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Measurement of plasma membrane potentials of yeast cells with glass microelectrodes

Milan Höfer [a] and Anton Novacky [b]

[a] *Botanisches Institut der Universität, Kirschallee 1, 5300 Bonn (F.R.G.) and* [b] *Department of Plant Pathology, University of Missouri-Columbia, 108 Walters Hall, Columbia, MO 65211 (U.S.A.)*

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Glass microelectrodes were used to measure the electrical potential difference ($\Delta\psi$) across plasma membrane of the yeast *Pichia humboldtii*. The cells were captured in the neck of a glass microfunnel and impaled with a glass microelectrode. The measurements were reproducible and stable for several minutes. The highest $\Delta\psi$ values were obtained in cells metabolizing glucose at pH 6. $\Delta\psi$ in cells deenergized by uncouplers or in dead cells was reduced to about one third of the maximal value. This residual $\Delta\psi$ probably represented Donnan potential. $\Delta\psi$ also was reduced by increasing concentrations of K^+ in the medium. Other monovalent cations were distinctly less effective: $Li^+ \ll Na^+ < K^+$, and Ca^{2+} was without effect. These experiments prove the applicability of the electrophysiological technique on yeast cells and thus open the way for direct determination of the electrical component of the plasma membrane electrochemical proton gradient.

Introduction

The energetic evaluation of a membrane transport process depends upon an accurate determination of the driving force. The predominant driving force of solute transport across the plasma membrane of yeast cells is the electrochemical potential difference of H^+, $\Delta\tilde{\mu}_{H^+}$ [1–3]. The electrochemical proton gradient across yeast plasma membranes is generated by an electrogenic plasma membrane-bound ATPase demonstrated in several yeasts [4,5]. It consists of two components, the electrical potential difference, (the membrane potential) $\Delta\psi$, and the chemical H^+-concentration gradient, ΔpH, the free enthalpy of which is $\Delta\tilde{\mu}_{H^+} = F(\Delta\psi + Z\Delta\text{pH})$, where F is the Faraday constant and Z is 0.058 V at 25°C.

Several different methods have been used to determine transmembrane ΔpH in yeast cells [2], including ^{31}P-NMR spectroscopy of the intracellular inorganic phosphate [3]. Membrane potentials are most dependably measured electrophysiologically using glass microelectrodes, but this method has been difficult to adapt to small, mobile cells such as yeast. Vacata and co-workers [6] reported a few successful insertions of a microelectrode into *Endomyces magnusii* cells but were unable to follow any physiological influence on the measured $\Delta\psi$ values.

In this paper we report experiments with the yeast *Pichia humboldtii*, which proved to be large enough to allow insertion of glass microelectrodes without destruction of cell integrity. We succeeded in puncturing many cells after capturing them in

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; TPP^+, tetraphenylphosphonium ion.

Correspondence: Dr. M. Höfer, Botanisches Institut der Universität, Kirschallee 1, D-5300 Bonn 1, F.R.G.

the neck of a fine microfunnel. We obtained reproducible measurements of $\Delta\psi$ under various physiological conditions. Preliminary results have been reported [7].

Materials and Methods

The obligatory aerobic yeast *Pichia humboldtii* (CBS 7197) was grown at 32°C in a synthetic medium routinely used in this laboratory [8]. Cells were harvested by centrifugation after 2 to 4 days of growth, washed three times in distilled water, and aerated as a 5% aqueous suspension for at least 2 h before use. The yeast grows in chains of 2–4 cells, the biggest of which reach a size of 12–15 μm.

Immobilizing the cells. Individual cells were immobilized in extremely fine microfunnels, which were pulled and blown from glass capillaries (WP-Instruments, New Haven, CT) with a microforge essentially as described in Ref. 9. However, the neck of the microfunnel was reduced to 7–10 μm. The growth of *P. humboldtii* cells in short chains proved to be of significant advantage, because individual chains were firmly anchored by the smaller cells inside the neck of microfunnel (Fig. 1).

Yeast cells in a drop of cell suspension were allowed to sediment on the slope of a wedge located in a pelxi-glass measuring chamber (Fig. 2), a modified version of a measuring cell used by Takeda and co-workers [10]. After the chamber was filled with bathing solution (see below), individual cell chains were sucked into the microfunnel. The microfunnel was advanced by means of a Leitz micromanipulator.

