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Intermolecular interactions in plant cells reflected by ultraweak luminescence phenomenon

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It is commonly known that photons necessarily participate in atomic and molecular interactions. On the other hand, plant, animal and human cells spontaneously emit electromagnetic radiation. The universality of ultraweak cell luminescence is inherently associated with fundamental processes in living organisms. The majority of researchers think that this very weak radiation (10–10^5 photons/(cm^2·s)) results from such radical reactions as, for instance, lipid peroxidation. Having at our disposal the spectra of ultraweak luminescence (UL) from intact *Characeae* cells and their particular cellular structures and fractions, we could confirm that the electron carriers of the mitochondrial respiration chain were active in cytoplasm and in the intact cells but not in the cell walls. We obtained a band of chlorophyll emission in UL spectra. The dark chlorophyll should not emit fluorescence, which we observed under illumination. Nevertheless in our experiments, in which plants were kept in darkness for ca. 12 hours prior to measurement, this emission was observed. The only way to account for this phenomenon is that the energy of excitation is transferred to chlorophyll by other molecules. In this ultraweak sensibilized luminescence, the excited carbonyl molecules $^{1,3}(=C=O^*)$ transfer their energy to chlorophyll molecules with a high quantum efficiency. As we found in the spectral analysis of UL, the intensity at the wavelength of 634 nm – corresponding to dimoles of singlet oxygen – markedly decreased when the cells were exposed to the action of ascorbic acid (AsA) and lignocaine, which are singlet oxygen quenchers.

Key words: *ultraweak luminescence; singlet oxygen; ascorbic acid; lignocaine; Characeae cells*

1. Introduction

Intermolecular interactions generate excited states in metabolic processes of living cells of animal and plant organisms. A reflection of such interactions is the phenomenon of electromagnetic radiation (along a broad scale of the spectra), well known as bioluminescence, photoluminescence, delayed luminescence and ultraweak luminescence (UL).

Detection and identification of excited states in well-defined chemical solutions or in model systems (biologically determined) are fairly easy to be accomplished. They

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are a spectacular example of the investigation of intermolecular interactions via the analysis of luminescence spectra.

This task becomes much more difficult, although not impossible in living systems. The essence of the phenomenon of chemiluminescence consists in the transformation of the energy of chemical reaction into the energy of electronic excitation of the product of reaction which then emits photon of energy $h\nu$. Effective electronic excitation in a condensed state, proper to biological systems, require that the energy of 100–640 kJ/mol (i.e., 1–6.6 eV/molecule) be supplied within a time period shorter than the mean time of oscillation or than the time of keeping the products in solvate surrounding (10^{-11} s). This corresponds to UL in the visible and ultraviolet range of wavelength (200–700 nm). Such high energies are triggered most often in free radical and chain reactions, and also those in redox reactions that run the course of radical mechanism (i.e., dismutation of peroxyradical, disintegration of the peroxides of the dioxyethan type, adiabatic transfer of electrons from donor to acceptor, chemical exchange of electrons).

Some authors ascribe a special meaning to this radiation, i.e. that it conveys some information [1–7]. The transfer of information from cell to cell, i.e. outside the organisation unit, as well as inside it, by means of photons, seems to be a very attractive and promising idea. In the self-steering and self-regulating living organisms, photons arise as a result of sublimated intermolecular interactions connected with a special group of processes in the definite phase of cell metabolism. These photons should initiate other processes as a result of the interactions triggered by photon absorption in the other parts of the structural cell. Today, because of understandable difficulties, there is no sufficient experimental evidence of the above mentioned thesis. Therefore, it is necessary to be satisfied with the study of electromagnetic radiation emitted from the cell into the external media. Growth [8, 9], cell division [10], differentiation [11, 12], the cell response to the environmental stressors [13–15], especially such that initiates protective mechanisms [16, 17], injury [18, 19] and death [20, 21] are manifested by changes in spontaneous UL intensity (most often by its increase). However, we can administer some specific substances and thereby obtain the back reaction (with photon emission) which reagents initiate in the cell. In this case, it is UL induced by the added reagent.

In this study we have applied such reagents of active biological group as radical scavengers and singlet oxygen quenchers. Then by means of spectral analysis we sought to obtain information which particles, strategically important in the cell, are in the terminal phase the most probable emitters of extra weak radiation.

