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Dissection of the frustules of the diatom *Synedra acus* under the action of picosecond impulses of submillimeter laser irradiation

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Abstract Diatom algae realize highly intriguing processes of biosynthesis of siliceous structures in living cells under moderate conditions. Investigation of diatom physiology is complicated by frustule (siliceous exoskeleton). Frustules consist of valves and girdle bands which are adhered to each other by means of organic substances. Removal of the frustule from the lipid membrane of diatom cells would open new possibilities for study of silicon metabolism in diatoms. We found that submillimeter laser irradiation produced by a free-electron laser causes splitting of diatom frustules without destruction of cell content. This finding opens the way to direct study of diatom cell membrane and to isolation of cell organelles, including silica deposition vesicles. We suppose that the dissection action of the submillimeter irradiation results from unusual ultrasonic waves produced by the short (30–100 ps) but high-power (1 MW) terahertz laser impulses at 5.6 MHz frequency.

Keywords *Synedra acus* · Diatoms · Submillimeter laser irradiation · Free-electron laser · Cytoplasmic vesicle · Cell organelles

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Introduction

Diatom algae are an intensively studied part of the phytoplankton, mainly because of their unique siliceous exoskeleton (frustule), which consists of valves and girdle bands (Fig. 1; Pickett-Heaps et al. 1990). These micro- and nano-ordered siliceous elements of the cell walls are adhered to each other by means of organic substances. The lipid membrane is located under the siliceous frustule. The exoskeleton protects diatoms from various effects but also complicates study of the cell membrane and subcellular organelles. Diatoms capture silicon from highly dilute (<100 μM) water solutions by an unknown mechanism. A membrane protein SIT (Silicon Transporter) was supposed to be a potential transporter of silicic acid (Hildebrand et al. 1997), but the localization of this protein, and also its existence, are debatable because the cell membrane of diatoms is inaccessible to direct investigation. A very important organelle is the silica deposition vesicle (SDV, Fig. 1), in which new siliceous valves and girdle bands are synthesized (Drum and Pankratz 1964; Reimann 1964). Molecular mechanisms of frustule synthesis are unknown, and a large obstacle in this regard is the lack of procedures to extract subcellular organelles from diatom cells. Corresponding methods are well known for cells having the usual lipid membrane only, including membrane disruption by mechanical or chemical action, including osmosis and ultrasound, followed by separation by sedimentation. In the case of diatoms, it is necessary to remove the siliceous cell walls before any further manipulation. There are several works devoted to study of diatom organelles and pigments; the methods used to destroy the siliceous frustules include high-pressure disruption (Mehard et al. 1974; Rossignol et al. 1999; Büchel 2003; Szabó et al. 2008), freeze–thawing (Nagao et al. 2010), and grinding with glass beads

