J. Membrane Biol. 103, 255–261 (1988)

The Journal of
Membrane Biology

Measurements of Electrical Potential Differences Across Yeast Plasma Membranes with Microelectrodes are Consistent with Values from Steady-State Distribution of Tetraphenylphosphonium in *Pichia humboldtii*

H.C. Lichtenberg, H. Giebeler, and M. Höfer
Institute of Botany, University of Bonn, 5300 Bonn, Federal Republic of Germany

Summary. Electrical potential differences across the plasma membrane ($\Delta\psi$) of the yeast *Pichia humboldtii* were measured with microelectrodes (filled with 0.1 M KCl) inserted into cells immobilized in microfunnels. The registered $\Delta\psi$ signals were reproducible and stable for several minutes. On attainment of stable readings for $\Delta\psi$ the specific membrane resistance R_{sp} was determined by applying square-current pulses to the preparation. Both $\Delta\psi$ and R_{sp} were pH dependent and displayed equal but opposite deflection, $\Delta\psi$ reaching its maximal value of -88 ± 9 mV ($n = 13$) and R_{sp} its minimal value of 10 k$\Omega \cdot$ cm^2 (maximal conductance) at pH 6.5. Uncouplers and the polyene antibiotic nystatin depolarized the cells, decreasing $\Delta\psi$ to -21 ± 15 mV ($n = 10$) with concomitant decrease of R_{sp}. Comparison of $\Delta\psi$ values from microelectrode measurements with those calculated from the steady-state distribution of tetraphenylphosphonium ions agreed within 10 mV under all physiological conditions tested, except at pH values above 7.0. During microelectrode insertion transient voltage signals (a few msec long) were detected by means of an oscilloscope. These voltage signals were superimposed on the stable $\Delta\psi$ recordings described above. These short voltage signals disappeared in uncoupled cells. The closely related $\Delta\psi$ values obtained by two independent methods (direct measurements with microelectrodes and calculation from steady-state distribution of a lipophilic cation) provide evidence that these values reflect the true membrane potential of intact cells.

Key Words electrical potential difference ($\Delta\psi$) · membrane potential · yeast electrophysiology · microelectrode · TPP^+ distribution · *Pichia humboldtii*

Introduction

Secondary active transport processes are energized by electrochemical gradients of ions, either as H^+ symport (West & Mitchell, 1973; Slayman & Slayman, 1974; Hauer & Höfer, 1978) or Na^+ symport (Bihler & Crane, 1962; Robinson and & Flashner, 1979). The ion gradients consisting of an electrical potential difference ($\Delta\psi$) and of an ion concentration difference (ΔpH or ΔpNa), are generated by plasma membrane-bound ATPases. Particularly for single-cell organisms the electrochemical proton gradient plays an important role in the energization of the uptake of many nutrients. Hence, the knowledge of accurate values of $\Delta\mu_{H^+}$ in these organisms is of great interest. Measurements of the electrical potential difference by means of electrophysiological techniques have been frequently described for animal cells (Schmidt, 1971), plant cells (Higinbotham, 1973; Bentrup, 1981) and for the fungus *Neurospora crassa* (Slayman, 1965; Slayman & Gradmann, 1975). However, because of the small size of microbial organisms direct methods of $\Delta\psi$ determination with microelectrodes have been rarely used (Felle et al., 1980; Vacata, Kotyk & Sigler, 1981; Bakker, Borst-Pauwels & Dobbelmann, 1986; Höfer & Novacky, 1986). Instead, estimation of the electrical potential difference across microbial plasma membranes was achieved by indirect methods, such as the use of fluorescent probes (Kovac & Varecka, 1981; Pena et al., 1984; Wolk & Höfer, 1987) or of lipophilic cations (Komor & Tanner, 1974; Hauer & Höfer, 1978; Boxman, Barts & Borst-Pauwels, 1982; Prasad & Höfer, 1986). In particular for yeast cells the use of tetraphenylphosphonium cation (TPP^+) has been well established (Hauer & Höfer, 1978; Höfer & Künemund, 1984; Prasad & Höfer, 1986). However, the reliability of these indirect methods has frequently been questioned (Eraso, Mazon & Gancedo, 1984; Boxman et al., 1982; Gimmler & Greenway, 1983; Wolk & Höfer, 1987).

