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Photoinduced pH and Membrane Potential Changes in the Alga *Pithophora pragensis*

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Abstract. Glass microelectrodes filled with antimony were constructed, the electrical potential of which linearly depends on pH and is not influenced by light. Recording at the surface and in the cytosol (the latter corrected for the transient response of the membrane potential measured with a conventional electrode) showed both intra- and extracellular alkalization brought about by irradiating the alga *Pithophora pragensis*. DIURON (DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea) in the concentration 10^{-4}M almost entirely abolished the photoeffect.

Photoinduced changes in the electrochemical potentials of protons play the main role in the process of membrane-bound photophosphorylation (Mitchell 1966). For this reason measurements of the electric membrane potential (EMP) and of pH gradients on both the plasmalemma and the membranes of subcellular organelles of plant cells are of interest.

Whereas the EMP can be measured directly with glass microelectrodes filled with KCl or choline chloride (Vredenberg 1976), the possibilities of pH recording in cells and subcellular organelles are more limited. Measurements with indicators may be affected by artefacts and the time response of the proton-selective microelectrodes is relatively high (1 to 5 s according to Bulychev 1980).

However, antimony microelectrodes sensitive to pH changes can be constructed (Vieira and Malnic 1968) and used to record pH changes in body liquids of animals (Satake *et al.* 1980). Gyenes *et al.* (1977) used antimony microelectrodes (SBM) to measure photoinduced pH changes in the vacuole of *Nitella*; however, they do not describe the properties or the fabrication of their electrodes.

The aim of the present paper is to describe a convenient way in which SBM can be prepared as well as measurements carried out with them on the alga *Pithophora pragensis*. Light-induced changes were measured at both the external and internal surfaces of the algal cell and in the latter case they were compared with the record obtained with a conventional choline-chloride filled microelectrode. The effect of 10^{-4} M DIURON (DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea) was also tested.

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MATERIAL AND METHODS

Preparation of Sb-Microelectrodes (SBM)

Antimony powder was melted under the atmosphere of nitrogen and introduced into preheated pyrex capillaries by the suction of a vacuum pump. At one end of the capillary the antimony was in contact with an outlet copper wire. From the antimony-filled capillaries microelectrodes were drawn with a semi-automatic micropipette — a puller (ME-4, USSR). SBM with tip diameters of 1 to 10 μm were obtained.

Calibration of SBM

A series of TRIS-HCl buffers (0.1 M) was used for calibration. The potential difference between SBM and a calomel electrode was measured with a pH meter/millivoltmeter (VEB-PRACITRONIC, GDR). The tip resistances of SBM were in the range of $5 \,.\, 10^7$ to 10^9 Ω.

Glass Microelectrodes

Microelectrodes used for membrane potential measurements were pyrex micropipettes with tip diameters of 0.1 to 0.5 μm, filled with 1 M choline chloride (VREDENBERG 1976).

Measurements of Photoinduced Changes

The measurements were performed with cells of the alga *Pithophora pragensis* ŠULA by the method described by DENESH *et al.* (1977). Potential differences between the microelectrodes and the reference electrode were monitored with a millivoltmeter of a high input resistance (10^{12} Ω) and registered with an oscilloscope. The time response of the experimental arrangement was 10^{-2} s. The alga was irradiated with "white light" (15 W lamp, NARVA, G.D.R.). The irradiance at the object plane was 30 000 lx. The cultivation medium was used in the measurements.

Cultivation of the Alga

The thermophilic alga *Pithophora pragensis* ŠULA (HINDÁK *et al.* 1975) was cultivated in a nutrient solution of the following composition: KNO_3 0.2 g l^{-1}, K_2HPO_4 0.4 g l^{-1}, $MgSO_4$ 0.03 g l^{-1}, $Ca(NO_3)_2$ 0.03 g l^{-1}, $FeCl_3$ 0.015 g l^{-1} (pH = 6.9).

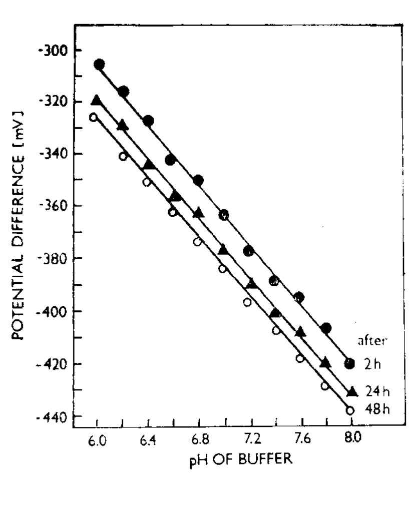

Fig. 1. The pH dependence of the potential difference between an antimony microelectrode and a reference electrode.