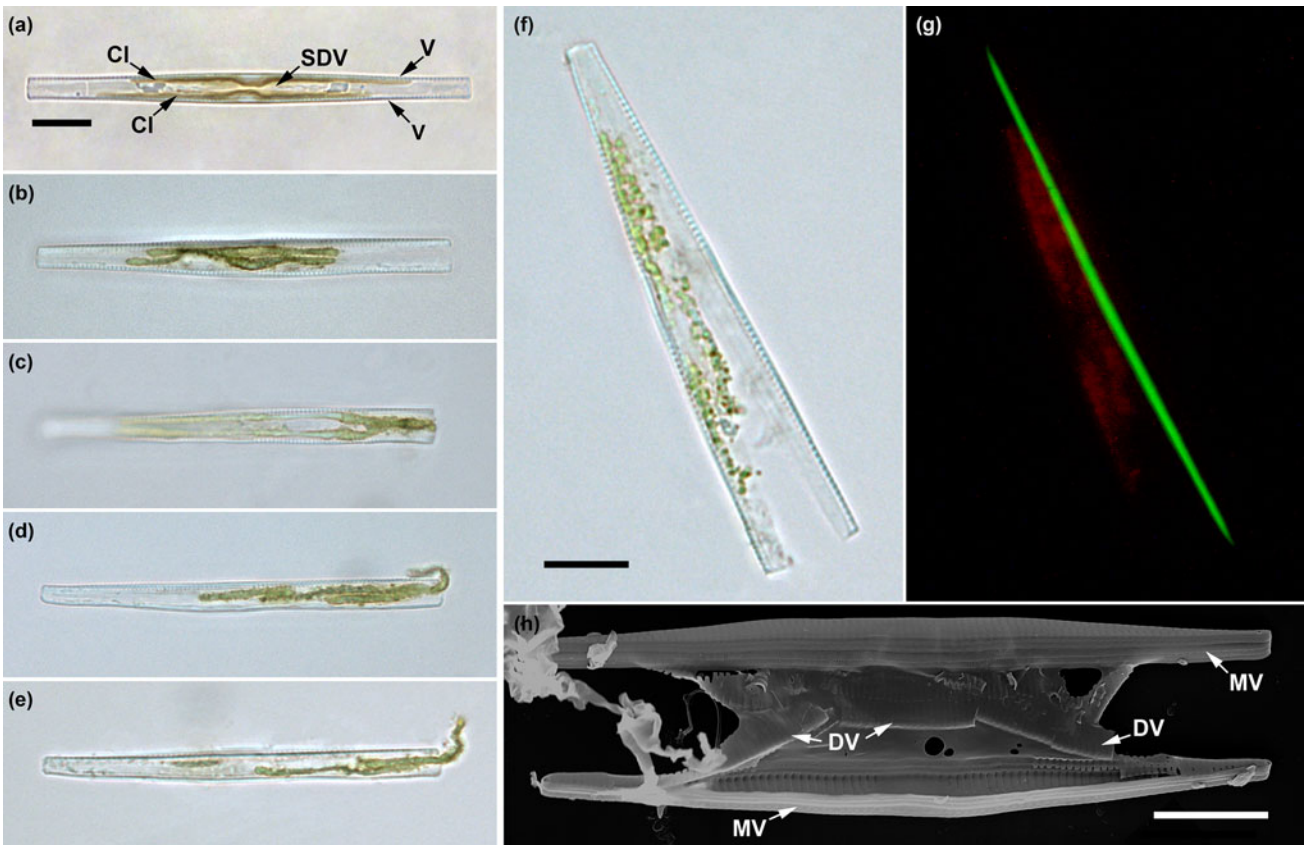

Fig. 1 Light (**a**–**f**), fluorescence (**g**), and scanning electron (**h**) microscopy images of *S. acus* cells before (**a**) and after action of SLI. The labels on the light image are as follows: *Cl* chloroplasts (*red* fluorescence), *V* valves of the parent cell, *SDV* two silica deposition vesicles in which new valves are formed (*green* fluorescence). Fragments of flat daughter valves (DV) synthesized in SDV are visible between two mature valves (MV) in the SEM image (**h**). *Scale bar* 10 μm

(Büchel and Wilhelm 1993; Winkler and Stabenau 1995). However, these methods allow one to obtain neither undamaged cells without frustules nor large organelles such as SDV.

Terahertz radiation can cause various effects in biological tissues, including destruction (Alexandrov et al. 2011 and references therein). High-power sources of submillimeter laser irradiation (SLI) recently became available due to the development of electron accelerators (Gavrilov et al. 2007). Radiation of 128–131 μm wavelength is close to vibrations of hydrogen bonds and causes nondestructive ablation of large molecules such as peptides, DNA, and hydrophilic polymers (Petrov et al. 2007; Kozlov et al. 2010; Annenkov et al. 2011). We supposed that the indirect action of SLI on water-based suspensions of diatom cells could weaken the binding of frustule elements with each other and thus facilitate removal of the siliceous frustule from the lipid membrane.

Materials and methods

We used a clonal culture of the araphid pennate diatom *Synedra acus* subsp. *radians* (Kützing) Skabichevskii isolated from the phytoplankton of Lake Baikal. Cultivation was performed in DM medium (Thompson et al. 1988), and the silicon concentration was 0.1 mM. The fluorescent dye NBD-N2 (Annenkov et al. 2010) was added in 1 μM concentration to culture medium 24 h before laser experiments to stain new siliceous valves synthesized in SDV.