2. Materials and methods

The objects of this research were plant cells from Characeae family, *Nitellopsis obtusa (Desv. In Lois J. Gr.)* taken from Zaglebocze Lake near Lublin in Poland. They were cultured in an air-conditioned laboratory in natural pond water in day/night re-

gime at room temperature. Before measurements they were kept in complete darkness for few hours in artificial pond water (APW) in the following composition (mmol/dm^3): KCl 0.1; NaCl 0.1; $CaCl_2$ 0.1and 20 mmol/dm^3 Hepes buffer. pH of APW was 7.5, achieved by adding 10% NaOH.

The cell walls were obtained by cutting one of the two cell nodes and gently squeezing out cytoplasm from the interior of the cell. Cytoplasm isolated from cells was obtained by the method described by Jaśkowska and Śpiewla [22]. In the isolated cytoplasm, one could observe rotating chloroplasts inside the drops and record action potentials generated on the membrane. The latter was formed as a new reconstruction on the cytoplasm surface. Microsomal fraction was obtained from the homogenate of cells (5 g fresh weight was homogenized in a cold buffer of 0.1 mol/dm^3 Tris–HCl, pH = 7.5 containing 1 mmol/dm^3 EDTA Na_2). After centrifugation at 14 000*g* for 15 min at 4 °C, the supernatant was again centrifuged at 68 000*g* for 2 h at 4 °C. The pellet obtained after the second centrifugation was treated as a fraction enriched in cell membranes. In order to obtain the microsomal fraction enriched in ribosomal components, the supernatant was once again centrifuged at 105 000*g* at 4 °C for 3 h [23].

Measurements of UL were performed by means of Single Photon Counting method (SPC) with the use of a very sensitive photomultiplier (EMI 9558). For spectral analysis we used a set of 20 Russian glass cut-off filters, marked as BC6, BC7, BC8, ZC10, ZC12, ZC16, ZC17, ZC18, OC11, OC12, OC13, OC14, KC10, KC11, KC13, KC14, KC15, KC17, KC18 and KC19 which transmitted electromagnetic radiation above required wavelengths. The scheme of the apparatus is shown in Fig. 1.

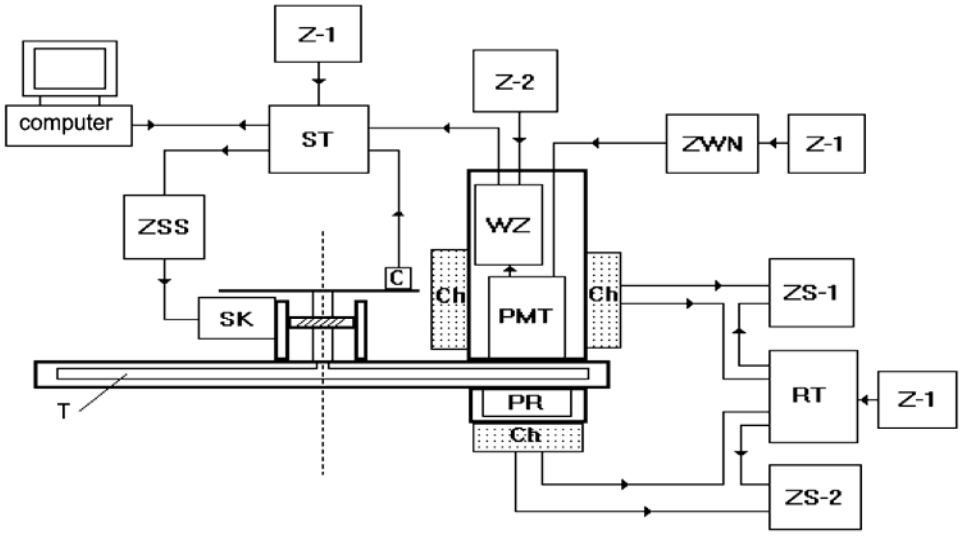

Fig. 1. Scheme of the filter spectrometer: PR – sample, T – turning round shield with cut-off filters, SK – stepping motor, C – controller of start position, PMT – photomultiplier, Ch – thermoelectric cooler, WZ – pulse amplifier, Z, ZS, ZWN, ZSS – voltage supplies, ST – interface, RT – temperature controller

The spectral distribution of UL was calculated and corrected for the total intensity changes with time, transmittance of filters, spectral sensitivity of the photomutiplier and long-lasting phosphorescence of some filters, as described elsewhere [24, 25]. Measurements of the spectral distribution were conducted following 0.5 h exposure to 1 mmol/dm^3 of ascorbic acid (AsA) and 2 mmol/dm^3 of lignocaine because the UL intensity was rather stable then. More details about the apparatus are enclosed in the previous article [26].

