Atomic Force Microscopy of the Interaction of Erythrocyte Membrane and Virus Particles

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The interaction of virus with cell is one of the key problems of virology. Virion adsorption on cell surface, penetration through a membrane and further development of infection in a cell – that are basic steps of virus infection. Atomic force microscopy (AFM) give advantage for a study of first two stages of virus-cell interaction. Nevertheless, AFM investigations of viruses are mostly devoted to examination of plant viruses having simple structure [1]. The studies devoted to examination of animal envelope virus, and especially to virus-cell interaction are absent. At the same time many articles describing AFM images of living and fixing cells and membrane properties examination have been published. The aim of the present study was to examine interaction of erythrocyte and virus particle.

Erythrocyte membrane serves as a good model for examination of virus-cell interaction. The membrane is well characterized on biochemical level, has relatively simple structure and is stable during preparation of the samples for atomic force microscopy [2]. Erythrocytes are widely used in virology for reaction of haemagglutination, which characterize virus containing suspension.

The changes of the membrane during virus-erythrocyte interaction was examined using human, rhesus monkey, goose and chicken erythrocytes by atomic force microscopy. We examined two different types of animal viruses (influenza virus and canine parvovirus (CPV)). The intermittent-contact modes in air and contact mode in water solution were used. Viral suspension was mixed with erythrocytes at 4-6^{o}C on glass slide. The reaction was stopped by addition of paraformaldehyde to final concentration 2%. After 48 h fixation erythrocytes were gently washed in distilled water (pH 6.8) and dried in air. Preparations were examined in atomic force microscope SolverP47BIO (NT-MDT, Russia).

Previous studies showed that erythrocytes were stable during drying, but were very sensitive to pH of fixing and washing solutions. It was possible to observe the membrane skeleton of control erythrocytes, which is similar to described previously [2] (Fig.3a). Exposure of erythrocytes with virus suspension change structure and physical properties of a membrane. These transformations depend on erythrocyte origination, type and concentration of viruses. Nucleated avian erythrocytes do not cause hemagglutination, and change under viral influence poorly and slowly. Monkey erythrocytes change very fast after virus adding,

and cause clear hemagglutination. They notably reshape during first one or two minutes of process (Fig.1) and then occur complete degradation (Fig.2). The fleetness of process prevent visualization of viral particles on the membrane. Virions quickly disappear from central area of erythrocyte. Their destiny is not clear. It is possible that virions fuse with cell membrane or move to peripheral regions of the erythrocyte. This process result in formation of humps on peripheral surface and pits in other parts (Fig. 4a,b). In contrast, relatively slow process in chicken erythrocytes allow to image influenza virions on cell membrane (Fig.3.b).

Conclusions: In was shown that AFM is a powerful technology for study of the virus-cell interaction. Virus moving and microdamages can not be visualized otherwise. Our future investigation will be devoted to measurement of membrane elasticity and interaction force of cell and virions.

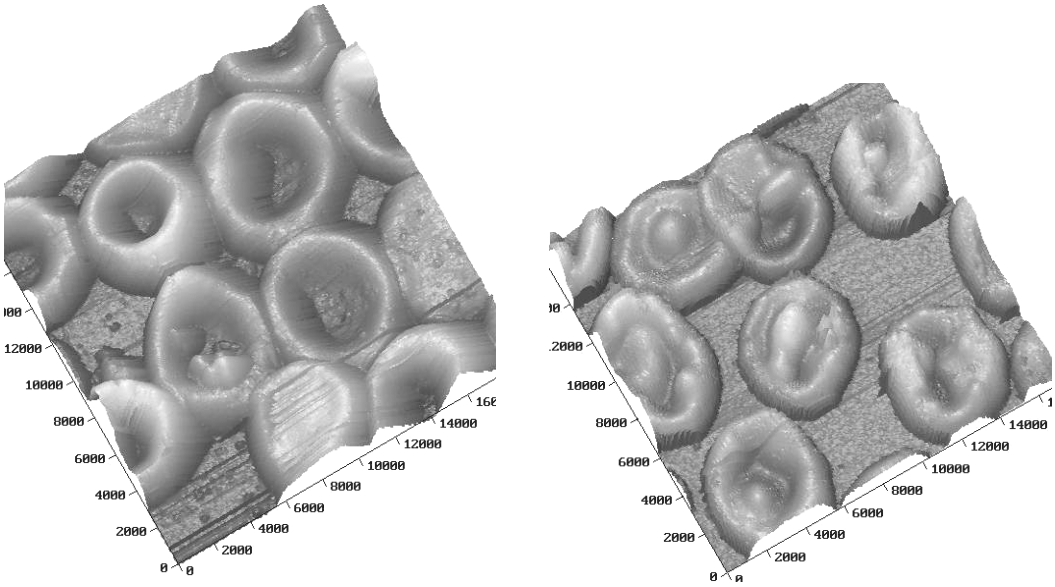

Fig.1. Erythrocytes of rhesus monkey: a – control; b- after virus interaction. intermittent-contact mode.

