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Bioelectrochemistry 58 (2002) 189–191

Bioelectrochemistry

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Two types of kinetics of membrane potential of water plant leaves illuminated by ultraviolet light ☆

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Received 1 June 2001; received in revised form 6 November 2001; accepted 12 November 2001

Abstract

The effects of ultraviolet-B (UV-B) radiation on plasma membrane of water plant (*Elodea canadensis*, *Vallisneria spiralis*) cells were investigated by using microelectrode methods. A fast and reversible depolarization of membrane potential occurs initially during exposure of leaf cells to UV on a white light background, after which a slow phase of depolarization sets in. On action series, UV is pulsed for 15 s, with dark interval of 3 min, no monotonous response of systems on the UV excitation is observed. The action spectrum of the fast UV response lies in the interval of 300–330 nm and that of the slow phase—in the interval of 280–300 nm. The input impedance of membranes remains unchanged during the period of exposure. It is concluded that the H^+-extruding complex of plant cell plasma membranes really consists of two types of interrelated electronic H^+-pumps: an H^+-pump of redox-active nature and the H^+-pump of the H^+-ATPase enzyme complex. Clearly, during the exposure of leaf cells to UV light, initially, the H^+-pump of redox-active nature and then H^+-ATPase are inhibited. It is proposed that the initial chromophore of UV-B light on plasma membrane can be one of the components of H^+-pump of redox-active nature. It is probably the molecular of quinone.

Keywords: UV-B radiation; Proton pump; Membrane potential; Plant cell

1. Introduction

At present, it is known that the plasma membrane of plant cells has two kinds of proton extruding pumps: an H^+-pump of redox-active nature and that of the H-ATPase enzyme complex. The results of many experiments show that both of this pumps are interrelated, although final proofs of this interrelation are so far lacking [1,2].

It is a problem to find specific factors for inhibition or separation of these different proton pumps. At present, all investigators working on the function of these pumps use chemicals. It is difficult to interpret the results of action chemical factors on plasma membrane, because chemical factors simultaneously affect many different processes of cells. That is why, to study the functions of proton pumps of plasma membrane, we chose a physical factor, such as UV irradiation.

Using the UV light, first, we investigated the initial mechanism of UV action on plant cells and second, we found out that it is possible to study the functions of plasma membrane proton pumps separately.

2. Materials and methods

The study was conducted on photosynthesizing cells of leaves of the higher water plants—Canadian waterweed (*Elodea canadensis* Rich.) and spiral wild celery (*Vallisneria spiralis* L.). The large cells of these plants make it possible to conduct experiments for a long time without disturbing their physiological state or intactness of the membranes. Moreover, electrogenesis of the cells of water plants has been thoroughly studied, and they are often used in electrophysiological experiments. Waterweed and wild celery were cultured in tap water, under laboratory conditions. The water was changed once a week.

☆ This paper was presented at the XVIth International Symposium on Bioelectrochemistry and Bioenergetics, Bratislava from June 1 to June 6, 2001. A collection of these papers has also been published in previous issues of this Journal 55/1–2, 56/1–2.

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1567-5394/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S1567-5394(02)00032-4

Before the experiment, the leaves were kept in a flow chamber in artificial pond water (APW), composition of which included 1.0 mM NaCl, 0.1 mM KCI, and 0.1 mM $CaCl_2$. A continuous flow of APW made it possible to preserve the normal physiological state of leaves.

The membrane potential and membrane impedance were studied using intracellular microelectrode technology, as described in detail by Khalilov and Akhmedov [3]. The microelectrodes constitute special glass capillaries filled with 3 M KCl. The main source of UV radiation was a DRT-230 high-pressure mercury lamp, which had a linear emission spectrum. The distance from the lamps to the exposed object was 0.25 m. Radiation intensity was 32 W m^{-2}. Glass filters (UFS and BS) were used in studying the action spectrum of membrane potential depolarization.

The UV-transmitting filters (UFS and BS) were obtained from Russian LOMO. The transmittance spectra of these filters are the same as filters of Toshiba Glass (UV-28, UV-29, UV-30, UV-31, UV-32). Each filter is characterized by reference to the wavelength at which 50% transmittance occurred [WL ($T=0.5$)].

The exposure procedure allow us to register membrane parameters of cell without interruption during typical recordings of membrane potential registration are presented on the figures below.

