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ULTRAWEAK BIOCHEMILUMINESCENCE OF DARK-ADAPTED *CHARACEAE* CELLS

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Characeae **cells should be kept in a complete darkness for about several hours before measurements of detectable ultraweak (10-10^4 photons/s cm^2) electromagnetic radiation within 340-700 nm. This ultraweak emission possesses a quasistationary character and the intensity of this radiation is dependent on vegetation period. In the case of damaged cells, an increase of ultraweak luminescence (UWL) is observed exactly from the damaged areas. It is not yet well recognized whether this emission enhancement is caused by some reactions related to degradating or to repairing processes. In our opinion this maximum is a sum-effect originated from both processes. However, when phase structures of cells and subcellular fractions of these cells were exposed to AsA action we could distinguish prooxidative and antioxidative function of this reagent on the basis of ultraweak luminescence intensity measurements from particular samples. Local anesthetics (procaine or lidocaine) „electrically" stabilized the cell membrane during first 1/2 hours, as it was shown by electrophysiological studies, while the enhancement of ultraweak emission occurred about 1/2-2 hours (dependent on concentration), as it was pointed out in luminescence research. These results call for simultaneous (coupled) experiments in electrophysiological and luminescence investigations.**

INTRODUCTION

It is commonly agreed that plant, animal and human cells emit ultraweak electromagnetic radiation (Chwirot, Cilento, Inaba, Nagl, Popp, Mei, Galle, Neurohr, Sławiński, Van Wijk & Schamhart, 1988; Sławińska & Slawinski, 1983; Van Wijk & Van Aken, 1991; Scholtz, Staszkiewicz, Popp & Nagl, 1988, Gasso, Grillo, Musumeci, Triglia, Rodolico, Cammisuli, Rinzillo, Fragati, Santuccio & Rodolic, 1992; Cadenas, 1984). This biochemiluminescence phenomenon, well-known in almost all living systems, has been confirmed in recent decades by means of single photon counting technique using highly sensitive photomultipliers (Inaba, Yamagishi, Takyu, Yoda, Goto, Miyazawa, Kaneda & Saeki, 1982). It is worth emphasizing that this method is absolutely noninvasive because only the photons emitted outside of biological organisms are recorded. For this reason it becomes a challenge to study *Characeae* algae cells, which are well recognized from the electrophysiological point of wiew but not by noninvasive methods at all. For a few years biochemiluminescence research of these plants has been conducted in our laboratory by means of photoemission technique. Native, dark-adapted *Characeae* cells emit measurable spontaneous ultraweak biochemiluminescence within the spectral range 340-700 nm (Jaśkowska, Milczarek & Śpiewla, 1987; Jaśkowska, Borc, Milczarek, Dudziak & Śpiewla, 2001) measurable as intensity several times higher than the emission level of the environment.

Following the level of luminescence intensity, the kinetics of these changes and the spectral distribution, it is possible to obtain some information about such metabolic processes in cells which are connected with the electronically excited species, generated during these processes in visible range. Cadenas (1984) proposed to group the forms of induced biological chemiluminescence into two categories. One category includes those systems in which the generation of excited species occurs as a consequence of interactions between oxygen-containing radicals; the excited species are formed in a secondary fashion and are in general by-products of oxidative free radical reactions. The other group includes those systems in which the excited species are generated in a primary fashion, as direct products of certain enzymatic reactions. A clear distinction between them cannot be made in living organisms, which can be confirmed by intricate spectral distribution from them. In this situation photoemission from biological systems appears to be more complicated light emission than that observed in the relaxation of the sole excited state to the ground state. Scientific efforts tend towards two directions; one is to exhaust the en-

