Colloid Journal, Vol. 66, No. 6, 2004, pp. 673–678. Translated from Kolloidnyi Zhurnal, Vol. 66, No. 6, 2004, pp. 750–755.

Study of the Peculiarities of Adhesion of Tobacco Mosaic Virus by Atomic Force Microscopy

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Received February 20, 2004

Abstract—The peculiarities of adhesion of tobacco mosaic virus to mica and graphite, in particular the mutual orientation of viral particles on the substrate and its possible effect on a virus, were studied using atomic force microscopy. Different versions of the chemical modification of a mica surface to enhance adhesion of viral particles were considered.

INTRODUCTION

The atomic force microscopy (AFM) that appeared in 1986 opened new opportunities for studying biological objects, in particular viruses. It is based on scanning the surface with sharp tip (probe) mounted on a cantilever whose deflection caused by the interaction with the surface is detected by a high-precision optical method [1]. Tip movement with respect to the surface is done by a piezoelectric scanner. Samples studied by AFM do not need contrasting with heavy metal atoms, as in the case of electron microscopy; in addition, scanning can be performed in a liquid medium that, as applied to biological objects, means the possibility of their study under native conditions.

Tobacco mosaic virus (TMV) is a classic object of virology whose isolation in 1898 paved the way to the development of this branch of science. The virus represents "rods" with a diameter of 18 nm and a length of 300 nm (Fig. 1) composed of a protein and ribonucleic acid (RNA) [2]. Modern virology owes to this virus not only its origin, but also main concepts developing up to now over the entire 20th century [3, 4]; hence, its further study, in particular with the use of novel techniques, is still urgent.

TMV was repeatedly studied with atomic force microscopy [5–8]. A high adsorption activity of viral particles with respect to the surface of highly oriented pyrolytic graphite (HOPG), compared to mica surface, was mentioned earlier [5] and explained by the hydrophobic interaction between the particles and the substrate. In this work, after the treatment of TMV virions with dimethyl sulfoxide (DMSO) or urea, we observed the "stripping" of viral particle and its escape from the RNA molecule. The AFM makes it possible to monitor the successive stages of the disassembly of other viruses that was illustrated with a potato X virus [9]. It should be emphasized that, in most of the similar studies, the choice of a substrate and the procedure of virus attachment to the substrate was a cornerstone problem.

The aim of this work is the comparative study of the adhesion of TMV particles to mica and graphite substrates using AFM both in air and liquid, the elucidation of the action of a probe on a sample, and the study of the possible modification of mica surface for the attachment of viruses to the modified surface.

MATERIALS AND METHODS

Atomic Force Microscopy

All measurements were performed with a Nanoscope IIIa atomic force microscope (Digital Instruments, USA) in tapping mode. For measurements in air, we used commercial silicon cantilevers with a nominal stiffness of 42 N/m. Resonant scanning frequency varied from 280 to 310 kHz. Measurements in liquid were

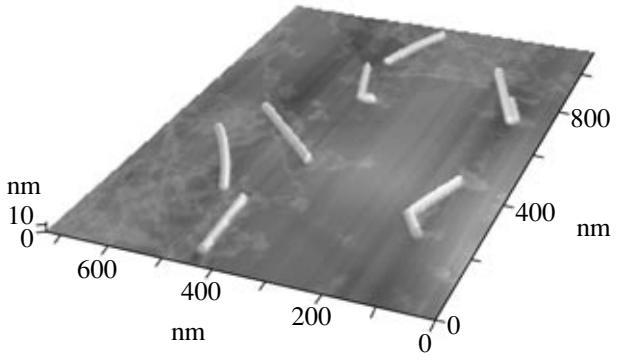

Fig. 1. 3D image of TMV taken with AFM.

