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Fluorescence in the Study of Diatom Ulnaria Ulna Cells

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Abstract

Luminescence microscopy with various modifications such as microspectrofluorimetry and laser-scanning confocal microscopy has been applied to the study of the fluorescence of living freshwater diatom Ulnaria ulna (Nitzsch) Compère (Bacillariophyta). Their fluorescence spectra recorded by microspectrofluorimeter showed the chlorophyll maximum 680nm in living cells and 520nm in dead ones or in isolated shells. Laser-scanning confocal microscopy demonstrated the appearance of green-yellow secretory products at the cell development. Histochemical staining of the diatoms for the biogenic amines (dopamine, histamine and serotonin) as stress indicators showed the presence of the compounds by fluorescent method at 460-470 nm in some cells.

Keywords: Biogenic Amines; Diatoms; Dopamine; Histamine; Laser-scanning Confocal Microscopy; Luminescence microscopy; Microspectrofluorometry; Serotonin

Introduction

Luminescence microscopy with various modifications such as microspectrofluorimetry and laser-scanning confocal microscopy rarely used for phytoplankton studies in laboratory yet. First microspectrofluorimeter of Valerii N. Karnaukhov recorded the fluorescence spectra of algae such as Peridinium depressum and diatom algae Nitzschia longisissima [1,2]. The attention was attracted to a fluorescence of sea diatoms as the oldest photosynthetic microorganisms that have two solid flaps (volves) impregnated with silicon forming a defensive shell. Its red chlorophyll fluorescence was supposed as an indicator of increased landscape changes due to human impact under a relatively less warm and humid climate.

Up to now technique of luminescence microscopy with modifications such as microspectrofluorimetry and laser-scanning confocal microscopy is rarely used for laboratory investigations of diatoms. Searching simple model for similar studies, our attention was paid to the freshwater diatom species Ulnaria ulna (Nitzsch) Compère (Bacillariophyta) previously been assigned to the large and heterogeneous genus Synedra Ehrenber [3]. The single-cell object has several rounded pyrenoids in the plastid, and has intussusceptions into the cytoplasm. The definition of freshwater diatoms based on chloroplasts and other cell organelles visible in a light microscope and used for the analysis of the development and reproduction [4,5].

Fluorescent methods for the analysis of Ulnaria cells were not used earlier and proposed to be useful for the laboratory cultivation and natural identification in the environment. Therefore, the aim of our study was the application of the methods to the object.

Materials and Methods

Object and cultivation. Objects of the study were samples of diatom algae Ulnaria ulna (Nitzsch) Compère such as the living cells and shells primary received from laboratory of Karadag Biostation (Pheodosia, Black Sea) [4,5]. Then the cells lines 0-419 and 0-903 were cultivated in Pushchino laboratory of microspectral analysis of cell and cellular systems on in the Petri plates - 3cm in a diameter on nutrient medium, which included K phosphate 6.63, $CaCl_2$ 6.51, NaCl 3.47, $MgCl_2$ 5µg/l, and silica-gel (Merk, Austria) 2µg/l as the source of silicon for the shells/valves' formation. The observation of diatoms was on cover glasses (slides), which are put on the Petri plates.

Fluorescence observation. The autofluorescence and fluorescence after the histochemical staining for biogenic amines was used as the test-reactions of the diatom cells on the object glasses (slides) as described earlier for unicellular probes [6,7]. All experiments were performed at room temperature 20-22 °C. The images of living cells and separated shells were recorded and photographed by luminescent microscopes Leica DM 6000 (USA-Austria), microspectrofluorimeter MSF-15 (LOMO, Sankt-Petersburg) with photocamera Levenhuk M300 Base (USA). (ultra-violet light 360-380 nm) excitation and laser scanning-confocal microscope Leica TCS SP-5 (Germany-Austria-USA) (laser- 488 nm).

Fluorescent histochemical determination of biogenic amines (dopamine, histamine and serotonin) within cells, was carried out according to the methods primary described for animal cells and applied for plant cell as well [8,9]. Microspores were put on object glasses (slides) and moistened by drops of 1% aqueous solutions of 0.5-1% solutions of glyoxylic acid for dopamine, or o-phthalic aldehyde for histamine or formaldehyde for serotonin. After 10-20 minutes of staining with the reagent, samples were dried at 50-80 °C during 5-10 min. Fluorescence reactions of forming products was studied under luminescence microscope Leica DM 6000 B or by camera Levenhook (USA) at the excitation by light 360-380 nm. The fluorescence spectra recorded by microspectrofluorimeter MSF-15 (LOMO, Sankt-Petersburg). Histochemical reactions repeated (up to 3-5 times). The results of the fluorescence intensity at 460nm were expressed statistically with a standard error of mean+SEM of 4

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replications (n=4 object slides) for each variant and control.