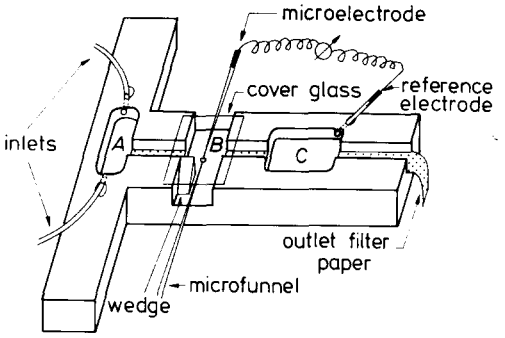

Fig. 2. Scheme of the measuring chamber: cells deposited on the surface of a wedge are collected in a microfunnel by suction. Constant flow of the bathing solution is obtained with strips of filter paper between vessel A, measuring chamber B and vessel C. Chamber B is filled to cover glass with bathing solution and is open at the sides.

The microfunnel and the measuring microelectrode (see below) were operated under a Leitz Dialux microscope which was fitted with a long-working-distance objective (20 ×), resulting in 250 × magnification.

Potential measurements. Glass micropipettes were pulled with a vertical puller (D. Kopf) from fiber-filled borosilicate glass capillaries (WP-In-

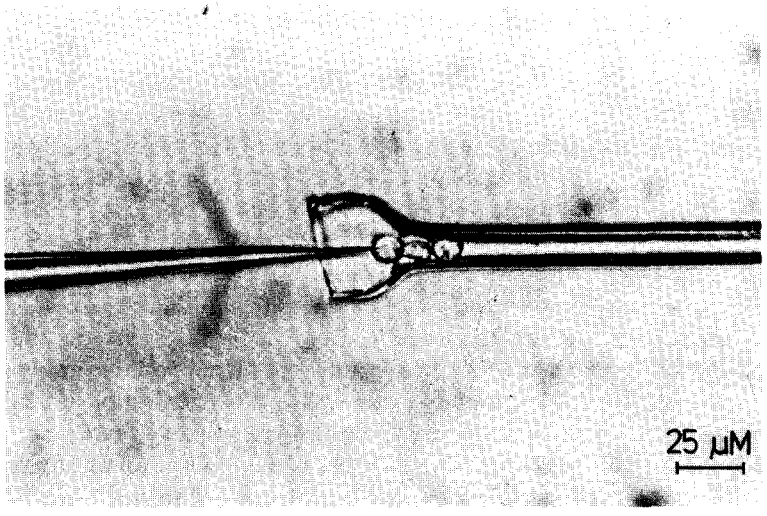

Fig. 1. Microscopic view of the microelectrode-microfunnel arrangement. A chain of three cells is anchored in the neck of the microfunnel and the outer cell is punctured with a microelectrode.

struments, New Haven, CT). Micropipettes with a tip diameter 0.5 μm were filled with 3 M KCl and used as microsalt bridges to silver/silver chloride electrodes. Tip resistance was 10–20 MΩ with a tip potential of less than −10 mV. The microelectrode was advanced by a hydraulic microdrive (Narishige MO-10) mounted to a Leitz micromanipulator. A polyethylene tube (2 mm diameter) filled with 2% agar containing 3 M KCl, in which a silver wire coated with silver chloride was inserted, served as a reference microsalt bridge. It was submerged in the bathing solution which contained: 10 mM Tris phosphate or citrate, 1 mM $Ca(NO_3)_2$, 1 mM $MgSO_4$ and 0.1 mM KCl. All other additions to the bathing solution are indicated in the legends to figures and tables.

The measuring and reference electrodes were connected to an electrometer-amplifier (WP Instruments Model M701) and to a line recorder. The values of membrane potential given in the figures and tables are arithmetic means ± S.E. (number of measurements). All measurements were carried out at room temperature (25–26°C).

For the purpose of comparison, TPP^+-uptake in *P. humboldtii* also was measured in order to calculate $\Delta\psi$ from the steady-state distribution of the lipophilic cation as described in Ref. 11.