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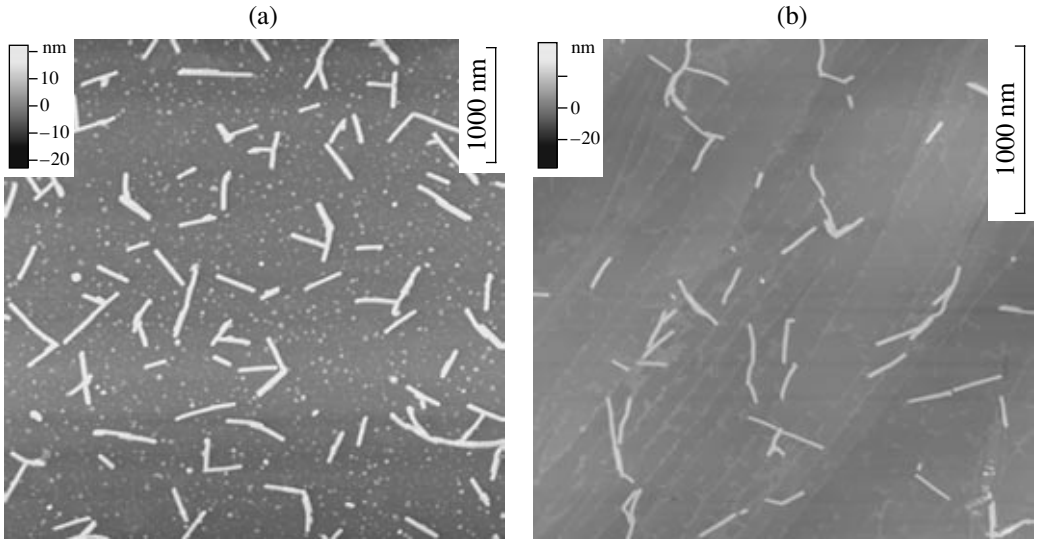

Fig. 2. AFM images in air of the TMV particles adsorbed on: (a) mica (size frame is 4.23 × 4.23 μm) and (b) graphite (size frame is 2.95 × 2.95 μm).

performed in a liquid cell (Digital Instruments, USA) in the tapping mode using commercial silicon nitride cantilevers at a scanning frequency of 8–10 kHz. FemtoScan Online software was used for the image processing [10].

Tobacco Mosaic Virus

The virus was grown on a *Tobacco nicotiana*. For the tobacco infection, the virus inoculum was rubbed into the leaves together with corundum. The virus was isolated from the infected leaves by the differential centrifugation [11]. The virus was purified additionally by a gradient-density centrifugation in a cesium chloride solution with a Beckman L8 centrifuge in an SW-41 rotor at a speed of 35 000 rpm for 3 h at 4°C. The TMV preparation with a concentration of 5 mM in a Tris-HCl buffer containing 50% of glycerol (pH 7.8) was stored at –20°C.

Preparation of Virus Samples for AFM Analysis

Prior to deposition onto the substrates, the aliquot of virus dispersion was centrifuged at 13 000 rpm on an Eppendorf 5415 C centrifuge, diluted with phosphate buffer (5 mM sodium phosphate, 150 mM NaCl, pH 7.3) until the concentrations of 1.3 mg/ml (for mica) or 0.325 mg/ml (for graphite) were achieved. Five microliters of thus prepared colloidal solutions were spread over fresh cleavages and left to stand in a humid chamber for 30 min on mica or for 15 min on graphite. Substrates with sorbed virus were rinsed with a triply distilled water (two or three times) and dried in a vacuum desiccator.

To observe virus in the liquid AFM cell, we used its dispersion in the phosphate buffer with a concentration of 0.065 mg/ml.

Mean height values (nm) of the TMV particles adsorbed on mica and graphite calculated from the AFM images obtained in air and liquid

Medium	Substrate	
	mica	graphite
Air	18.5 ± 0.9	18.8 ± 0.9
Liquid	17.6 ± 0.9	17.5 ± 0.9

Modification of Mica Surface

Ten to fifteen microliters of undiluted isobutyl alcohol, triethylamine, diethylenetriamine, or aqueous 1% cetyltrimethylammonium bromide (CTAB) solution were spread over mica and left to stand for 15 min; then, substrates were twice rinsed with water.

RESULTS AND DISCUSSION

Viral particles deposited onto the substrates of two types (mica and highly oriented pyrolytic graphite) were studied with the AFM technique. The number of particles, deposited onto the HOPG cleavage from the dispersion with a concentration of 0.065 mg/ml, was about 200 μm^{-2}, whereas upon the deposition onto mica cleavage from the dispersion with a 20-fold higher concentration, this number was equal to 3–6 μm^{-2}.