We used a Leica DM 5000 microscope (with HBO 50 W/AC ASRAM mercury lamp) and a 100× immersion objective. Excitation was performed at 450–490 nm.

Scanning electron microscopy (SEM) was performed using an FEI Quanta 200 instrument. A suspension of cells fixed with formaldehyde/glutaraldehyde mixture (Tsuji and Yanagita 1981) was placed on aluminum sample holders, freeze-dried, and then sputter-coated with gold using an SDC 004 (BALZERS) device.

The free-electron laser (FEL) of the Siberian Center for Photochemical Research (Gavrilov et al. 2007) was used as the terahertz radiation source, with laser wavelength of 128–131 μm. Liquid water absorbs strongly in the whole THz region, and 128–131 μm is the "transparency window" of the gaseous water in laboratory air (Hale and Querry 1973; Chaplin 2012). FEL irradiation is

quasicontinuous and consists of 30–100 ps impulses emitted with 5.6 MHz frequency. This frequency corresponds to the maximal impulse power; the dissection effect was observed at other frequencies (2.8 and 11.2 MHz) but was less pronounced. The impulse power can reach 1 MW. More detailed characteristics of this FEL are described in Gavrilov et al. (2007). A suspension of diatom cells was placed into a 50-μL hemispherical cavity in a steel bar with the aim of heat removal during the action of SLI. The average power of the laser irradiation was 20 W. The irradiation was focused onto the sample with exposure time of 3–10 s. A scheme of the experimental setup is presented in Supplementary Fig. 1.

A piezoelectric microphone was used to detect acoustic vibrations. The signal was recorded with two independent channels (signal and background) using a Tektronix TDS 3034B digital oscillograph (Tektronix Inc., USA). The scanning duration was 40 μs with 10,000 sampling points. The obtained records were converted into frequency spectra by Fourier transformation.

Results and discussion

The action of SLI on a water suspension of diatom cells results in opening of the frustules (Fig. 1; Supplementary Fig. 2). Optical and fluorescent microscopy showed stability of chloroplasts during the frustule splitting. SEM allowed observation of fragments of flat daughter valves synthesized in SDV. The addition of surface-active substances (0.1 % sodium dodecyl sulfate) increases the fraction of dissected cells up to 35 %.

SLI is effectively absorbed by water in a 50–70 μm surface layer, and so these electromagnetic waves are not able to directly influence cells in the water column. On the other hand, we must take into account that the free-electron laser generates irradiation as impulses at 5.6 MHz frequency. Absorption of this irradiation by water can be accompanied by the formation of ultrasonic waves in the megahertz range. Indeed, we found ultrasonic waves in the water under the action of SLI: the frequency of 5.6 MHz and the double frequency of 11.2 MHz were clearly detected (Supplementary Fig. 3). Similar formation of ultrasound was found recently in experiments with a femtosecond laser (Dehoux et al. 2013). Our experiments with conventional ultrasound sources, including 44 kHz, 50 W devices, did not result in any dissection of diatom frustules. The conventional sources produce sine waves in contrast to FEL which gives very short (30–100 ps) impulses separated at 180 ns intervals. We suppose that the ultrasound produced by SLI is the cause of its opening action on the diatom frustules, but this assumption must be verified in the future.

The action of SLI on human erythrocytes (Munzarova et al. 2013) and snail neurons (Olshevskaya et al. 2009) has shown stability and survival of such cells lacking a solid exoskeleton. Thus, organic cell membranes are not destroyed under SLI. We suppose that the found phenomenon of the influence of SLI on diatom cells will allow the elaboration of an approach for nondestructive isolation of diatom subcellular organelles, including SDV. Certainly, this is a highly complicated task, which must include optimization of the SLI dissection and the development of procedures for destruction of the organic membrane and separation of subcellular organelles. Nevertheless, the obtained results open the way to study of diatom lipid membrane, including the search for special membrane proteins, e.g., SIT.

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Conflict of interest The authors declare that they have no conflict of interest.

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