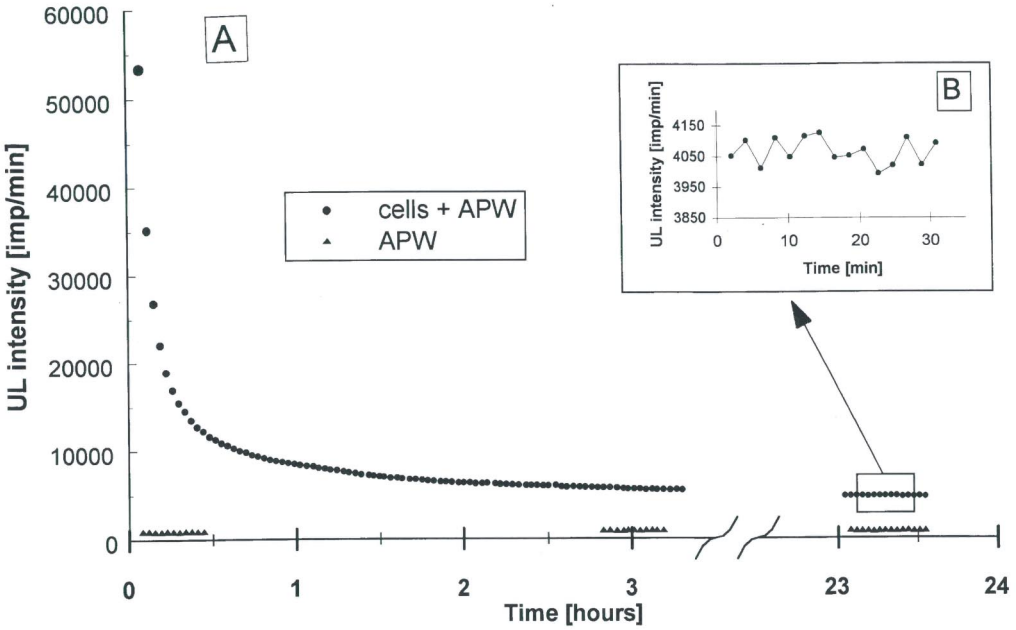

Figure 1. (A) The decrease of photon emission recorded just only 3 min. after moving the cells from daylight to UWL measurement conditions (average of 12 series), (B) - the terminal fragment of UWL intensity of the signal shown at

dogenous quenchers, the other is to reduce the complexity of the intact system by subfractioning or by examining the enzymatic model systems.

In this paper, it is shown that the reduction of complexity by sample subfractioning does not lead to better readability of ultraweak luminescence because the overlapping emission that originates not only from degradative processes but also from the repairing ones.

MATERIALS AND METHODS

Algae Characeae; Nitellopsis obtusa cells (Desv, in Lois) J.Gr. taken from the Zaglebocze lake near Lublin in Poland were cultured under laboratory conditions in natural pond water at room temperatures with natural day/night light regime. Before the luminescence measurements were taken, the encorticated cells were kept in dark about 20 hours after being rinsed with tap water and kept in antibiotics (10 μg/cm^3 streptomycine and 20 μg/cm^3 cristalline peniciline) for 30 min. The standard medium (artificial pond water - APW) for these cells was composed: 0.1 mmol/L of each: KCl and NaCl and $CaCl_2$ at pH 7.4 using 20 mmol/L Hepes and 10% NaOH.

Ultraweak luminescence measurement method was described in details earlier (Jaśkowska *et al.*, 2001).

Table 1 Dependence of the electrical parameters and UWL intensity on vegetation period for *Nitellopsis obtusa* cells.

Unit membrane resistance (kΩ cm^2)		Ultraweak luminescence intensity (imp/min)	
June	9.5 ± 1.4	June	500 ± 50
November	22.8 ± 3.1	October	2000 ± 150

RESULTS AND DISCUSSION

The time dependence of ultraweak luminescence intensity obtained from *Nitellopsis obtusa* cells is represented at Fig.1. These data were recorded from the cells that had been kept before measurements in normal daylight conditions without adaptation to darkness. The registration of emission was started just only about 3 min. after taking plants from the daylight place to apparatus light-tight camera. From this curve it clearly appears that there are three steps of decay. The first step, that of fast decay, in which the emission intensity drops by about 6 times, lasts about ½ h. The second phase, with much