The use of contact scanning mode, at which the cantilever with a probe scans across the sample surface, being in a "physical contact" with the sample, is the basis of a popular AFM technique of measuring the elasticity or Young's modulus of a studied object, for example, viral particles (e.g., see [12]); as the contact force between the tip and the surface increases, the

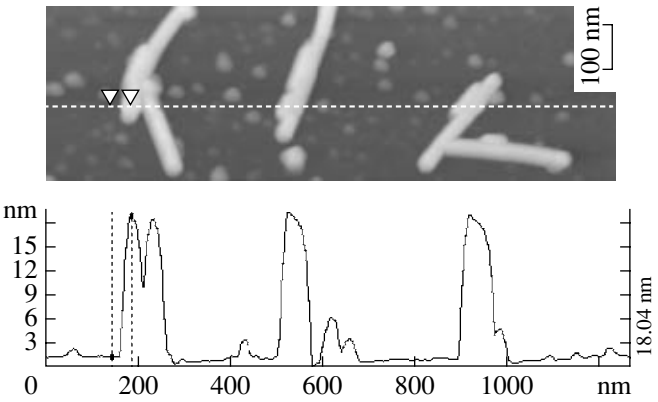

Fig. 3. Fragment of the AFM image of TMV on mica and cross-section illustrating the possibility of using this virus as a standard for the calibration of piezoelectric scanner in a vertical direction.

height of imaged viral particle diminishes. The minimization of effective cantilever action on the object to zero is rather problematic due to capillary forces. Therefore, on images recorded in contact mode, the heights of soft objects, such as a viral particle, can often be underestimated. Taking this into account, we performed measurements in tapping mode when the presence of capillary forces does not play a significant role.

The AFM images of tobacco mosaic virus deposited onto mica and graphite obtained in air in tapping mode are represented in Fig. 2. The height of viral particle calculated from the obtained images was equal to 18.5 ± 0.9 and 18.8 ± 0.9 nm for mica and graphite, respectively, that agrees well (within the error limits) with the diameter of TMV particle measured by another techniques. Mean heights of TMV particles deposited onto both substrates measured in air and liquid (in buffer solution) are listed in the table. Tobacco mosaic virus deposited onto mica can be used as a standard for the calibration of the piezoelectric scanner of a microscope in a vertical direction, because such a procedure ensures stable height values (see Fig. 3) (main part of the error in height values is related with the nonideal feedback, the hysteresis, and the creep of piezoceramics, as well as with the temperature drift and the noises in a microscope).

Figure 4 demonstrates the viral particle length distributions for both studied substrates. Their analysis is indicative of a noticeable deviation of the lengths of TMV particles adsorbed on graphite from their common values: the distribution maximum fits the value equals 250 nm, whereas TMV virion length is 300 nm (that is actually obtained for viruses on mica). The reason for such a difference in lengths is not completely clear to us; possibly, this is associated with the effect of the graphite substrate on the TMV particles that leads either to the partial destruction of the protein shell of the virus or to the mechanical deformation (compression) of virus adsorbed on the substrate. Partial destruc-

