OSCILLATORY CHARACTER OF CHANGES IN ULTRAWEAK LUMINESCENCE FROM *NITELLA* CELLS

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ABSTRACT

Characteristics of changes in ultraweak luminescence of *Nitella* internodal cells under physiological conditions and after exposure to the action of biologically active substances are determined. The results presented here supplement the picture of oscillatory behaviour of *Nitella* cells worked out by us regarding changes in the electrical and transport properties.

KEY WORDS: *Nitellopsis obtusa* cells, ultraweak luminescence, oscillations.

INTRODUCTION

During our studies carried out over last many years (Śpiewla 1979; Śpiewla and Tokarska 1990; Tokarska and Śpiewla 1994; Śpiewla 1994b), oscillatory changes in electrical and transport properties of cell membranes of Characeae plants have been discovered and characterized. The appearance of a clear dependence of parameters of these changes on the functional state of cells and their vegetation period was also found (Śpiewla et al. 1983; Paszewski and Śpiewla 1986; Śpiewla and Tokarska-Schlattner 1994; Śpiewla 1994a).

The relation between the quality of characteristics of the oscillatory changes with the state of cell metabolism suggested to undertake investigations to understand whether ultraweak biochemiluminescence (UL) of these cells also change periodically and whether the parameters of these changes are correlated with electrical changes found by Usa et al. (1989, 1990) in germinating soybean root systems including the hypocotyl. The biochemiluminescence phenomenon in plant cells is identified almost universally with processes of cyclic transport of electrons in photosynthesis (Hideg and Inaba 1991) and with the outflow of electrons from the respiratory chain (Lavorel 1980; Silva et al. 1991). According to our concept, UL is sensitized by chlorophylls (Jaśkowska et al. 1999). Thus, search of emitters whose activation and deactivation may elucidate the oscillatory character for luminescence photon emission is an important aspect of our investigations.

MATERIALS AND METHODS

Measurements and observations were made on internodal and rizoidal cells of uncorticated *Nitella* plants, especially the species *Nitellopsis obtusa* (Desv. in Lois) J. Gr. The plants were collected from Zaglebocze lake near Lublin and cultured under laboratory conditions in natural lake water at tempera-

tures between 17°C and 19.5°C, with natural day/night light cycle.

Before measurements, the cells were selected appropriately, their pseudoleaves were trimmed and rinsed in a solution of artificial pond water (APW of composition: distilled water + 0.1 mM KCl + 0.1 mM NaCl + 0.1 mM CaCl₂) with antibiotics (10 µg/cm³ streptomycine and 20 µg/cm³ crystalline peniciline) for 15 min. The cells were then stored for about 20 h in dark (in light-tight camera of the measurement arrangement) in APW solution (at a pH 7.4 maintained constant using 20 mM HEPES and 10% NaOH). The purpose of rinsing the cells in the solution with antibiotics was to kill bacteria which could appear at cell walls and emit mitotic radiation synchronized with their breeding cycle.

Measurements were made on series of plants: freshly collected, laboratory cultured and collected during different periods of their vegetation. Changes in the intensity of UL were observed and determined by the single photon counting method, using an arrangement built locally with an EMI-9598 photomultiplier (dark-current photomultiplier: 2 nA) as its most important element.

The system serves as an automatic filter spectrometer with a set of glass filters (limiting filters), the so-called cut-off filters (Jaśkowska et al. 1993; Sławiński 1989; Vassiliev 1967) and enables to make measurements also of the total flux of photon radiation. In the mobile part of the measurement arrangement there are six light-tight chambers in each of which 30 test cells are placed. UL measurement of the cells in each of the chamber lasts one minute while the entire cycle of measurements takes more than 3.5 h.

The total photon radiation in 345-700 nm range was calculated using a computer and presented graphically as illumination impulses per minute (or per 10 s). Part of the results of the measurements was presented using an analytical method of "moving average" for 3-4 periods. Some of the data were normalized by the following method: average values of UL intensities obtained from ten samples, each of thirty cells,

Fig. 1. Selected examples of oscillatory changes in the intensity of spontaneous ultraweak luminescence of two samples (a and b) containing 30 *Nitellopsis obtusa* cells each.

treated with ascorbic acid (minus the average value of background counts by APW) were divided by average values of UL intensities from intact cells which were measured before AsA treatment.

In investigations of the effect of local anesthetic procaine on the intensity and kinetics of UL, the procaine solution was injected to the measurement chamber directly in quantities which enabled to obtain expected concentrations.

RESULTS AND DISCUSSION

Figure 1 illustrates selected examples of changes in UL intensities for two samples each containing 30 *Nitellopsis obtusa* cells. The oscillatory character of these changes is evident while the UL intensity level from cells exceeds that of the background from the solution by four times.

UL radiation of two *Nitellopsis obtusa* internodal cells connected by a node is presented in Fig. 2. UL clearly exceeding that of the solution background has not been successful so far for individual cells.

Figure 3 shows UL oscillatory changes in *Nitellopsis obtusa* cells exposed to the effect of 15 mM procaine. From the curve it is clear that a sudden increase in the photon emission intensity, recognised by us as "necrotic peak", appears after about 120 min. exposure. The intensity of the UL emission decreases during the following minutes. This decrease also occurs in an oscillatory fashion, but there is a clear change in oscillation period. A majority of the studied samples confirmed this observation.