Results and Discussion

The work with river-living single cell diatoms like Ulnaria ulna is very hard. It needs constant observation in transmitter light of usual microscope at high magnification or special staining with dyes because objects look as transparent and badly seen at all. To keep in mind that the algae have photosynthetic activity, we tried to study their autofluorescence related to chlorophyll using luminescence microscopy with modifications such as microspectrofluorimeter and laser-scanning confocal microscopy (Figure 1).

Autofluorescence. Living stick-looked cells fluorescent in red (a) are fine seen in luminescence microscope (Figure1), and one can differ dead cells emitted in green (b) here. At UV excitation 360-380 nm fluorescence in blue was insignificant both in living and dead cells. Autofluorescence of first diatoms has maximum 680nm peculiar to chlorophyll. These effects permit to differ living cells from dead ones, which fluoresce in green. In second case, chlorophyll is destroyed, and the emission with maximum 520nm belong to the shells of diatoms. Individual isolated shells also demonstrated maximum 520nm in their fluorescence spectra. These structures consist from phenolic compounds in mixture with silicon (Figure 2).

When we used laser-scanning confocal microscopy (Figure 2), red emitted diatoms were seen as singles (a) or multiplied cell population (b). Fluorescent images were observed in red and green channels, and summa of the emission images was combined with the image in transmitted light (c). Within dead cells there are yellow-fluoresced chloroplasts, lack of chlorophyll. Here red-emitted cells mixed with green-emitted ones that confirm our effects seen in luminescence microscope. Individual Ulna cell form was also clear at higher multiplication (d). The cells are capable of both ordinary meiosis and sexual reproduction. Up to now a practical identification of diatoms at the species and generic levels is based on the morphology of the shell valves [4,5]. During the vegetative phase of the life cycle, the cells of diatoms divide in two, and since they carry a protective silica shell consisting of two flaps, one of which covers the other like a lid in a box (“box” model), each of the daughter cells gets one half. The second missing part is being completed, and this happens inside the existing sash. As a consequence, after division, one cell has the same size as the parent, and the second is slightly smaller. The cells are capable of both ordinary meiosis and sexual reproduction.

In confocal microscope the observer can see more details from diatom life (Figure 3).

Besides emissions of chlorophyll within cells in red and shells in green, confocal microscopy permitted to see possible secretory products and the formation of auxospores. In our case, spherical forms may also relate to auxospores that need special study. Auxospora (from the Latin auxi - to expand, to increase) is a stage of the life cycle of unicellular algae from the class diatoms, usually representing an overgrown zygote. Some secretions looked as yellow drops. Figure 3 shows the comparison of cell images in transmitted light (a) and in green channel (b) with released possible auxospores.

Fluorescence after histochemical staining for biogenic amines. By histochemical fluorescent method we had recorded also the presence of biogenic amines as stress indicator in Ulna cells (Figure 4). As seen from Figure 4, dopamine, histamine and serotonin are present in the individual cells that demonstrated the fluorescence at 460-470 nm (fluorescence spectra for example of dopamine), and spectra for histamine and serotonin staining were analogous in maximum 460nm) after the histochemical staining with the reagent’s glyoxylic acid, ortho-phthalic aldehyde and formaldehyde, relatively [7-9]. The treatment completely masked the chlorophyll emission at 680nm. The fluorescence was observed mainly in blue spectral region. As a whole in this experiment, the fluorescence intensity higher, than in control before the staining, and was maximal for dopamine. Although some cells in the population also had high concentration of histamine. There is first approach to study biogenic amines in the diatom by fluorimetry of individual cells, and we suppose that following investigations of the stress amines in diatoms. A presence of the amines known as neurotransmitters in animals and also found in plants and microorganisms [10,11] is the field of great interest for the evolution of the irritability, beginning from ancient unicellular diatoms, where role of the compounds never studied. The luminescence microscopy permits to follow the investigations.