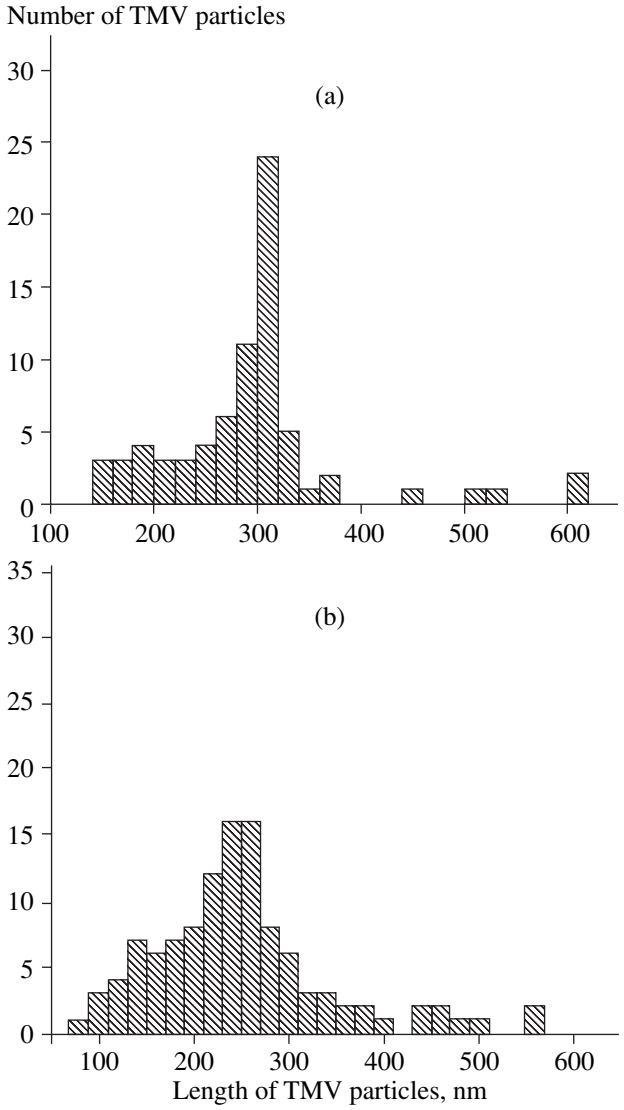

Fig. 4. Histograms of the lengths of the TMV particles adsorbed on: (a) mica and (b) graphite.

tion of rodlike virions on the hydrophobic surfaces was observed in [8]. The presence of the TMV particles, whose length approximately twice as large as the most probable value, indicates the ability of virions to joint in the end-to-end manner. As the concentration of TMV preparation increases, the aggregation of viral particles in the side-to-side manner, along with end-to-end contacts, is also observed (Fig. 5); moreover, up to ten particles can participate in such an interaction. The presence of such aggregates in the form of islands suggests the cooperative mechanism of assembly of viral particles.

Yet one more difference in the adsorption of TMV on mica and graphite is the orientation of individual viral particles on these substrates. The TMV particles on HOPG are characterized by a specific orientation coinciding with the direction of steps that are always

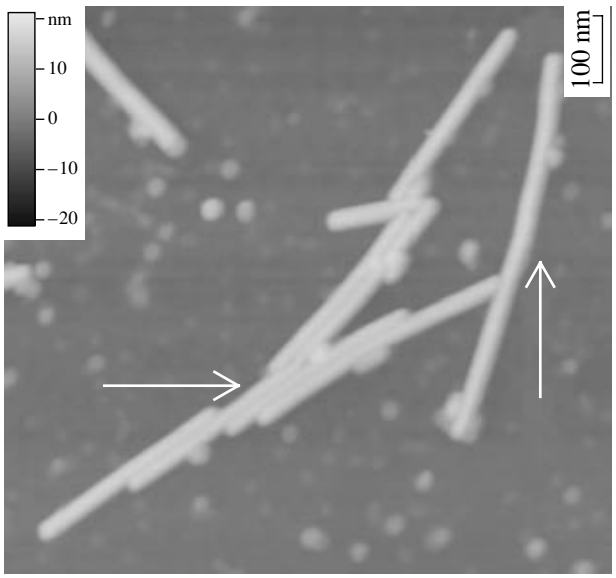

Fig. 5. AFM image of the TMV particles on mica illustrating the particle aggregation in side-to-side manner (horizontal arrow) and their end-to-end binding (vertical arrow).

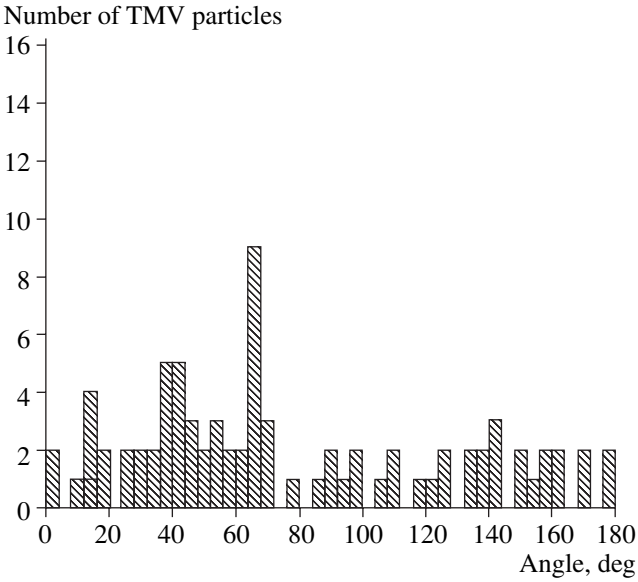

Fig. 6. Distribution of TMV particles adsorbed on graphite over the angles. The angle was counted counterclockwise from horizontal direction.

present on graphite due to defects in its lattice. Figure 6 shows the histogram of HOPG particle distribution over the angles counted counterclockwise with the reference to the horizontal direction. The maximum in the region of 63° just corresponds to the direction of steps.