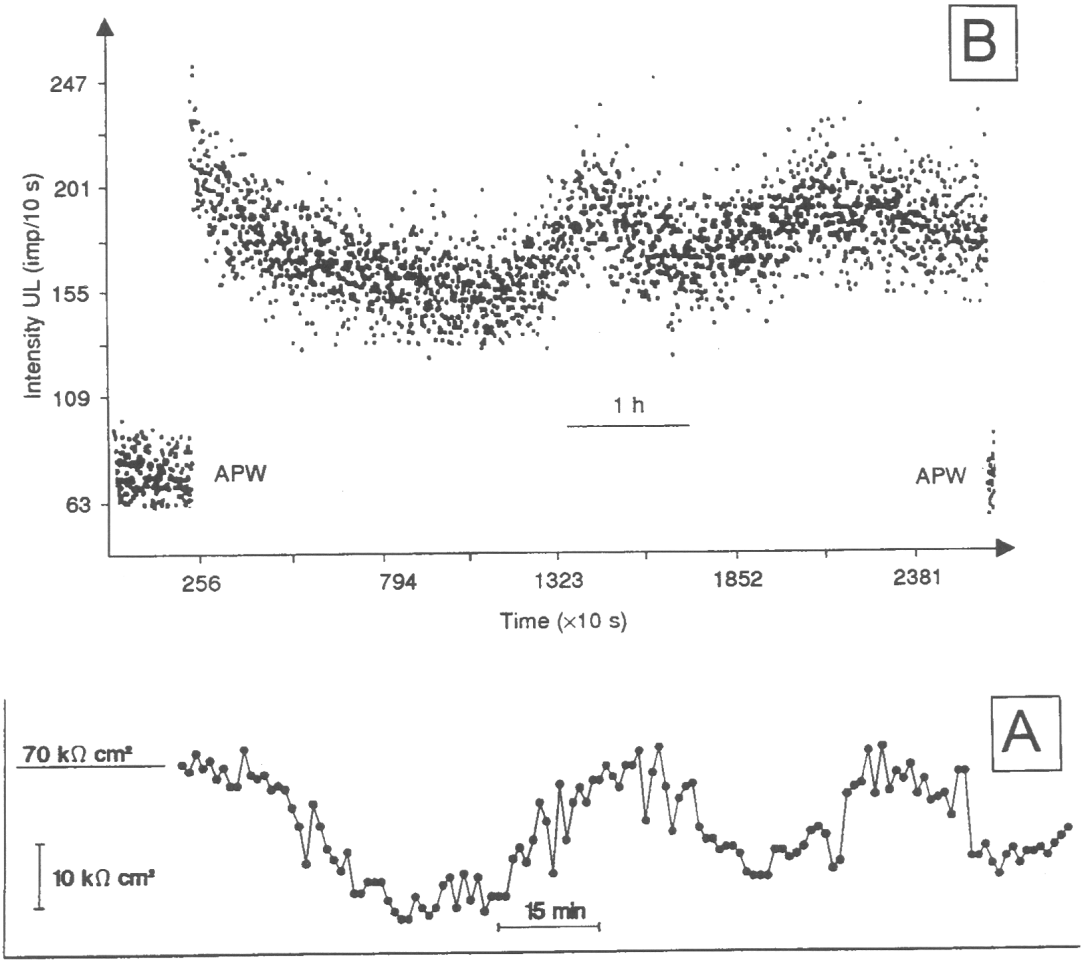

Fig. 2. (A) Spontaneous oscillation (with period time about 1h) of membrane resistance obtained by the use of outer electrodes method for *Nitellopsis obtusa* cell immersed in natural pond water, (Tokarska, Jaśkowska, Śpiewla 1990), (B) - oscillatory changes in the intensity of spontaneous UWL from part of the plant composed from two internodal cells connected by a node (Jaśkowska, *et al.* 1999)

slower decay lasting a few hours shows the intensity drop by a half. In the third stage it passes into the quasi-stationary level of emission intensity in the interval time of several tens of hours. (Fig. 1B shows ultraweak luminescence of the terminal fragment of the signal obtained at Fig. 1). We recognize exactly this quasi-stationary emission as ultraweak biochemiluminescence from the examined cells after the decay of delayed photoluminescence from their chloroplasts. Similar data of emission decay, but for chloroplasts isolated from spinach leaves, has been obtained by Hideg *et al.* (1991). According to their observations, photoluminescence of chlorophyll is negligibly small after a few hours. This means that ultraweak luminescence in our experiments, which is a few times higher than the level of emission from the environment medium and which is observed from our cells during several tens of hours, must be the biochemiluminescence of the systems under study.