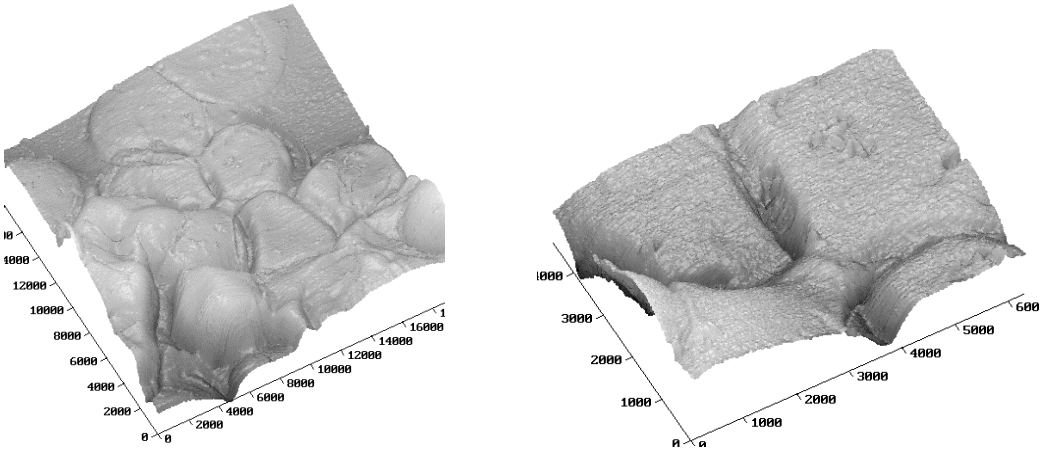

Fig.2. Degradation of rhesus monkey erythrocytes after 90 min interaction with CPV.

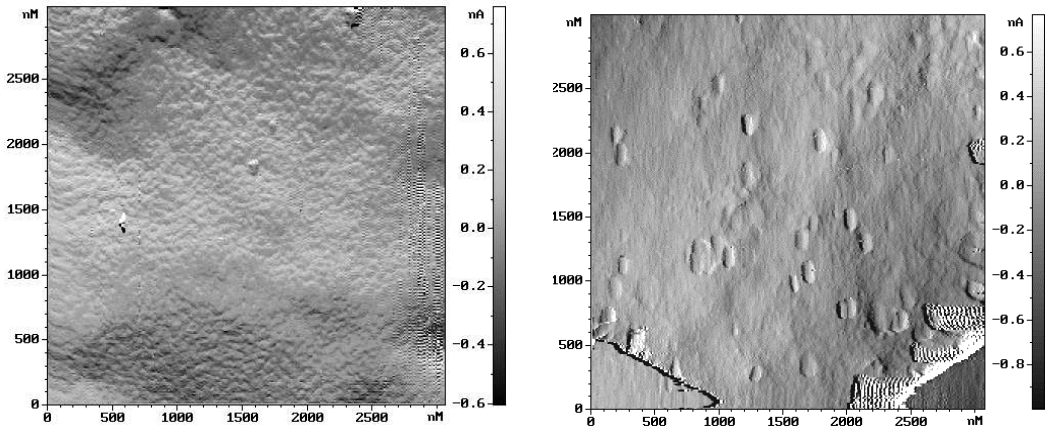

Fig.3. Membrane of chicken erythrocyte. a – control sample; b – influenza virus particles on membrane. Feedback error mode.

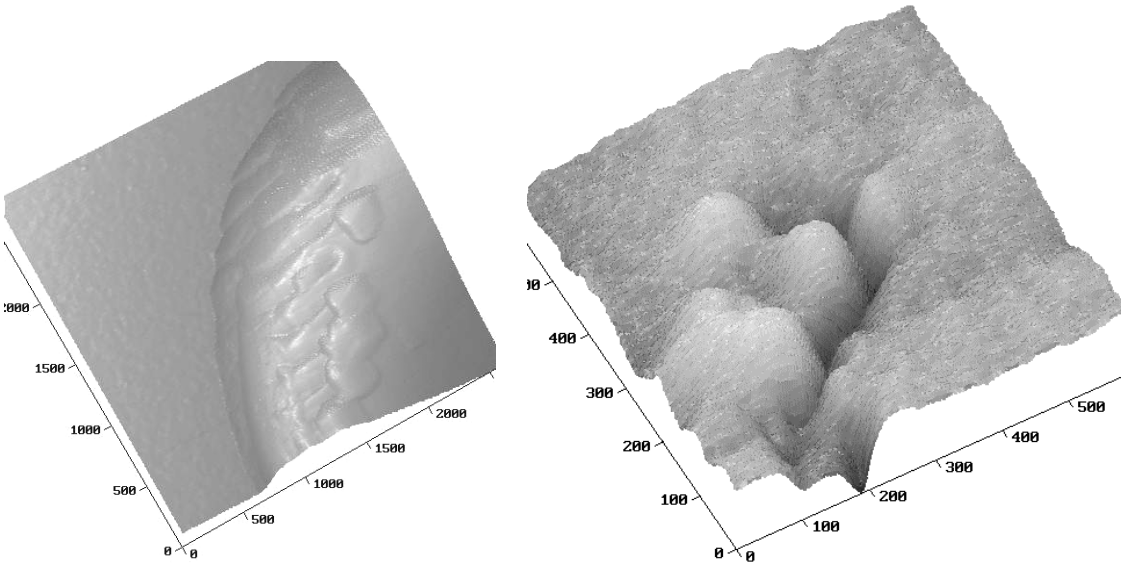

Fig.4. Damages on rhesus monkey erythrocytes membrane after CPV addition.
a – edge of erythrocyte; b – pits on membrane surface.

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2. Takeuchi M., Miyamoto H., Sako Y., Komizu H. and Kusumi A. Structure of the Erythrocyte Membrane Skeleton as Observed by Atomic Force Microscopy. *Biophys J, May 1998, p. 2171-2183, Vol. 74, No. 5*

The work was supported by the ISTC grant #1802