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Application of UHV-AFM for investigation of structure of plant viruses and their interaction with Si (1 1 1) surface

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Summary. In this research we applied ultrahigh-vacuum atomic force microscopy (UHV-AFM) for investigation of tobacco mosaic virus (TMV) and alfalfa mosaic virus (AMV) interaction with Si (1 1 1) surface. Changes of virion conformation and different degree of order on the investigated surface were determined. It was demonstrated that adsorption of TMV virions is accompanied with formation of predominantly single-layer ordered films while AMV adsorption is accompanied with formation of unordered accumulations of the virions and more abrupt change of the height of viral particles. It was determined that the change of virions' height depended on the quantity of negatively-charged amino acid residues on the outer surface of capsid.

Keywords: UHV-AFM, plant viruses, capsid, Si (1 1 1), conformation.

Viruses consist of nucleic acid and globular molecules of protein and are the simplest forms of living organisms. Viral particle doesn't have own reproductive system, but penetrating into the cell it keeps own replication at the expense of synthesis of viral proteins by infected cell. The dimensions of viral particles are in the range of 15-600 nm [1]. Besides biological features (ability to reproduction, infectivity) viruses possess many useful properties. In particular, viral particles are able to form crystals with advantageous optical properties. The presence of the great amount of charges at internal and external surfaces of the virion helps to receive nanoparticles of different materials with the use of biomimetic approach [2, 3]. The ability of viruses to penetrate into the cell may be utilized for the development of novel intracellular nanoprobes and nanosensors. The most perspective viruses for the use in nanotechnologies are plant viruses because they are safe for humans and animals, can be received in great amounts and are able to stand modifications [4].

In this work we used the method of high-resolution probe microscopy for investigation of the behavior and ultrastructure of rod-like viruses with spiral symmetry of capsid (Fig. 1), in particular, of alfalfa mosaic viruses (AMV) and tobacco mosaic viruses (TMV) on the Si (1 1 1) surface. Previously, the best resolution of 22 nm was achieved using transmission electron microsco-

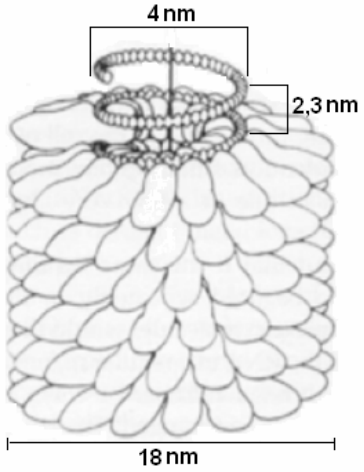

Fig. 1. The model of packing of protein subunits in the tobacco mosaic virus.

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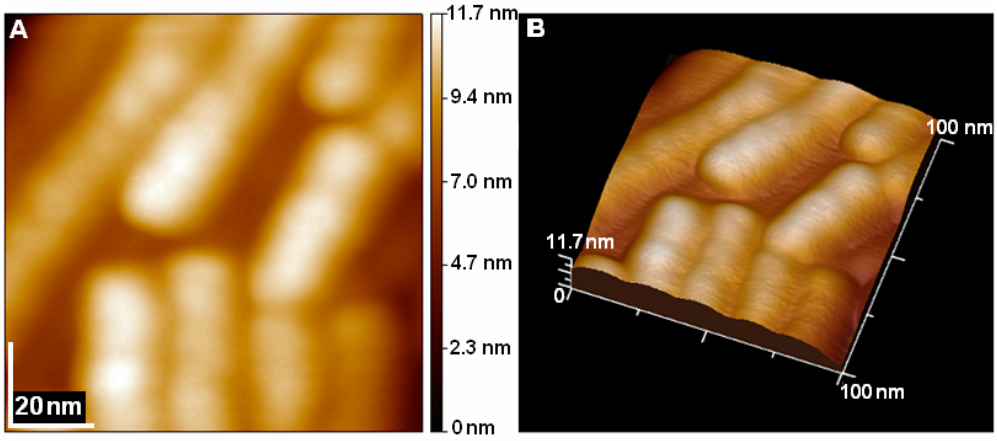

Fig. 2. TMV virions on the Si (1 1 1) surface after deposition of fresh suspension. (A) Two-dimensional image of TMV virions. (B) Three-dimensional image of TMV virions.

py and resonant X-Ray diffraction microscopy [5]. We investigated not only the ultrastructure of viruses but also their behavior on the surface of semiconductor.