3. Results and discussion

The first step of our investigation was to decide whether the UL spectral analysis allows us to distinguish individual bands in the spectra related to the metabolic processes in the plant cells. For this purpose, UL spectra of intact cells and their walls as well as of isolated cytoplasm from the cells have been recorded. The results are presented in Fig. 2 and one can see the difference in the spectral range 450–610 nm when comparing UL spectra from living samples, i. e. intact cells and cytoplasm, with the ones from the cell walls.

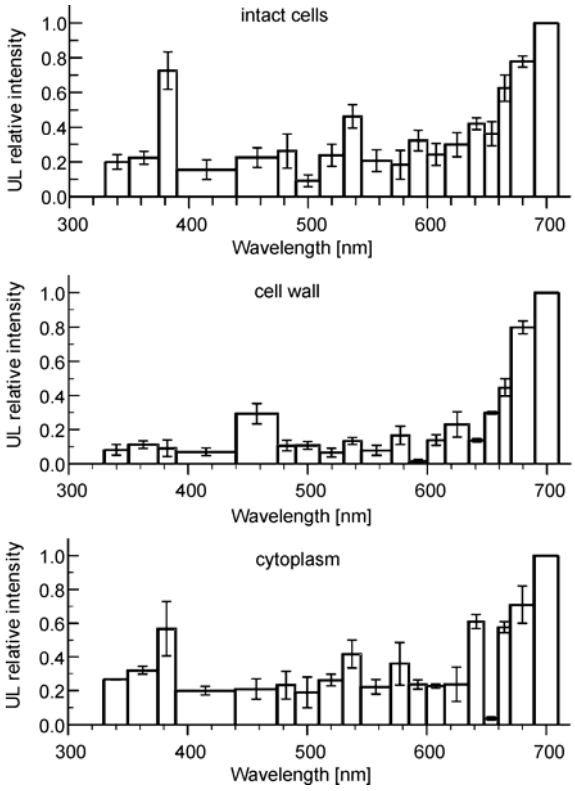

Fig. 2. Spectral distribution of UL from intact *Nitellopsis obtusa* cells and from cell walls and cytoplasm isolated outside the cells (averages of three series of measurements; vertical bars indicate standard errors)

Within the region of wavelengths mentioned above, the electron transfers of mitochondrial respiration chain emit electromagnetic radiation and probably they are responsible for UL within that spectral range. The essential elements of respiration chain are NAD and its reduced form – NADH, FMN and ubiquinone. For this reason, it was worth to compare fluorescent spectra of the mentioned compounds (Fig. 3) with the spectra of the objects under investigation. We would like to remark that the spectra of NADH and FMN are taken from literature [27, 28], whereas the spectrum of ubiquinone was obtained in our own measurements and published earlier [29].

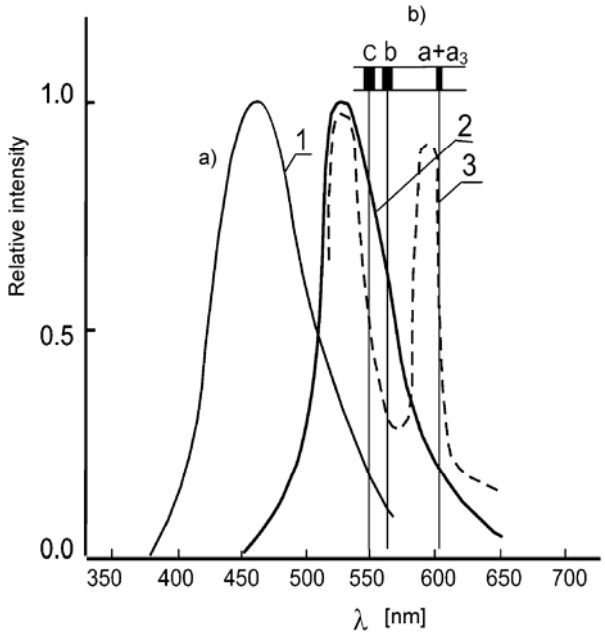

Fig. 3. Normalized fluorescence spectra (a): 1) NADH in 0.05 mol/dm^3 sodium phosphate, excitation at 351 nm [27], 2) FMN in neutral aqueous solution, excitation at 450 nm [28], 3) ubiquinone in benzene, excitation at 505 nm [29]. Light absorption by cytochromes (b): c – 550 nm, b – 563 nm, a + a_3 – 605 nm