Note that graphite is not a perfect crystal, but consists of crystallites, the carbon atom ordered regions whose sizes depend on a graphite type. Therefore, to describe the mutual arrangements of the TMV particles on graphite, we measured the acute angles formed by the pairs of only neighbor virions adsorbed on one crystallite. The histogram obtained for TMV on graphite is characterized by a well pronounced maximum at 55° and a less pronounced maxima at 35° and 77° (Fig. 7). Because the graphite lattice has a hexagonal structure, one can explain (within the error limits) the obtained values of maxima: viruses are adsorbed along the crystal directions shown in Fig. 8 where angles *1* an *2* are equal to 60° and 30°, respectively. The third maximum turned out to be very broad; we believe, that it corresponds to an angle of 90° which is designated in Fig. 8 as angle *3*. Note that similar results were obtained when analyzing orientations of the DNA molecules on graphite [13] where the bendings of these molecules were 30°, 60°, and 90°.

The ability of viral particles to be deposited in a large amount on graphite does not mean, however, that there is a strong adhesion interaction between the substrate and TMV: when scanning in liquid, even in tapping mode when the tip action on a sample is minimized, one can observe a sharp decrease in the number of the TMV particles on two consecutive images (Fig. 9). Similar results were obtained also for scanning (in liquid) of the TMV particles adsorbed on mica treated

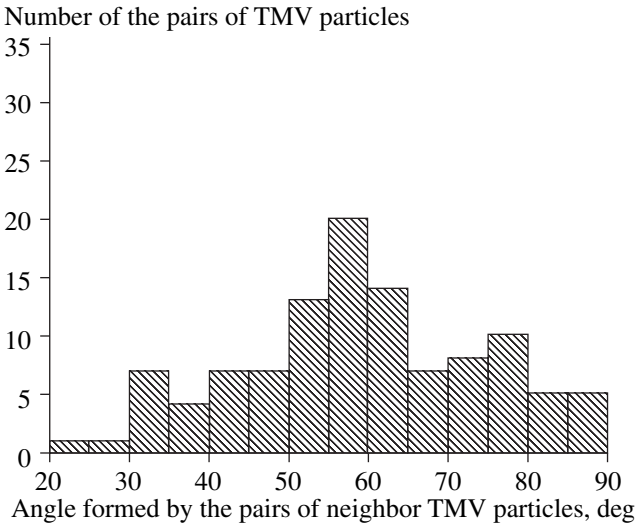

Fig. 7. Histogram of the distribution of the pairs of neighbor TMV particles adsorbed on graphite over the angles formed by these pairs of particles.

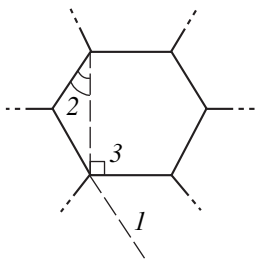

Fig. 8. Scheme illustrating the fragment of hexagonal crystal lattice of highly oriented pyrolytic graphite. Angles *1*, *2*, and *3* are equal to 60°, 30°, and 90°, respectively.