Materials and metods. We used the TMV suspension in concentration of 12 mg/ml and AMV suspension in concentration of 10 mg/ml. Both suspensions were dialyzed against distilled water in order to remove salts available in the buffer. The suspensions were deposited on the surface of silicon monocrystal Si (1 1 1) using the micro syringe. The Si (1 1 1) surface was dried under nitrogen gas and the specimen was transferred to the atomic-force microscope (AFM) chamber (ultra-high vacuum scanning probe microscope «JSPM-4610», «Jeol», Japan). The residual pressure in the chamber was 3.0×10^{-8} Pa. For the scanning of the surface we used silica nitride probes. The investigation of virus on the surface of monocrystal was carried in the non-contact AFM mode with atomic resolution.

During the experiment the interaction of TMV with the Si (1 1 1) surface was studied after deposition of fresh suspension of viruses and after 4 months after preparation. Obtained results were compared with the results of studies of the AMV interaction with the identical surface.

Results. The received specimens of plant viruses were analyzed by ultra-high vacuum non-contact AFM. Uppermost we investigated behavior of TMV virions after adsorption on the Si (1 1 1) surface.

As it can be seen from the Fig. 2 the diameter of TMV virions is about 18 nm. If we see virions' shape as cylindrical then its height on the surface must coincide with its width from the AFM data. You can see that the width of the virions on the Si (1 1 1) surface corresponds to the electron microscopy data (Fig. 2) but the virions' heights are significantly less (about 11.4 nm). This could be the evidence of significant interaction with the silicon surface. The authors of the the work [6] also indicate the decrease of TMV virions' heights from 18.0 nm on the pure pyrolytic graphite to 15.0 nm after deposition on the pyrolytic graphite modified with carboxyl and acylchloride groups.

Such decrease of the virion's height could be explained with the virion-probe interaction (what happens under contact AFM probing), but this interaction cannot result in so significant changes espesially under non-contact AFM conditions.

The deposition of fresh suspension of TMV virions on the Si (1 1 1) surface was accompanied with formation of ordered monolayers. Virions' textures which can be seen on the surface of silicon appeared due to strong linear interaction of viruses. After detailed examination of received images besides «texturing» crosscut lines were observed. This may be caused by the metameric structure of virions, which can be explained with existence of basic structural unit which is repeated and consists of 49 capsid subunits (capsomers) and have the length of 69 Å (corresponds to three turns of helix) while the full virion's length is about 300 nm. This segmentation can be seen more clearly from three-dimensional image (Fig. 2b).

Studies of the effect of the term of storage of the suspension after its preparation on the

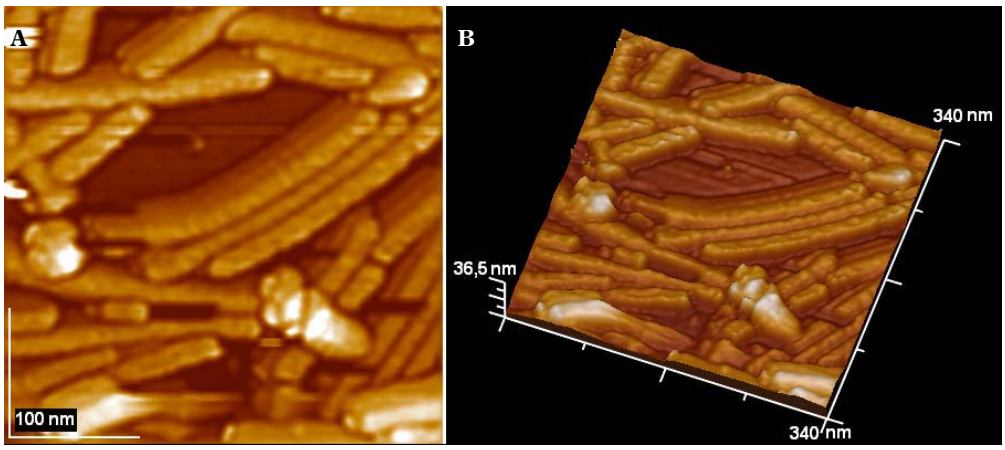

Fig. 3. TMV virions on the Si (1 1 1) surface after deposition of virions' suspension (4 months after preparation). (A) Two-dimensional image of TMV virions. (B) Three-dimensional image of TMV virions.

structure and properties of TMV virions showed that along with monolayer formation the layup of virions was observed at some zones, which can be caused by aggregation of the virions at the room temperature. The diameter, length and the height of virions remained unchanged (Fig. 3).