It should be added that a 15 mM procaine concentration is the critical level for the investigated material as after an 2 hour exposure all cells in the samples died. For comparison,

Fig. 2. Oscillatory changes in the UL intensity from a part of the plant composed from two *Nitellopsis obtusa* internodal cells connected by a node.

Fig. 3. Oscillatory changes in the UL intensity for *Nitellopsis obtusa* cells exposed to the action of 15 mM procaine. The sample was composed of 30 cells.

at 800 μM concentration the survival rate of the cells was at a level of about 35%.

In connection with the effect of procaine the results presented in Fig. 4 appears interesting. This figure shows the values of UL intensity, averaged and normalized with respect to the "background", for 10 samples of *Nitellopsis obtusa* cells, exposed to the action of 10 mM ascorbic acid (AsA). The lower curve illustrates the results obtained during winter while the upper curve during autumn. After exposure to the action of AsA (exposure 3.5 h) the survival rate of the cells was 100% and the UL intensity increased by 16 times.

Figure 5 illustrates a gradual decrease in the intensity of UL emission in relation with the adaptation of plants after their collection and transport from the lake to the laboratory conditions of temperature and light exposure. A clear increase in the UL intensity, observed immediately after their collection from the lake, suggests that these plants compensate the conditions of generation of large amounts of chlorophyll during weak light exposure at the bottom of the lake and at low temperatures. After bringing the cells to the laboratory, this large amount of stored chlorophyll increases the UL intensity according to the principle of the so-called long lasting lumi-

nescence. After a couple of days the amount of chlorophyll decreases and the UL intensity is reduced.

The results obtained in this work enable to formulate some conclusions of a general nature. On the basis of the results of more than a dozen series of measurements it was established that the UL intensity of *Nitella* cells and the character of oscillatory changes in the UL intensity depend on vegetation period. The UL intensity is distinctly higher for the cells freshly collected from the lake, but within a couple of days it decreases to a particular, relatively stable, level. The cells emit UL radiation most intensively during autumn while most weakly during winter. On the average 10% of the studied samples (with 30 cells in each one) reveal oscillatory changes of the UL intensity. Their relative changes lie between 15-25%.

The results obtained hitherto allow to distinguish one distinct type of oscillations of a period of ca 1 h. Electrical potential and resistivity (Hansen 1978; Śpiewła and Tokarska 1990) as well as respiration (Edmunds 1988) also show the same duration of oscillatory changes in these plants. The synchronous behaviors of biophoton emission intensity changes and surface potential variations with shorter duration of oscil-

Fig. 4. UL intensity, averaged and normalized relative to the background, from ten samples of *Nitellopsis obtusa* cells, calculated during their exposure to the action of 10 mM ascorbic acid at different periods of the plant vegetation. Each sample was composed of 30 cells.

Fig. 5. UL intensity of *Nitellopsis obtusa* cells measured during successive days after their collection from the lake. Each point represents the average value obtained from four series of measurements.

latory period originating from a soybean root system including the hypocotyl was also found by Usa et al. (1990).

It should be emphasized that the observed UL oscillations from our cells represent an effect of synchronisation which accomplishes on the principle of additive coupling of changes occurring only in parts of cells entering in the composition of specimen and that the periodicities of oscillatory changes of particular cells are most probably different. It may be accepted that if, for example, an overwhelming number of cells present in the specimen shows an oscillatory variation, then the behaviour of the specimen as a whole also has an oscillatory character. However, when a decisive minority does not show oscillatory variation, the specimen as a whole does not show oscillations.

It is highly probable that changes in the UL intensity also have shorter periods, as observed for electrical potential, electrical conductivity or pH. However, under the present technical conditions we encounter objective difficulties in their identifications.

It is universally accepted that in green plants an equilibrium occurs between the processes of respiration and photosynthesis. The point of compensation of this equilibrium may shift, for example, in the direction of more intensive photosynthesis and hence to increasing of the oxygen amount flowing from cells. This leads to an increase in respiration and therefore to an intensification of the activity of carriers of respiratory chain. As shown earlier (Milczarek et al. 1994), these carriers are main UL emitters from the cells of Characeae algae.

Under the conditions of plant incubation in the dark where photosynthesis does not occur and processes of nonphotosynthetic phosphorylation take place, chlorophylls fulfil their functions and emit UL in the red range of the spectrum. Mühling (1967) showed oscillations in changes of absorption of ring (band) of cytochrome c, measured in isolated (individual) mitochondria of living yeast cells. The oscillations observed here are in some way connected with the results of Mühling (1967), but understanding oscillations in cases of cell groups incubated in the dark needs further careful investigations.

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OSCYLACYJNY CHARAKTER ULTRASŁABEJ LUMINESCENCJI KOMÓREK *NITELLA*

STRESZCZENIE

Określono charakterystykę zmian ultrasłabej luminescencji (UL) międzywęzłowych komórek *Nitella* w warunkach fizjologicznych oraz po ekspozycji na działanie substancji biologicznie czynnych. Otrzymane wyniki w zasadniczy sposób uzupełniają obraz oscylacyjnych zachowań komórek *Nitella* opracowany przez nas w aspekcie zmian właściwości elektrycznych i transportowych.

SŁOWA KLUCZOWE: komórki *Nitellopsis obtusa*, ultrasłaba luminescencja, oscylacje.