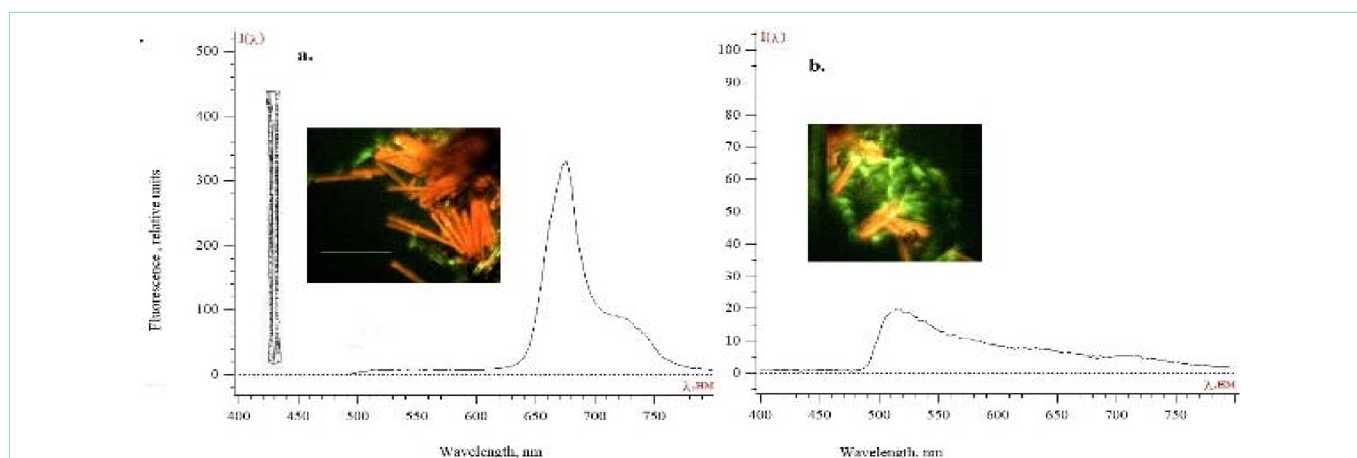

Figure 1: The fluorescent images and fluorescence spectra Ulnaria ulna recorded by luminescence microscope Leica DM6000B and microspectrofluorimeter MSF-15, relatively. Excitation by light 430nm. Bar=75µm. (a) The population of cells emitted in red with maximum 680nm and schematic form image of the algae (left); (b) Dead cells emitted in green and lack of chlorophyll. The spectra were recorded from one single cell.

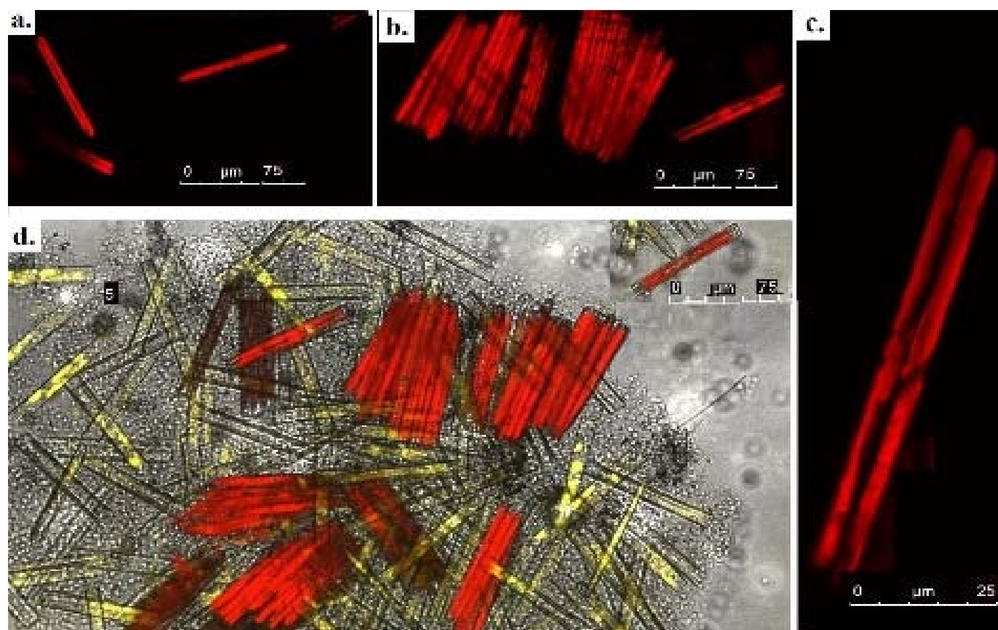

Figure 2: Fluorescent images under laser-scanning confocal microscope. Laser - 488nm. (a) and (d) Single cells in red channel; (b) Populations of cells in red channel and (c) Summa of the images in red and green channels basing on image in transmitted light.