The deposition of suspension of AMV virions on the Si (1 1 1) surface was accompanied with aggregation of virions into dense clusters and with decrease of their heights from 19 to 9 nm. The formation of unordered multilayer clusters was predominant (Fig. 4).

Also we examined the behavior of AMV/anti-AMV antibody complexes. The adsorbed antibodies were localized on the virion surface. The adsorption of antibodies was accompanied with disaggregation of virion's clusters and decrease of their height (Fig. 5). Also it can be clearly seen that adsorption of antibodies happens only in several regions of virion's surface. This can be explained with the specificity of antigen (AMV)/antibody interaction.

Discussion. There are two types of adsorbtion: 1) physical — takes place at the expense of dispersive (Van-der-Vaals) forces, formation of hydrogen bonds and other interactions with electrostatic nature; 2) chemical — takes place at the expense of formation of chemical bonds between adsorbate and adsorbent. The outer and inner surfaces of viral capside are oppositely charged because of presence of positively and negatively charged groups in aminoacid's radicals. But the Si (1 1 1) surface isn't charged and, obviously, the interaction of the capsid with this surface isn't caused by electrostatic attraction. So the role of such interactions is excluded.

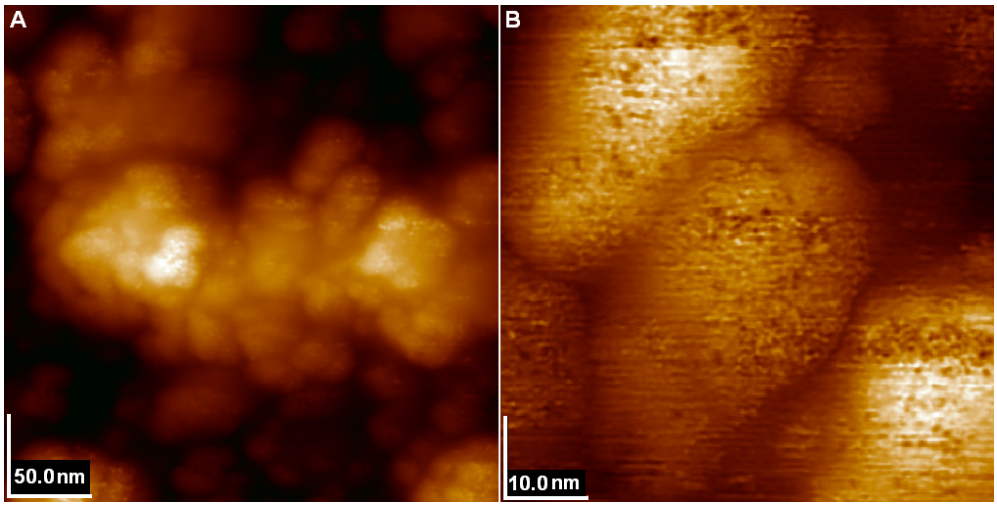

Fig. 4. AMV virions on the Si (1 1 1) surface after deposition of fresh suspension of virions. (A) The image of AMV virions' aggregations; (B) The enlarged image of AMV virions.