On the basis of these observations we have concluded that plant cells must be kept for several hours in complete darkness before the biochemiluminescence measurements are taken, so that the processes connected with delayed photoluminescence of chlorophyll are over. Lavorel (1980) has found that this radiative decay of chlorophyll singlet states formed at the expense of electron-hole pairs recombines at the site of photochemical centers. Lavorel attributed the steady, low background level of luminescence from fully relaxed *Chlorella,* to a quasi-permanent, very low-level reverse electron flow through the System II reaction center.

In our investigations ultraweak luminescence could be observed for several tens of hours when *Characae* cells were kept in complete darkness. One question necessarily arises whether the cells

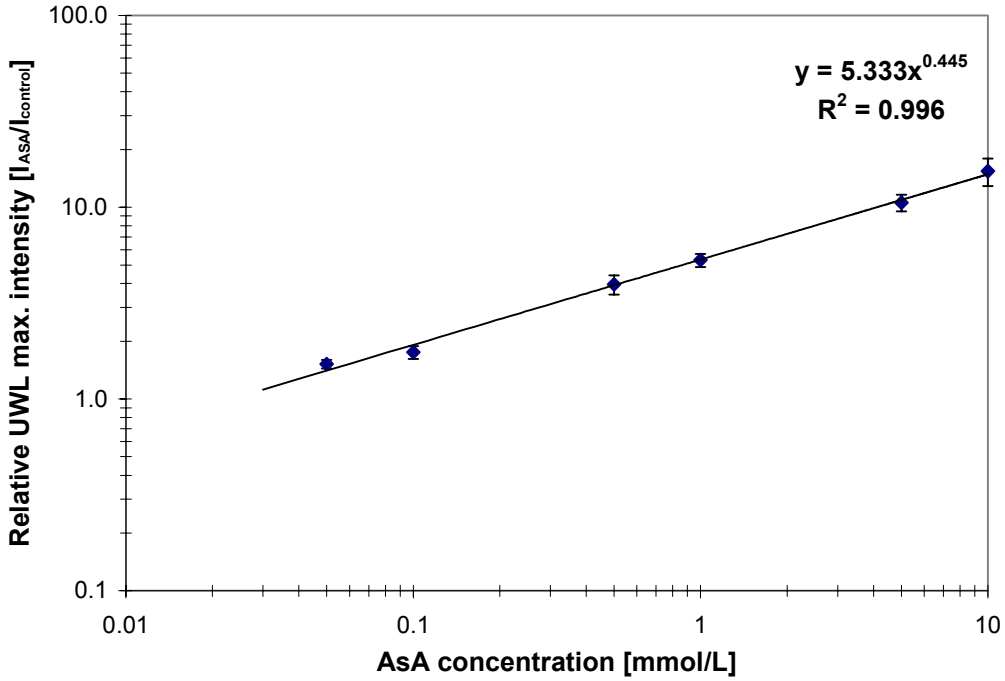

Fig. 3. Maximum intensity of UWL as a function of AsA concentration.

can be kept for such a period in complete darkness without suffering a loss in their metabolism. The research on cytoplasmic motion and respiration rate, performed by Gabryś (1979), has shown that these plants can be kept for several weeks in darkness without any change in their condition. Probably they are used to these conditions because these algae live in lakes at a depth of a few meters.

Different levels of ultraweak luminescence intensity depend on the vegetation period that is in correlation with earlier electrophysiological findings, in which values of electrical parameters were different in different seasons. An exemplary comparison of data is presented in Table1.

We suggest that this correlation of behavior can albo be seen in the fact that only in 10% of cells population under study, the oscillatory character of spontaneous ultraweak luminescence can be discovered (for a possible explanation of this, see Jaśkowska *et al*., 1999). Similarly, in electrophysiology research, in 10% of the cell series under study there have been found changes of electrical parameters which are oscillatory in character (Fig. 2) (Tokarska, Jaskowska & Spiewla, 1990). The similarity in the amount of cells with oscillations among all the examined ones, appears not accidental, and in relation to this there are some plans for quasi-parallel electrophysiological and luminescence research.