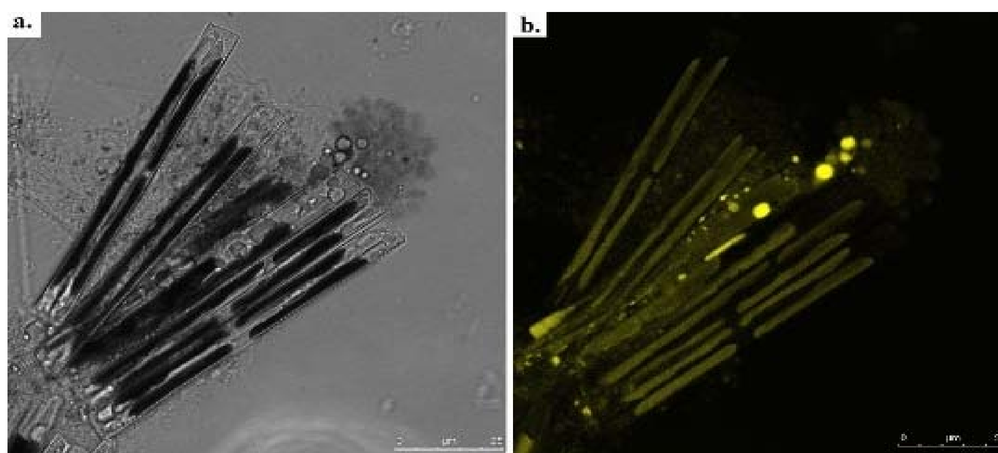

Figure 3: Confocal microscopy of Ulna cells in transmitted light (a) and in green channel (b) Bar=25µm. Laser 488nm.

Thus, applicated fluorescent methods open new possibilities in the study of the diatom algae that permits to indicate the ecological state of the concrete water reservoir.

Conclusion

Our study shows that fluorescent methods may be applied in practical work with diatoms if a laboratory has elementary luminescence microscope. Red emitted cells of Ulnaria ulna demonstrated that the object has chlorophyll and can live. Damaged or dead cells appear to observe in any cases due to weak reddish color or green color of their emission, relatively. If diatom losses chlorophyll only shell may fluoresce in green. Histochemical staining after the formation of blue fluorescent products with biogenic amines is also useful for stress indication of the water inhabitants.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

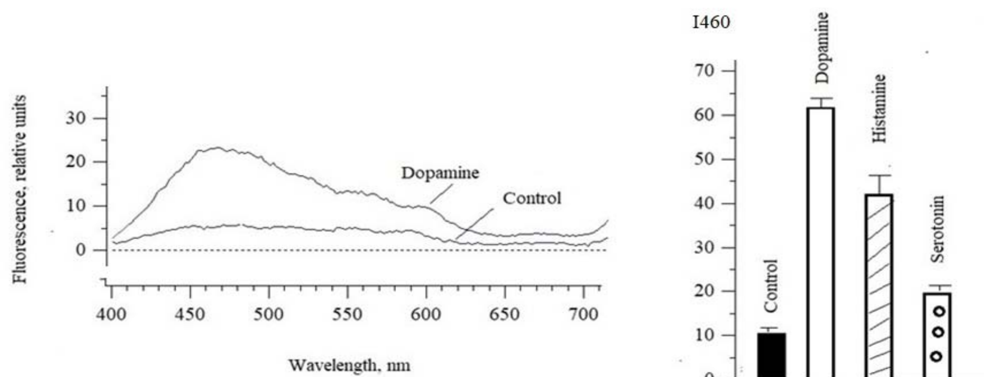

Figure 4: The fluorescence spectra (on the example of dopamine left) and the fluorescence intensity at 460 nm (I 460 right) after the staining with reagents for biogenic amines. Excitation 360-380 nm. The spectra were recorded from one single cell.

Author Contributions

Victoria V. Roshchina, the author of main conception of the work, receiver of all experimental data, and she has written the paper. Valerii A. Yashin, leading specialist in optical methods and laser-scanning confocal microscopy, Dr. Yulia A. Podunai-cultivation of the original samples of diatoms and consultations for their use.

Data Availability

The datasets generated during and/or analyzed during the current study are available in the [NAME] repository, [PERSISTENT LINK TO DATASETS].

Ethics Approval

There is no research involving animals, their data or biological material.

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