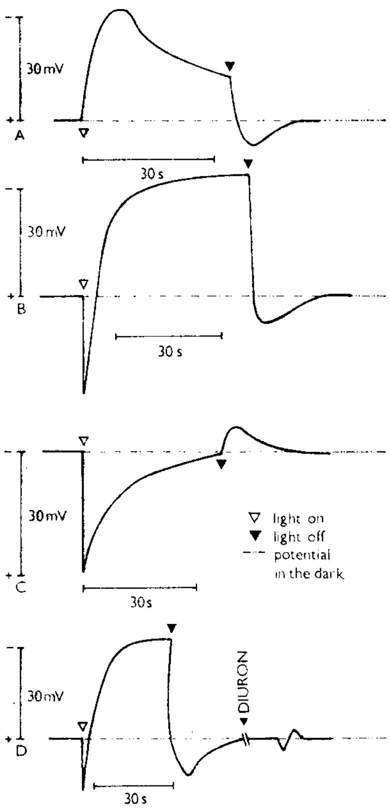

Fig. 2. Photoinduced changes as measured: (A) with an antimony microelectrode (SBM) close (less than 10 μm) to the external surface of the cell of the alga *Pithophora pragensis*, (B) with the tip of SBM inside the cytosol, (C) with a glass microelectrode filled with choline chloride solution, (D) with the tip of SBM in the presence of DIURON.

RESULTS

Fig. 1 shows the pH-dependence of the electrical potential difference between SBM and the reference electrode. With electrode aging the absolute value of the SBM increased, but its calibration curve remained linear.

Photoinduced changes of the SBM potential were already observed outside the cell, when the microelectrode tip approached the external surface at a distance of less than 10 μm (Fig. 2A), after illumination the SBM potential was shifted by −10 to −30 mV with respect to its dark level. After switching off the light the potential relaxed to the original value (Fig. 2A).

With the tip of the SBM inside the cytosol (Fig. 2B) a deflection of +50 to +80 mV may be observed within the first 10 ms. A similar phenomenon occurred with the microelectrode filled with choline chloride (Fig. 2C). However, whereas the potential registered by SBM reached within 3 to 5 s a value shifted by −20 to −40 mV with respect to the dark level (Fig. 2B), the potential measured with the glass microelectrode filled with choline chloride returned to its original value (Fig. 2C). In the dark the two potentials returned to their original values after transient overshoots (Fig. 2B, C). An addition of DIURON (DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea) at the concentration 10^{-4} M (Fig. 2D) showed that the photoeffect measurable with SBM was almost entirely abolished by the inhibitor.

DISCUSSION

Photoinduced proton translocations across the membranes of plant cells are rather fast and for this reason H^+-selective glass microelectrodes filled with ion-exchange material are of little use in their registration — their response time is of the order of seconds (BULYCHEV 1980).

Antinomy microelectrodes (SBM), however, are more suitable for the purpose. Moreover, their pH-dependence is linear and they are not light-sensitive. On the other hand, the SBM-potential is affected by the presence of various solutes (salts, proteins, *etc.*) in the medium (SATAKE *et al.* 1980).

The present study demonstrated that SBM are suitable for monitoring light- and dark-induced pH changes at the surface and in the cytosol of plant cells, such as *Pithophora pragensis*. Close to the external surface of the cell SBM registers the photoinduced pH change only (glass microelectrode filled with choline chloride shows no change in the same position). In the cytosol, however, the two microelectrodes show the same fast initial response, which thus corresponds to the light-induced change of the *Pithophora pragensis* membrane potential. Then the potential measured with the conventional microelectrode falls to the original value and the potential measured with SBM demonstrates alkalization of the *Pithophora pragensis* cytosol after irradiation. The inhibitory effect of DIURON (inhibitor of photosystem 2 of photosynthesis) shows that both the initial membrane potential change and the alkalization in the cytosol as well as in the proximity of the cell are coupled to photosynthesis.

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