3. Results and discussion

A complex change of membrane potential was detected when leaves of the water plants were exposed to UV. Fast and strong depolarization of the membrane potential occurred during the first minutes of exposure. Regardless of continuing exposure, the membrane potential returned to the starting level, after which a slow phase of depolarization set in (Fig. 1). Thus, UV can evoke two types of depolarization, rapid and reversible depolarization at first, and then slow depolarization. It was interesting to study the action spectrum of these two types of depolarization. To this end, we used different sources of UV and UFS and BS glass filters to investigate the kinetics of membrane potential changes during exposure to UV.

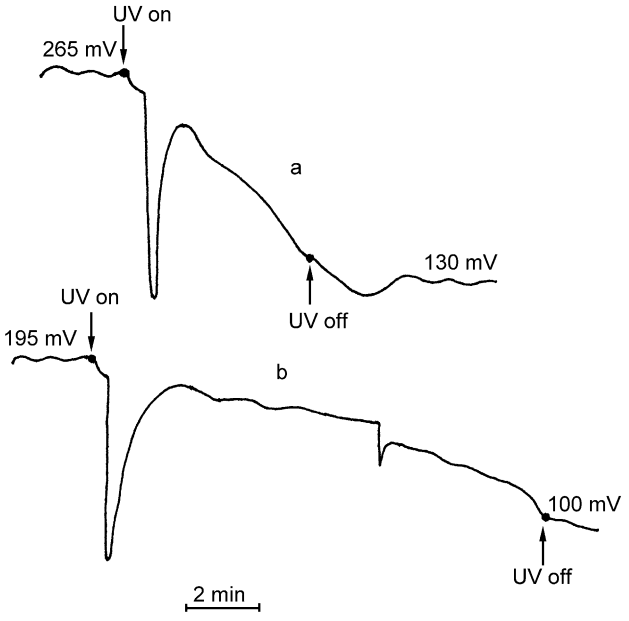

Fig. 1. Changes in membrane potential of waterweed leaf cells (a) and wild celery leaf cells (b) during UV exposure.

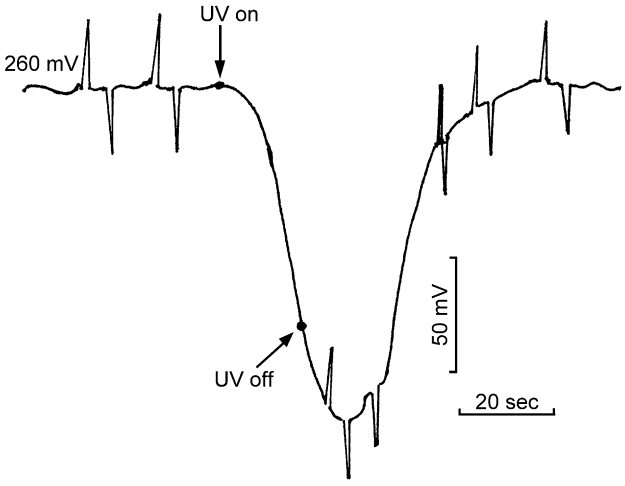

Fig. 2. Fast UV response of waterweed leaf cells during UV exposure (15 s). Vertical lines indicate shift of membrane potential during passage of dc-impulses (5×10^{-9} A, duration of 2 s).

Detailed study of this fast phase of membrane potential changes indicates that a fast and reversible depolarization occurred in cells during brief (15–25 s) exposures to UV with 290 nm wavelength. Depending on the starting membrane potential level and the time of exposure, the depth of depolarization here attained more than half the value of the membrane potential. It is interesting to note that input impedance of the membrane and intercellular electrical couplings (conductance of plasmodesmata) did not change during depolarization development or after repolarization processes (Fig. 2). We identified that the action spectrum of fast depolarization of membrane potential at the level of 300–330 nm means that, in this case, UV affects the component of a redox chain of the plasma membrane directly. The action spectrum of the slow phase of depolarization at the level 280–300 nm means that it affects a protein natural component of plasma membrane, which is H^+-ATPase complex.