Chemicals. Carbonylcyanide *m*-chlorophenylhydrazone (CCCP) was purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals were analytical grade, commercially available compounds. Solutions were made using distilled deionized water.

Results

A typical recording obtained with the microfunnel apparatus is reproduced in Fig. 3. The indicated membrane potential was stable for several minutes and it was reproducible even in two consecutive insertions of a microelectrode into the same cell. Injury by the microelectrode, however, sometimes caused a rather rapid decrease of the measured $\Delta\psi$ so that peak-like responses of the microelectrode were recorded. In few of such cases the injured plasma membrane resealed, leading to recovery of the initial $\Delta\psi$-peak response. Hence, peak-like responses of microelectrodes also represented true values of plasma membrane potential (Fig. 4).

The membrane potential depended on the metabolic activity of the yeast cells. At pH 7 cells metabolizing glucose displayed a $\Delta\psi$ of -52.8 ± 2.0 mV ($n = 10$), whereas in cells without added substrate $\Delta\psi$ was -41.3 ± 1.4 mV ($n = 22$). In cells deenergized by an uncoupler $\Delta\psi$ dropped to values of around −23 mV, independent of pH (Table I). Cells fatally injured by the microelectrode averaged a membrane potential of −19 mV. This residual membrane potential, which was independent of pH between 6 and 4, probably represents the Donnan potential. The observation criteria for cell death were: first, a sudden appearance of a clearly recognizable net-like structure throughout the cytoplasm, and second, the lost of cell turgor. Many cell chains under continuously applied suction vanished inside the microfunnel neck when the largest cell was injured and lost turgor.

Earlier estimates of $\Delta\psi$ across microbial plasma membranes using the indirect method of steady-state distribution of lipophilic cations such as tetraphenylphosphonium (TPP^+) [11–13] indicated that the membrane was depolarized by increasing concentrations of H^+ (or other permeant cations) in the medium [14–17] due to increased back-diffusion of H^+ into the cells [18]. The data of Fig. 5 demonstrate the pH dependency of $\Delta\psi$ in *P. humboldtii*. The highest membrane potential was measured at pH 6, it was increasingly depolarized by lowering the pH of the medium. The membrane potential was also diminished at pH values above 6, correlating with the reduced cell respiration at these pH values (unpublished results).

The depolarizing effect of increasing concentrations of K^+ on $\Delta\psi$ in *P. humboldtii* cells is depicted in Fig. 6. Addition of 100 mM KCl to the bathing solution at pH 6 depolarized the membrane to values of the Donnan potential (cf. Table I). Applications of other monovalent cations at 100 mM concentrations were significantly less effective: $Li^+ \ll Na^+ < K^+$ (Table II). There was no detectable effect of Ca^{2+} (50 mM) on the plasma membrane potential. The lipophilic cation TPP^+, frequently used in μM concentrations as an indicator of $\Delta\psi$ in biological systems (see above), caused plasma membrane depolarization at 5 mM concentrations as efficiently as 100 mM K^+.