Taking into consideration the emission at 450 nm, which may be caused by NADH, we did not record any essential changes between different cell structures. However, the emission at this wavelength from cell walls is a little stronger than that from intact cells and cytoplasm, but differences are not statistically essential. The ultraweak radiation from the cell walls at 530 nm and 590 nm, which can be associated with FMN and ubiquinone fluorescence, is markedly lower. It is justified because mitochonria are absent in the wall structure, which means that the respiration processes does not take place here. However, a relatively higher emission at 450 nm can reflect a large amount of reduced form of NAD, i.e. the first compound of the electron transfer in the respiration chain. This may be caused by the presence of damaged mi-

tochondria from cytoplasm retained in the cell walls. Electron transfer processes related to mitochondrial respiration however, do not occur in the walls, as in intact cells and cytoplasm, which is confirmed by relic emission from the other electron carriers of respiratory chain (FMN, ubiquinone).

It is puzzling that a large intensity of UL of living samples is observed at 380 nm, whereas this peak does not occur for cell walls which are not a living part of the cell. The radiation emitters at 380 nm, pointed out in literature, are oxygen dimoles $2[^1\Sigma_g^+]$ (with a very low emission probability) and carbonyl compounds which most often transfer their energy into chlorophyll. That is why a big intensity of radiation emission at 680 nm (chlorophyll band) is observed in all cases in Fig. 2.

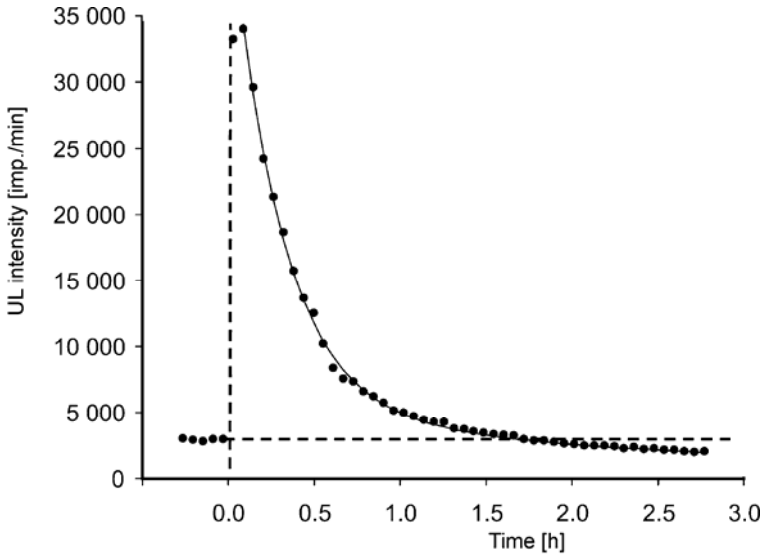

Fig. 4. Decay of the UL from isolated cytoplasm exposed to 1 mmol AsA. The horizontal dashed line shows the level of UL intensity from cytoplasm before AsA treatment. The standard errors for the points before and after AsA action are not more than 4% and 8.5% respectively. The continuous line shows the best fit of UL decay to the double-exponential curve: $y = 39181\exp(-3.32x) + 4760\exp(-0.39x)$; $T_{(1/2)1} = 12.5$ min, $T_{(1/2)2} = 106.3$ min, $R^2 = 0.999$

Ascorbic acid (AsA) is well-known as hydrophilic antioxidant and free radical scavenger (Vit C). When *Nitellopsis obtusa* cells were exposed to its action, first we observed a considerable increase of UL intensity, and then its decrease, but never below the starting level [26]. If UL intensity had fallen below the beginning level, it would have confirmed the antioxidant properties of AsA [30], something that we would like to obtain. Following that, we studied particular subcellular structures and fractions. The kinetics of UL intensity for cytoplasm isolated from cells treated by 1 mmol/dm^3 of AsA (Fig. 4) is almost the same as for the intact cells, but after about 1.7 h UL intensity begins to fall below its starting level. Among the fractions under investigation, i.e. membrane, lipid and microsomal enriched in ribosomes, only in the

last case UL intensity fell below the starting level when ribosomal fraction was treated by 5 mmol/dm^3 of AsA. When these samples were exposed to 1 mmol/dm^3 AsA, there was no change at all (Fig. 5). The period of time after which a decrease of UL intensity is observed (for 5 mmol/dm^3 of AsA), equals about 0.5 h. This decrease is deeper than that for the cytoplasm isolated from the cells.