It appeared that a comparison of spontaneous luminescence intensity from particular parts of *Characeae* cells and their subcellular units is also very interesting. The data are shown in Table 2. The methods of obtaining subcellular fractions from *Characeae* are described in the paper of Jaśkowska *et al.* (2001). The extreme value of ultraweak emission was recorded from cytoplasm isolated from internodal cells after the cutting of nodes. This example testifies strongly, that the method of ultraweak emission monitoring can plag a specific role in the examination of the degree of a biological system degradation. To achieve this it is necessary to find, ”to catch”, a difference between the beginning of disintegration in cells and the end of the efforts made by the living systems during their full mobilization, the efforts which are to prevent disintegration. This must be possible because in a special environmental medium an isolated cytoplasm can form drops which contain vibrational chloroplasts and what is more, they can reconstruct cytoplasmic membrane on their surfaces (Jaśkowska & Śpiewla, 1979; Inoue, Ishima, Horie & Takenaka, 1971; Takenaka, Inoue, Ishima & Horie, 1971; Koppenhofer, 1974). That is an evidence for large repairing possibilities inherent in an isolated cytoplasm. For this reason, however, we do not know which process is predominant and which is responsible for such an extremely high level of ultraweak luminescence intensity. A similar risk to make mistakes appears when one studies cells that are homogenated and subcellular fractions separated from these homogenates.

Table 2 Spontaneous ultraweak luminescence intensity from different parts of *Nitellopsis obtusa* plants, from the cell structures, from the subcellular fractions and model from system: liposoms + 0.1 mmol/L of FeCl3 + 1.5 mmol/L of AsA [Leyko, Ertel & Bartosz, 1991]

Source of UWL	Average UWL intensity (imp./(min.•gram dry mass)
Internodal cells	5532 ± 60
Rhizoids	9556 ± 166
Nodes	23839 ± 219
Cell wall	156609± 1131
Isolated cytoplasm	285047 ± 1070
Membrane fraction	1250*
Mitochondria fraction	8600*
Rybosomal fraction	1500*
Model system	1854* ± 6
Background	392* ± 2

* - imp/min – absolute impulses frequency

We had the same problem with the interpretation of data obtained by means of Molecular Light Imager method with the use of CCD camera (Sławińska, Górski & Sławiński, 1998) in which it is possible to obtain the unique ultraweak luminescence map with different regions of intensity (measurements were made in Radio- and Photochemistry Department at Poznań University of Technology, Poland). Analysing this map of ultraweak emission from particular parts of samples we observed maxima of emission intensity in places where neighbouring cells and „pseudoleaves" were cut off. In this case also two kinds of processes overlapped: the disintegrative one, which arose in cytoplasm flowing out, and the repairing one, which took place in a post-injury situation during healing of plasmodesmata (Śpiewla, 1982; Śpiewla & Matusiewicz, 1982).

Apart from this research, the kinetics of ultraweak luminescence changes elicited by the exposition of samples to the action of different biological active substances has been studied. At Fig. 3 the dependence of maximum UWL intensity in the function of concentration of ascorbic acid (AsA) is shown. This reagent, which was given exogenously, did not appear antioxidatively active. On the contrary, the prooxidative character of this reagent is revealed when the concentration of AsA increases. At Fig.4 the results are shown from which it appears that when AsA concentration increases, maximum emission is reached faster. The decay of this luminescence is also faster