The common character of response of membrane potential is preserved during pulsed action of UV-radiation: the duration of the phase of depolarization is not dependent, evidently, on dose (or duration) of the UV-impulse. In one-act pulse excitation, however, the slow phase of depolarization, which happens due to enduring long time irradiation, does not occur. However, in a series of UV, pulsed with duration of 15 s, with dark interval of 3 min, there was no

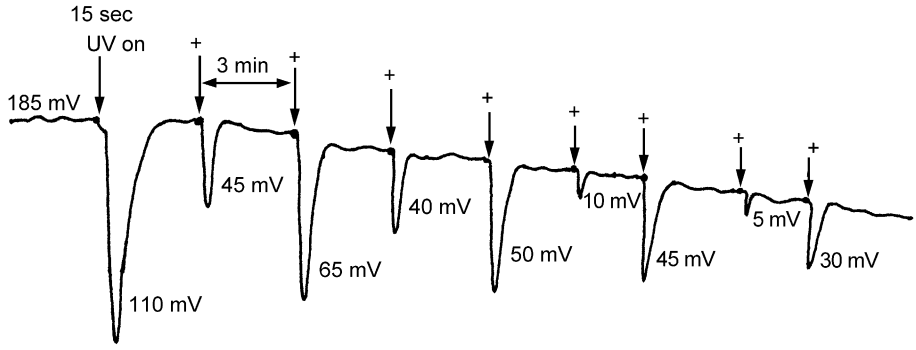

Fig. 3. The kinetics of membrane potential change of wild celery leaf cells during UV irradiation by impulses in series.

observed monotonous response of systems on the UV-excitation: the depth of depolarization is greater in the first and the following odd impulses than in the second and the following even impulses in series (Fig. 3) [4]. This original character of response develops on the basis of common monotonous depolarization; obviously, it happens for the same reasons as those happening under continuous irradiation. When the interval between impulses in series is decreased, the depth of depolarization in response to the add impulses also decreases, but is still greater in comparison with the even impulses (See Fig. 3). Results of the conducted experiments (Fig. 1) indicate that two types of membrane potential depolarization are clearly isolated during exposure to UV light. Characterizing these two types of depolarization, we can say that the fast response of leaf cells is largely similar to an action potential (Fig. 2). Duration of the response is 50–60 s, while the depolarization rate does not depend on the exposure dose and is characterized by the presence of a threshold and saturation. Only the depth of depolarization depends on the exposure dose. The membrane potential is depolarized by 100–120 mV over the seconds measured. The dependency of depolarization depth on exposure dose has an S-shaped form.

Adhering to the idea of parallel existence of the H^+-ATPase and redox-active types of H^+-pump on the plant cell plasma membrane, we suggest the following explanation for the effect we obtained during the above-mentioned UV exposure. The action spectrum of the fast UV response at the level of 300–330 nm means that UV in this case directly affects to the nonprotein component of the plasmalemma. This may be a component of a redox chain. Evidently, UV with wavelength of 300–330 nm alters the function or structure of a component of the redox system. This component is probably molecular quinone. By exciting and altering the form of quinone, UV brings about inactivation of the redox system. Inactivation of the redox system in turn leads to membrane potential depolarization and acidification of the cytoplasm, which stimulates a pH_i-dependent H^+-pump of the H^+-ATPase type. The initial strong membrane potential depolarization during the development of the fast UV response therefore undergoes repolarization and returns to the starting level irrespective of stoppage or continuation of the exposure. As for the slow depolarization phase at the wavelength level of 280–300 nm, it coincides with the absorption spectrum of protein molecules. It may therefore be assumed that the H^+-pump of the H^+-ATPase type is inactivated at the same time as the redox system remains under UV exposure.

What occurs during irradiation by series of short UV pulses? The first impulse inactivates redox-type pump that results in activation of H^+-ATPase. The second impulse, acting in the condition inactivating redox pump does not cause intrinsic depolarization, as relative contribution of redox pump in this phase is small after the action of the first impulse. The more remote is the third impulse from first impulse, the more continuous is the condition of reactivation of redox pump, thus ensuring a cyclic process.

As a conclusion, the results of these experiments are in agreement with the idea that the H^+-extruding complex of the plant cell plasma membrane consists of two types of interrelated electrogenic proton pumps: an H^+-pump of redox-active nature and that of the H^+-ATPase enzyme complex [5]. So, initially, the ultraviolet-B (UV-B) radiation inactivates the plasma membrane redox system and then occurs the inactivation of H^+-ATPase.

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