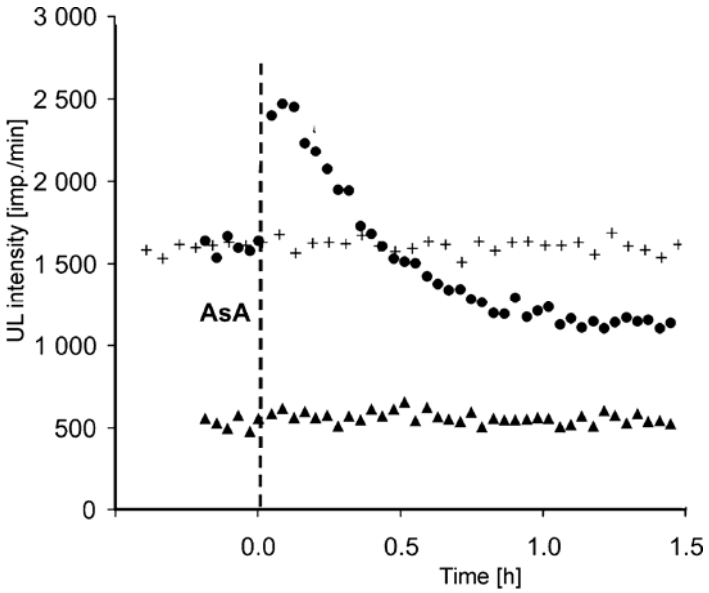

Fig. 5. Time dependence of UL of the ribosomal fraction obtained from *Nitellopsis obtusa* cells exposed to AsA: 5 mmol (black circles) and 1 mmol – (crosses). The standard errors for the points before and after AsA treatment are not more than 5% and 10%, respectively. Triangles show the levels of UL from APW solution before and after exposure to the action of 5 mmol/dm^3 AsA as a function of time (no changes are clearly seen)

Antioxidant action of AsA is represented by the following reaction (Eq.1):

$$AAH^- + O_2^{-\cdot} + H^+ \rightarrow AAH^{-\cdot} + H_2O_2 \quad (1)$$

while the prooxidant action by Eq. (2):

$$AAH^- + O_2 \rightarrow AAH^{-\cdot} + O_2^{-\cdot} \quad (2)$$

where: AAH^- – ascorbate, $AAH^{-\cdot}$ – free radical of ascorbic acid.

In spite of the increase in total UL intensity from the cells exposed to AsA action, we observed in the spectra (Fig. 6) a decrease of radiation at $\lambda = 634$ nm, a fact that can be related to photon emission from the dimoles of singlet oxygen represented by the following equation:

$$2O_2(^1\Delta_g) \rightarrow 2O_2(^3\Sigma_g^-) + hc/\lambda \quad (3)$$

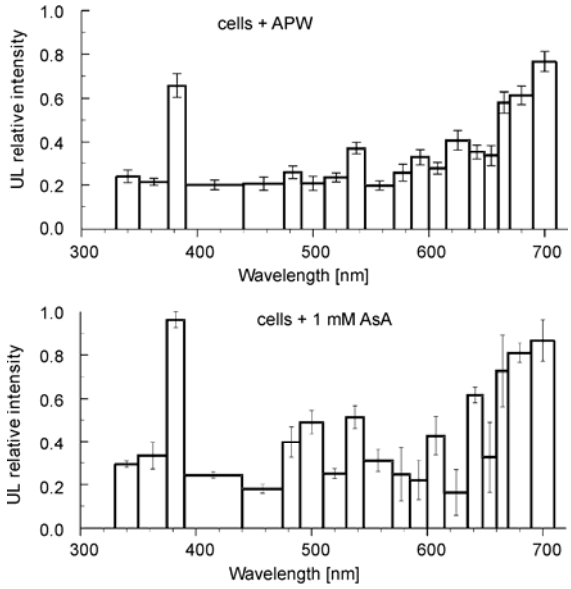

Fig. 6. Spectral distribution of UL from intact *Nitellopsis obtusa* cells before and after exposure to 1 mM AsA action (average of three series of measurements)

AsA is a quencher of singlet oxygen $^1\Delta_g$ at λ = 1270 nm [31]. In our measurements we have shown that AsA is also the quencher for the dimoles of singlet oxygen. In Fig. 7, the scheme of electronic transitions for singlet oxygen molecules and their dimoles is shown.