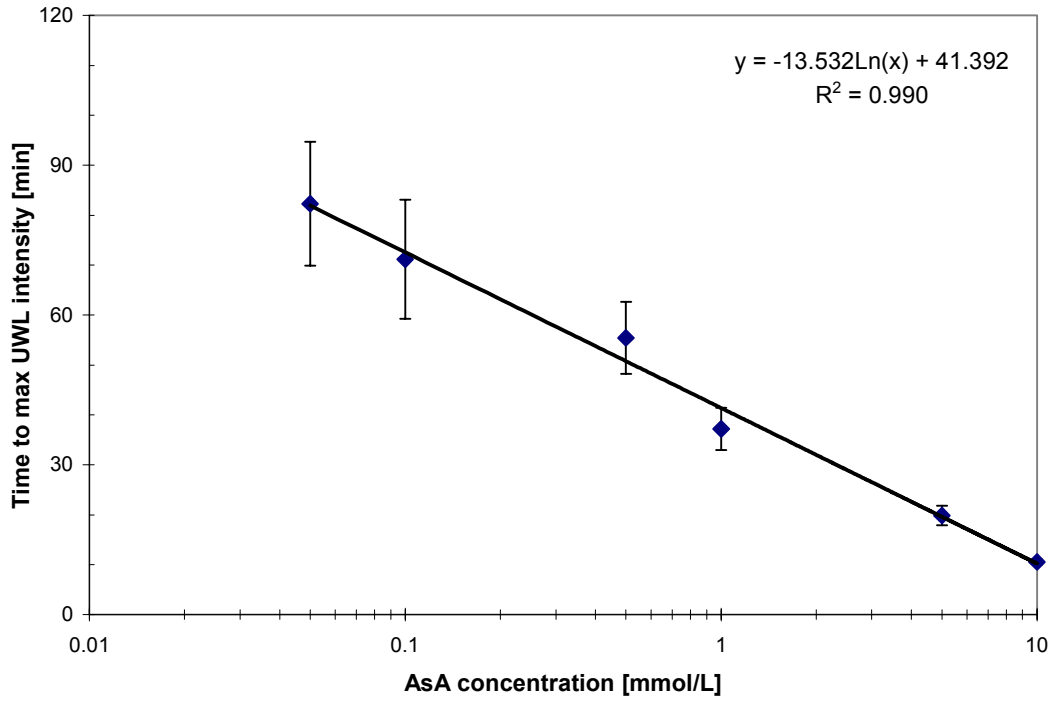

Fig. 4. Dependence of the time after which the max.intensity of UWL is observed, as a function of AsA concentration

with the increase of concentration. The reason for these changes in emission kinetics might be accounted for by a more effective penetration of AsA deep into the cell by facilitated diffusion, so there are better possibilities to reach the potential emitters of ultraweak emission. Subfractioning *Characeae* cells, we have found that only at the ribosomal level at 5 mmol/L of AsA concentration, this reagent shows its antioxidative character and prooxidative on the isolated cell walls, isolated cytoplasm, and membrane fraction (Jaśkowska *et al.*, 2001). We have pointed out with these results that in spite of a similar degree of disintegration while obtaining particular subcellular fractions, it is possible to distinguish certain essential differences in reagent action by means of noninvasive registration of UWL intensity and the kinetics of changes in emission.

Studies of UWL induced by local anaesthetics are also quite promising. It is characteristic of these reagents that responses of cells in form of UWL emission to the action of procaine or lidocaine (7-15 mmol/L concentration) follow after lag time: 0.5-2 hours, respectively. It might appear that penetration is so difficult, but changes in membrane electrical parameters are observed in first 10 minutes to 0.5 hour after exposing the sample to the anaesthetic action. Generation of a series of action potentials is observed with a decrease of their amplitude and with an increased length of time of these potentials. After 0.5 hour the membranes become "electrically stabilized" and do not responded to stimulus exceeding threshold. Membrane resistance decreases in this time. We suggest that the "electrically stabilized" membranes interfere with the cellular response to stress.

Concluding, in the luminescence research on dark-adapted *Characeae* cells we state the following:

- quasistationary level of UWL intensity several times higher than intensity of emission from environmental medium is an inherent attribute of the living processes undergoing in cells;
- UWL intensity is dependent of on vegetation period;
- in 10% examined population spontaneous UWL has changes of oscillatory character;
- action of some reagents (AsA) can be distinguished in different subcellular fractions by ultraweak emission decay kinetics;
- Single Photon Counting method allows us to find and estimate (especially Molecular Light Imager allows us to localize) injury of biological systems, which can be helpful in diagnostics;
- results of the research with local anaesthetics which act on the excitability of membrane and delay plant response in luminescence measurements thereby making it possible to combine electrophysiological and biochemiluminescence investigations.

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