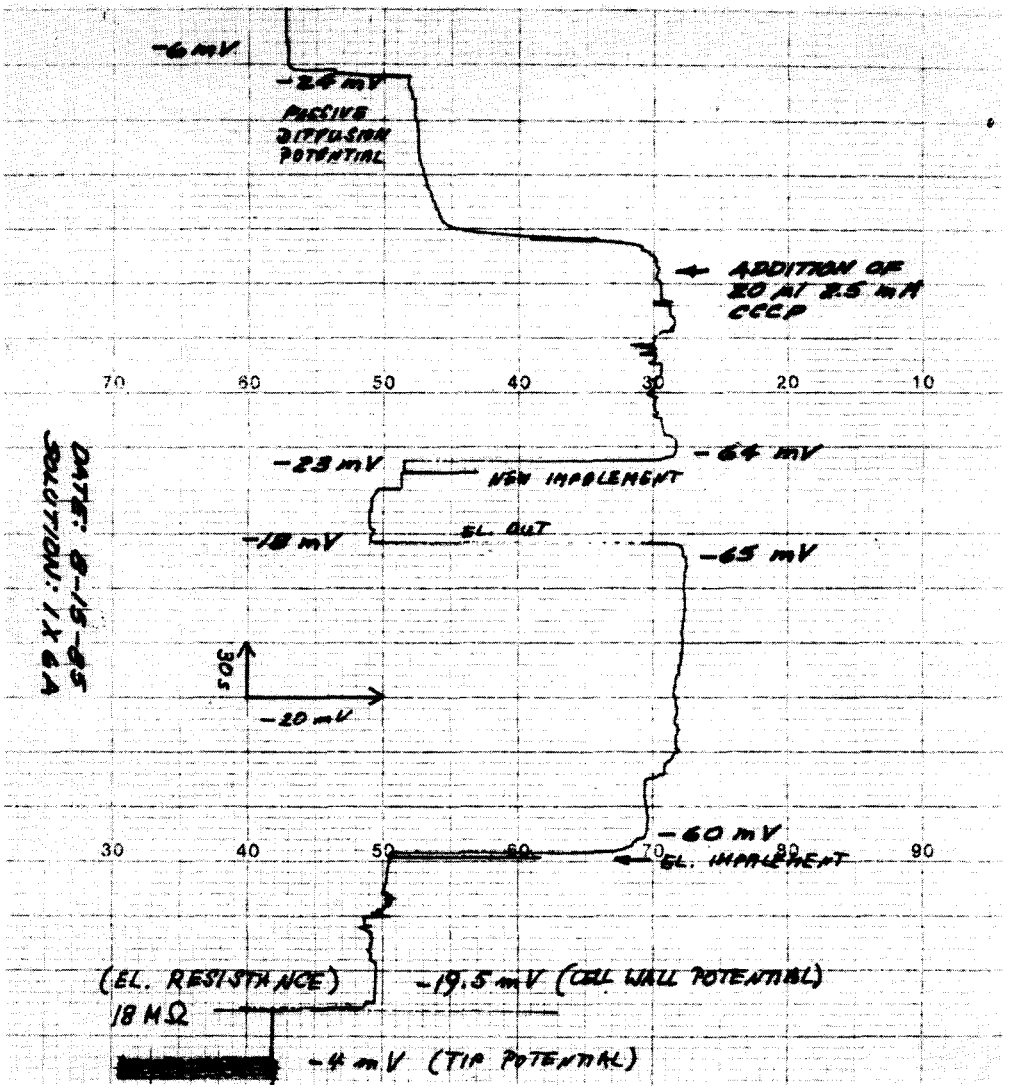

Fig. 3. Reproduction of an original recording, showing two consecutive insertions of a microelectrode into one *P. humboldtii* cell. Addition of 50 μM CCCP led to depolarization of the membrane. Chart movement is from bottom to top. Values of $\Delta\psi$ are as indicated on the chart. The bathing solution contained phosphate buffer, pH 6, and 5 mM glucose.

Attempts to use TPP^+ as an indicator of $\Delta\psi$ in *P. humboldtii* have failed. When added in 2 μM concentrations both at pH 4 and 6 in the presence of glucose, TPP^+ was absorbed from the medium within 30 s and did not respond to applied uncouplers (not shown). Experiments with other mono- and divalent cations have already demonstrated a high cation-exchange capacity of *P. humboldtii* cell wall (unpublished results). Hence, TPP^+ also may be bound to cell wall structures and partially exchange for other cations. Consequently, TPP^+ cannot be used as $\Delta\psi$ indicator in *P. humboldtii*, and therefore, a comparison between $\Delta\psi$ values measured directly with microelectrode and calculated by means of an indirect method was not possible.

