong adsorption of APS-mica to linear molecules played a very important role in stretching them.[23,26,35–38] We can see from Fig. 1(a) that phage viruses were distributed randomly on the surface and they looked like a piece of soft thread. Most of them were in randomly curved or coiled shapes and this was in agreement with the nature of phage virus and has been verified by other observers.[24,25]

The phage viruses changed their shapes, however, after they were treated by a modified molecular combing method as shown in Fig. 1(b). The viruses became straight and were aligned in the same direction, indicating that there was an efficient stretching force exerted on the phage during combing. The principle of stretching in this method can be described briefly as following. During combing, the solution flew along the surface of APS-mica and phage virus ran with the fluids. If one point on a phage strand occasionally touched the surface, it became fixed because of the adsorption force. The other parts of the strand kept moving forward. If the adsorption was strong enough, this would give a stretching force along the strand in the direction of the flow. The viscosity of the flowing fluid would also produce a force in the same direction along the strand when it was slowed down by adsorption. It was in this way that the phage viruses were straightened and aligned in one direction.

One interesting question arises here. The first adsorption on the phage strand may often happen in the middle but rarely at the ends. If the first adsorption occurred often in the middle, ''U'' or ''V'' shape strands would be the most expected shape in the images according to the stretching mechanism introduced above. However, we found that most of the viruses were stretched from one end to another in our experiments. Here we give a possible interpretation and it can be understood by Fig. 2. In Fig. 2(a), one end of the phage strand was first adsorbed on the surface. One can expect that a short of the following parts of the strand could be adsorbed immediately in some cases. If the adsorbed length was long enough, the stretching force exerted on the free parts was smaller than the adsorbing force which was an integration of point adsorption along the whole adsorbed length. Then the strand was fixed in a straight shape. In the case of Fig. 2(b), one middle point B of the strand was first adsorbed onto the surface. The stretching force along the following part BC may follow the same rule as that in Fig. 2(a) and BC could be stretched and fixed on the surface. However, the stretching force on the former part AB would easily overcome the adsorbing force on B which was only a value of point adsorption. The strand was then driven away from the surface. Even though the first adsorption may often happen in the middle, the viruses could not be fixed in this case and we could not see them. This is why in most cases we only observed straight strands from one end to another.

Another interesting phenomenon in Fig. 1(b) is that one end in almost every strand was straight and another a little curved. And the direction from the straight end to the curved was always against the direction of the fluid flow. We also found a similar phenomenon when stretching short DNA fragments. Detailed discussions of this will be published elsewhere.

IV. CONCLUSION

In summary, we have straightened and aligned phage viruses on an atomically flat surface of APS-mica by molecular combing. It was found that in many cases the phage strands were stretched from one end to another. Related mechanisms were also discussed.

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