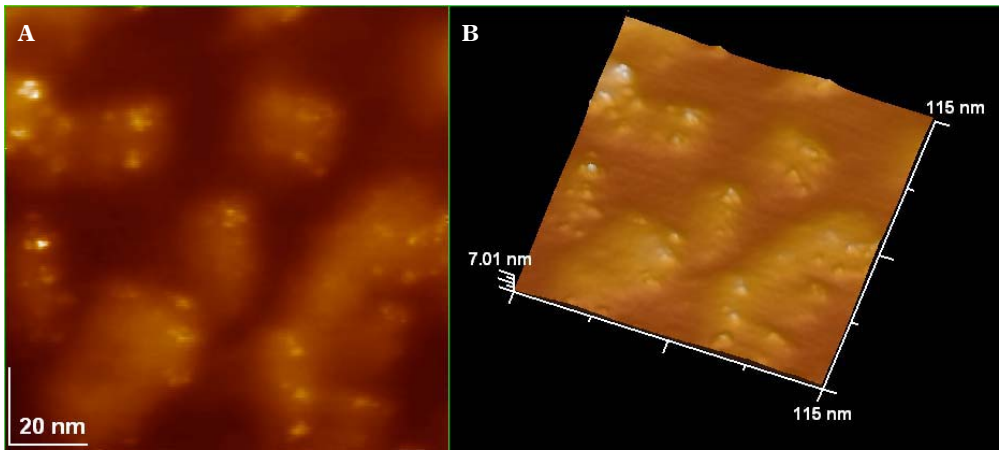

Fig. 5. Adsorption of anti-AMV antibodies on the surface of AMV virions. (A) Two-dimensional image of AMV/anti-AMV antibody complexes. (B) Three-dimensional image AMV/anti-AMV antibody complexes.

Chemical adsorbtion also is excluded as far as silicon does not form chemical bonds with any components of viral capside. So the most probable processes during virions' adsorption on the Si (1 1 1) surface are Van-der-Vaals interactions and formation of hydrogen bonds with the surface. Van-der-Vaals interactions are prevalent during adsorption of the virions on the graphite surface as far as this material is hydrophobic and formation of more stable hydrogen bonds is imposible. Since the energy of such interactions is the lowest (about 2 kJ) Van-der-Vaals interactions are very weak and aren't accompanied with significant changes in molecule conformation. Consequently, there are no changes of TMV virion's height on the graphite surface and vice versa the adsorption of TMV virions on the Si (1 1 1) surface is accompanied with significant changes in their conformation and decrease of virions' height up to 11.7 nm. Similar result was obtained after studies of AMV behavior on this Si (1 1 1) surface (decrease of virions' height from 19.0 to 9.0 nm).

So it could be concluded, that such conformational changes of virions are caused by amino acid compositon of the outer surface of the capsid. Whereas negatively charged amino acid residues containing hydroxyl and carboxyl groups (serine, threonine, tyrosine, asparagic and glutamic acids) are concentrated on the outer surface, they enable virion adhesion through the formation of hydrogen bonds with the surface. If we compare the availability of amino acids that carry above-mentioned groups in their side chains in capsid proteins of AMV and TMV, then we will see that the content of these amino acids is larger in capsid protein of AMV (Table 1).

Table 1

Amino acid residues, available in TMV and AMV capsid protein

AMINO ACID	AMV	TMV
Serine	15	16
Threonine	13	16
Asparagic acid	11	8
Glutamic acid	11	6
Tyrosine	4	4

Conclusions. Thus the adsorption of tobacco mosaic virus and alfalfa mosaic virus on the Si (1 1 1) surface is accompanied with conformational changes of virions through the formation of hydrogen bonds and Van-der-Vaals interactions. The conformational change resulted in decrease of the virions height is due to interaction of amino acid residues on the outer capsid surface with atoms of Si on the Si (1 1 1) surface. The adsorption of TMV virions is accompanied with formation of monolayer films while after adsorption of AMV on the Si (1 1 1) surface the layup of several virions is observed. The adsorption of antibodies on the AMV virions was accompanied with disaggregation of virion's clusters and decrease of their height and was site-specific, meaning that anti-AMV antibodies adsorbed only in several regions of the virion's surface.

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Застосування ультрависоковакуумної атомно-силової мікроскопії для дослідження структури вірусів рослин та їхньої взаємодії з поверхнею Si (1 1 1)

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Резюме. Для дослідження взаємодії вірусів тютюнової мозаїки (ВТМ) і мозаїки люцерни (ВМЛ) із поверхнею Si (1 1 1) застосовано метод ультрависоковакуумної атомно-силової мікроскопії (УВВ-АСМ). Виявлено зміни конформації віріонів і різний ступінь упорядкованості на дослідженій поверхні. Продемонстровано формування переважно одношарових упорядкованих плівок при адсорбції віріонів ВТМ, тоді як адсорбція ВМЛ супроводжувалась утворенням невпорядкованих скупчень віріонів і більш різкою зміною висоти вірусних частинок на поверхні Si (1 1 1). Встановлено, що зміна висоти віріонів залежить від кількості негативно заряджених залишків амінокислот на зовнішній поверхні капсиду.

Ключові слова: УВВ-АСМ, віруси рослин, капсид, Si (1 1 1), конформація.

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