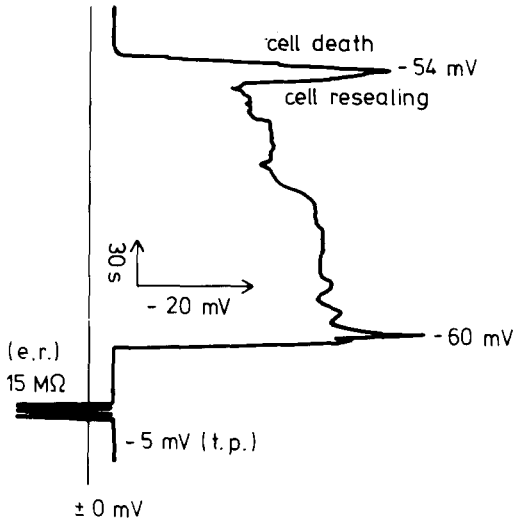

Fig. 4. Recording to the plasma membrane resealing process. After insertion of the microelectrode, $\Delta\psi$ rapidly dropped and recovered again to the initial $\Delta\psi$-peak value due to spontaneous cell resealing before the cell died and was sucked into the microfunnel. Chart movement is from bottom to top. The bathing solution contained phosphate buffer (pH 6), 1 mM KCl and 5 mM glucose. t.p., tip potential; e.r., electrode resistance.

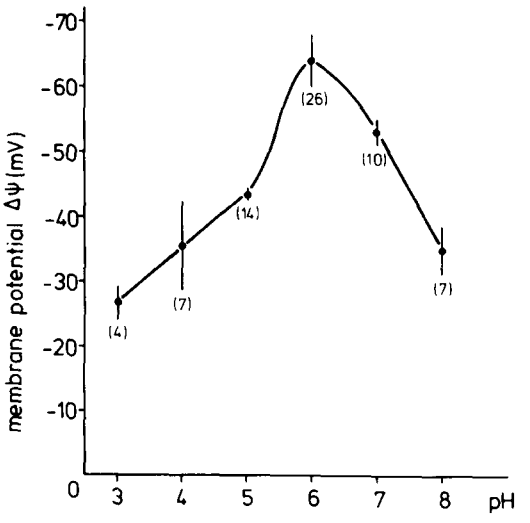

Fig. 5. pH dependence of the plasma membrane potential of *P. humboldtii* cells. Measurements were carried out in citrate buffer containing 5 mM glucose. Vertical bars give the standard error of the mean. The numbers of measurements are in parentheses.

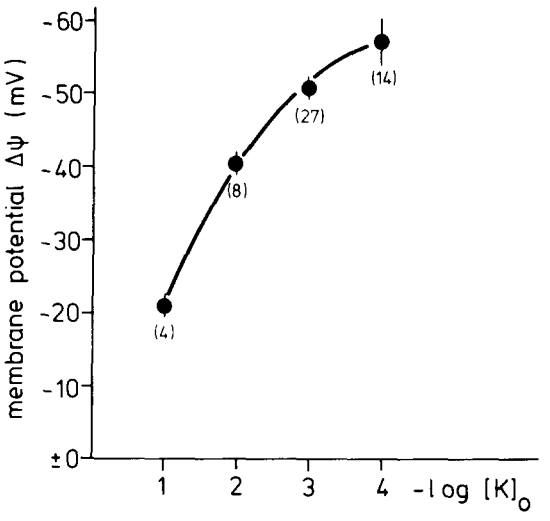

Fig. 6. Depolarization by K^+ of the plasma membrane potential of *P. humboldtii* cells. Measurements were carried out in phosphate buffer (pH 6) containing 5 mM glucose. Vertical bars give the standard error of the mean. The numbers of measurements are in parentheses.

TABLE I

MEMBRANE POTENTIAL $\Delta\psi$ IN DEENERGIZED *P. HUMBOLDTII* CELLS

The bathing solution contained phosphate buffer of given pH, 1 mM KCl and 5 mM glucose.

pH	Additions	$\Delta\psi$ (mV)	Number of measurements
6.0	Live cells	-50.9 ± 1.6	27
	Live cells + 25 μM CCCP	-22.5 ± 1.2	15
	Dead cells	-19.7 ± 1.0	6
	Dead cells [a]	-17.2 ± 1.9	10
	Dead cells + CCCP	-20.2 ± 1.1	6
4.0	Live cells	-39.4 ± 2.9	6
	Live cells + 25 μM CCCP	-24.2 ± 1.9	6
	Dead cells	-19.1 ± 1.2	12

[a] 0.1 mM instead of 1 mM KCl.

