APPLICATION OF NEW FLUORESCENCE-TAGGED AMINES AND POLYMERS IN STUDY OF DIATOM PHYSIOLOGY

Vadim V. Annenkov, Tatjana N. Basharina & Elena N. Danilovtseva

Limnological Institute of the Siberian Branch of the Russian Academy of Sciences

We present the results of the growth of the fresh-water diatom *Synedra acus* subsp. *radians* (Kützing) Skabichevskii in the presence of new fluorescence-tagged amines containing one or two tertiary amine moieties and high-molecular polymers with carboxylic, amine and imidazole groups. The following observations and conclusions have been made:

- Poly(acrylic acid) (PAA) bearing fluorescence groups (one group *per* chain) was found in silica frustules after *S. acus* growth in the presence of PAA. Non-tagged PAA was also detected in the frustules after removing of organic components of the cells Such neutral polymer as poly(1-vinylimidazole) and positive charged poly (vinyl amine) are not included into the frustules. We consider this behaviour of PAA is explained with (macro)pinocytosis hypothesis [Vrieling E. G. et al. PNAS 104:10441]. The most probable cytoplasmic form of silicon is slightly condensed silicic acid [Annenkov V.V. et al. Biomacromolecules 12:1772] and PAA is similar with poly(silicic acid) in acidity, so we can suppose that during some stages of silicon assimilation the diatom cell does not discriminate these substances and PAA penetrates into growing valves.

- Fluorescence-tagged short-chain amines penetrate into the diatom cell and stain new frustules similar to the known lisotrackers PDMPO and HCK-123 [J. Desclés et al. New Phytol. 177:822]. Formation of dyed submicron cytoplasmic vesicles was observed on the first stage of new valve growth. These vesicles disappear after the formation of a two-dimensional valve, which takes 5–10 min for individual cells. Further formation of the three-dimensional valve structure takes 10–12 h, following which cell division occurs. We speculate that the small cytoplasmic vesicles observed are related to silicic acid assimilation via a (macro)pinocytosis mechanism which could take place on the first stage of the valve formation.
- Fluorescence-tagged short-chain amines are useful substances for monitoring processes in diatoms. We have found that the introduction of the dyes into a synchronized culture under silicon starvation is a good way to estimate fraction of cells stopped in G_2 phase (synthesis of new valves) – these cells contain stainable vesicles as opposed to good synchronized cells. The ability of new dyes to stain SDV (Silicon Deposition Vesicle) was used to monitor splitting of diatom frustules under the action of terahertz laser irradiation. We have found that this irradiation allows to remove silicon valves from the organic membrane without deterioration of chloroplasts and SDV.

The study was partially supported by Project # 11-04-00707-a of the Russian Foundation for Basic Research.