Atomic force microscopy for investigation of ribosome-inactivating proteins' type II tetramerization

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Ribosome-inactivating proteins type II (RIPsII) bind to carbohydrate residues of glycoproteins and glycolipids present in different clasters of cellular membrane. This property of RIPsII determines their binding to the variety of cell-surface receptors and probably participates in their endocytosis and retrograde transport [1].

Biology of the toxins violently depends on their carbohydrate-binding centres' organization. A number and three-dimensional localization of such centres per one molecule strongly influence on toxins' biological properties [2]. Many RIPsII were earlier shown to exist as dimers and under defined conditions some of them, for example viscumin, were observed to be tetramers. High concentration can be such a condition [3]. Toxin tetramerization can lead to both increasing of lectin-binding centres' number and changes in their structural organization. Such increase promotes changes in valency and avidity of toxin binding to cellular receptors. These changes result in multiple toxin binding to various both cell-surface and intracellular receptors and finally lead to changes in cytotoxic RIPsII action [2].

In spite of many investigations concerning biological properties of RIPsII it is not known if the toxins bind to their receptors in dimeric form or the binding requires tetramerization of the proteins. Atomic force microscopy (AFM) techniques allow to investigate conditions of RIPsII tetramerization.

For our study we used commercial instrument Solver P47H (NT-MDT, Russia). High resolution "golden" silicon cantilevers (NSG11S, NT-MDT, Russia) with two rectangular springs with typical force constant 5.5 N/m and 11.5 N/m were used to perform the experiments. All the experiments were performed in air at room temperature and semicontact AFM mode was used. The cantilever was oscillated with free amplitude in the range of 5-10 nm.

Using AFM techniques that allow to treat the object of investigation gently we obtained high-resolution images of RIPsII molecules sorbed on mica (Fig. 1).

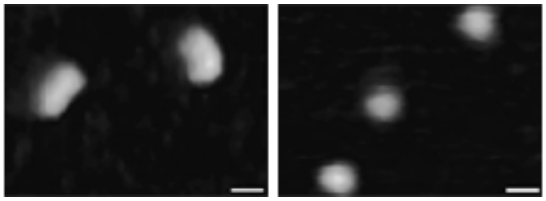

Fig. 1 *AFM image of tetrameric ricinus agglutinin (left) and ricin (right). Ricinus agglutinin always exists as a tetramer. Ricin has dimeric structure both in water solutions and being sorbed on solid phase. Bar, 10 nm*

Employment of AFM techniques in combination with scanning electron microscopy, transmission electron microscopy, confocal laser scanning microscopy and fluorescence life-time imaging as well as immunochemical, biochemical and molecular biology approaches allows us to study intracellular events including such processes as receptor-mediated and fluid-phase internalization of macromolecules, their endocytosis, retrograde and anterograde transport, and translocation of proteins through biological membranes.

References

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