Discussion

This paper reports on electrophysiological measurements of plasma membrane potential under various physiological conditions of yeast cells. Impalement of the microelectrode into cells immobilized in the neck of a microfunnel led to reproducible recordings of $\Delta\psi$, which were stable for

TABLE II

DEPOLARIZATION OF $\Delta\psi$ IN *P. HUMBOLDTII* CELLS BY CATIONS

The bathing solution contained phosphate buffer (pH 6), no added substrate.

Additions	$\Delta\psi$ (mV)	Number of measurements
None	-48.2 ± 1.8	18
KCl (100 mM)	-21.1 ± 1.5	13
NaCl (100 mM)	-37.3 ± 3.7	8
LiCl (100 mM)	-45.0 ± 1.7	9
$CaCl_2$ (50 mM) [a]	-46.5 ± 3.4	6
TPP^+ (5 mM) [b]	-20.1 ± 1.3	8

[a] Citrate instead of phosphate buffer.
[b] 1 mM KCl and 5 mM glucose present.

several minutes. In comparison to $\Delta\psi$ values estimated in other yeasts by indirect methods [16–18] or measured by means of microelectrodes either in the fungus *Neurospora crassa* [19] or in giant cells of *E. coli* [13], the values for *P. humboldtii* may appear somewhat low. However, $\Delta\psi$ values above -100 mV were determined in yeasts at pH values between 7.5 and 8.5, at which *P. humboldtii* plasma membrane was already partially depolarized. At pH 6 the plasma membrane potential in other yeasts was always around -70 mV.

A reason for lower values of $\Delta\psi$ measured in *P. humboldtii* with microelectrodes may be the leakage of KCl from the impaled microelectrode. Page and co-workers [20] as well as Blatt and Slayman [21] observed a considerable outflow of KCl through the tip of microelectrodes filled with 1–3 M KCl, when the electrode tip remained unoccluded. Consequently, recorded $\Delta\psi$ decreased gradually with time [21]. However, in root cells [20] no alterations of membrane potential consistent with KCl leakage were detected. Similarly, many $\Delta\psi$ measurements in *P. humboldtii* were stable for several minutes (the longest was 23 min) indicating no significant leakage of electrolyte out of the microelectrode. Moreover, recent experiments with microelectrodes filled with 0.1 M K_2SO_4 produced $\Delta\psi$ values (to be published elsewhere) consistent with the present results.

Recently, Bakker and co-workers [22] reported experiments with *Endomyces magnusii* using the technique of Vacata and co-workers [6] in which $\Delta\psi$ pulses of 1 ms duration were recorded by an oscilloscope. The authors extrapolated the measured peak values of -124 mV at pH 4.5 and -146 mV at pH 7.1 from the time-course of recorded microelectrode potential to $\Delta\psi$ values as high as -190 mV and -275 mV, respectively. The rapid membrane depolarization was assumed to be the result of ion fluxes at the site of cell injury by the microelectrode. In comparison, even peak-like responses of $\Delta\psi$ in *P. humboldtii* cells injured by insertion of the microelectrode were monitored over periods of at least several seconds. However, $\Delta\psi$ pulses of ms duration also may occur in *P. humboldtii* cells following microelectrode impalement which could not be detected by the used apparatus.

P. humboldtii cells exhibited a residual $\Delta\psi$ of about -20 mV when deenergized by an uncoupler or when fatally injured by the microelectrode. We ascribe it to the Donnan potential, generated by an unequal distribution of ions across the plasma membrane due to the presence of charged macromolecules inside the cells. In contrast, the distribution of TPP^+ ions in the yeast *Rhodotorula glutinis* (TPP^+ could not be used as $\Delta\psi$-indicator in *P. humboldtii*) indicated no plasma membrane potential under comparable physiological conditions. The lipophilic cation was completely expelled from preloaded cells following the application of uncouplers [16]. This discrepancy may be an artifact of the indirect method of $\Delta\psi$ measurement due to dissipation of the Donnan potential, which is incapable of performing chemiosmotic work, by even very low TPP^+ concentrations used.

This work opens electrogenic plasma membrane-bound processes of the simplest eukaryotic organisms to electrophysiological investigation. At present, it is limited to large yeast cells such as those of *P. humboldtii*, in which plasma membrane transport is little understood. Further experimental data are necessary to develop a causal relationship between membrane potential-driven transport processes and their driving force, which now can be measured directly.

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