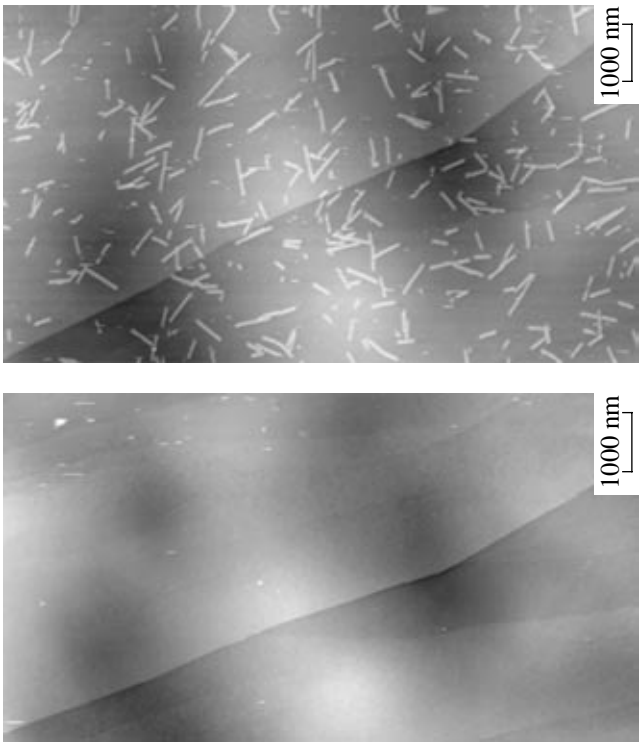

Fig. 9. Successive AFM images of TMV on graphite obtained on scanning in liquid. Frame size is 10.7×5.9 μm.

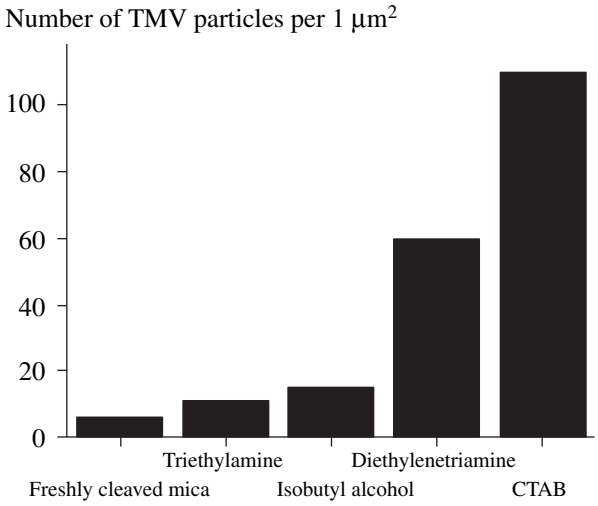

Fig. 10. Histogram demonstrating the amount of viral particles adsorbed per unit surface of mica modified with various chemical compounds.

with γ-aminopropyltriethoxysilane [14]. Thus, for the efficient study of TMV in liquid, it is necessary to preliminarily modify the substrate surface to more strongly attach viral particles to the substrate.

As was already mentioned, the difference in the values of virion adsorption on HOPG and mica is, most likely, associated with the hydrophobic interaction between the graphite surface and the TMV particles. Thus, the number of these viruses adsorbed per unit substrate surface area can act as a peculiar index of the surface hydrophobicity.

In order to enhance the hydrophobicity of mica surface, its properties were changed by the modification with various chemical compounds (see above): isobutyl alcohol, triethylamine, diethylenetriamine, and cetyltrimethylammonium bromide. Figure 10 shows the histogram demonstrating the number of the TMV particles adsorbed on mica whose surface was treated with each of the aforementioned compounds. The most effective modifier was cetyltrimethylammonium bromide, which increased the TMV adsorption on mica by 10 times larger compared to unmodified mica.

CONCLUSIONS

Tobacco mosaic virus can serve as a standard for atomic force microscopy [15]. Hydrophobic surfaces better adsorb the TMV particles; in this case, their partial destruction or deformation take place. Mutual orientation of the TMV particles on graphite correlates with the structure of its crystal lattice.

The studies performed testify the necessity of the development of the procedure for the attachment of the TMV particles to the substrate for their AFM study in liquid: the unmodified mica and graphite substrates do not allow to obtain reproducible images upon the repeated scanning even in tapping mode.

ACKNOWLEDGMENTS

This work was supported by the Russian Academy of Sciences (Program "Creation and Study of Macromolecules and Novel Macromolecular Structures," project "Mechanics of Single Macromolecules and Their Complexes Using Plant and Bacterial Viruses as Examples"), by NATO Research Program (project no. LST.CLG.980194 "Analytical Nanoscopy of Nucleic Acid Dynamics"), and by INTAS (project no. 01-0045).

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