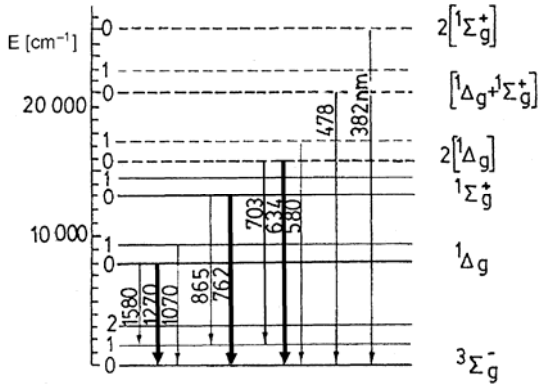

Fig. 7. Scheme of energetic levels and electronic transitions in singlet oxygen molecules [32]

The increase of UL intensity at 450–610 nm, induced by AsA, can suggest that redox processes in mitochondrial respiratory chain appear when the electronic transmitters are excited. The increase at λ = 680 nm (chlorophyll emission) may suggest scav-

enging of a certain pool of free radicals in chloroplasts by AsA. This can intensify the electron reverse transport, which results in generating excited chlorophyll molecules and eventually light emission.

Another reagent being a biologically active substance, i.e. lignocaine from local anaesthetics group, is also a quencher of singlet oxygen – physical and chemical as well. Zanocco [33] found that the main path for the interaction of lignocaine with singlet oxygen corresponds to physical quenching and can be explained in terms of a reversible formation of an exciplex via charge–transfer interactions due to the electrophilic attack of the excited oxygen on the amino group.

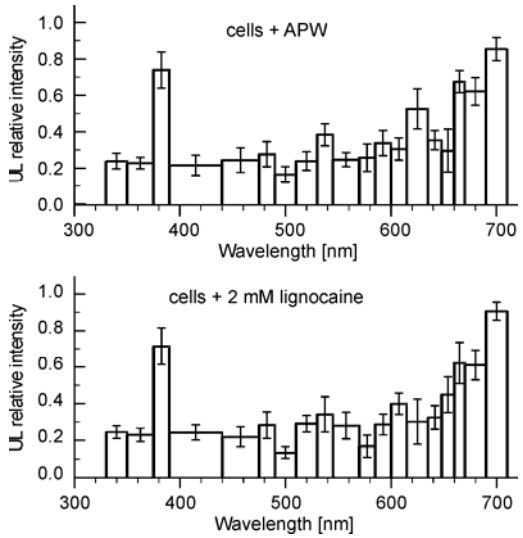

Fig. 8. Spectral distribution of UL from intact *Nitellopsis obtusa* cells before and after exposure to 2 mM lignocaine (average of three series of measurements)

In the spectra obtained in this study, when the reagent influenced the intact cells, we observed UL intensity decrease at $\lambda = 634$ nm (Fig. 8). This means that lignocaine is also a quencher of singlet oxygen dimoles ($2O_2\ ^1\Delta_g$). However, light intensity at the wavelengths related to luminescence of NADH, FMN, ubiquinone, chlorophyll and oxygen dimoles did not change essentially. It is worth to notice that in this case the measurements of UL spectra were performed in the course of 1.5 hours after anaesthetic addition when total UL intensity did not change, what we described in our previous paper [34].

5. Conclusions

One of the main and ubiquitous emitters of ultraweak luminescence from plant cells of *Characeae* is chlorophyll with fluorescence at 680 nm. Even a small amount

of it (e.g., in isolated cytoplasm) is visible in spectra of samples which were kept for several hours in darkness. Excitation of chlorophyll, visible in all investigated samples spectra of UL, is also enhanced by the action of ascorbic acid.

NADH, FMN and ubiquinone are other essential cell emitters, which is an evidence that there are interactions in mitochondrial respiration chain. But the intensity of their radiation in the UL spectra of the cell walls is relic. It can prove that UL spectra reflect metabolic processes undergoing in living organisms.

The biologically active substances used in our research (ascorbic acid and lignocaine – known as singlet oxygen quenchers) caused decrease of UL intensity from Characeae cells at 634 nm. This may be interpreted as the quenching of singlet oxygen dimoles.

The existence of peak in UL spectra at 380 nm may be connected with the excitation of singlet oxygen dimoles $2[^1\Sigma_g^+]$, but because of extremely low probability of it this concept requires further investigations.

Single Photon Counting method equipped with a set of cut-off filters is helpful in the identification of intermolecular interactions existing under the influence of exogenous reagent, as we had pointed it out for *Characeae* plants.

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