In the present study we used the electrophysiological approach to determine $\Delta\psi$ in the yeast *Pichia humboldtii*. Both the membrane potential and the membrane resistance were determined simultaneously. In addition, extremely short $\Delta\psi$ pulses were observed during microelectrode insertion, which were superimposed on the stable $\Delta\psi$ record-

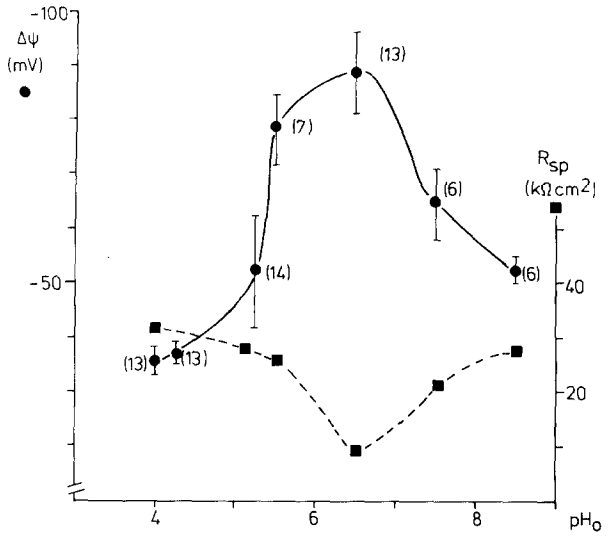

Fig. 1. Dependence of $\Delta\psi$ and R_{sp} on extracellular H^+ concentration (pH_o) as sensed by microelectrodes. (●) membrane potential. (■) specific membrane resistance. Experimental conditions as described in Materials and Methods. Data are given as mean values ± SEM; number of measurements in brackets

ings, monitored over several minutes. These stable $\Delta\psi$ values agreed well with those calculated from the steady-state distribution of TPP^+ in *P. humboldtii* cells.

Materials and Methods

Cells of the obligatory aerobic yeast *Pichia humboldtii* (CBS 7196) were grown in a medium containing Bacto-peptone and yeast extract as described recently (Höfer & Novacky, 1986). The cells were harvested in the stationary phase and washed twice with distilled water.

For electrophysiological measurements the cells were suspended in a medium containing (mM): 0.1 KCl, 1.0 $CaCl_2$, 1.0 $MgCl_2$ and 5.0 HEPES. Single-cell chains were captured in the neck of a microfunnel fabricated as described by Höfer and Novacky (1986). Microfunnels and microelectrodes were pulled from microcapillaries (Hilgenberg, Malsfeld, FRG) using a vertical electrode puller (Kopf-Instruments, Model 720, Tujunga, California). Tips of glass microelectrodes had diameters less than 1 μm. They were filled with 0.1 M KCl and had tip resistances of 20–90 MΩ. Both membrane potential and membrane resistance were measured by the one-electrode method according to Etherton, Keifer and Spanswick (1977). Microelectrode resistance R_{el} was also determined both before and after impalement to ensure that the electrode was not plugged with cell material during the measurement (*cf.* Fig. 2). The Ag/AgCl/KCl microelectrodes were connected to a patch-clamp amplifier (LM/EPC 7, List Medical, Darmstadt, FRG) having one output to an oscilloscope (Model 3091, Nicolet, München, FRG) and another one to a dual-pen recorder (PM 8245, Philipps, Düsseldorf, FRG). Square-current pulses were applied with a function generator (Model 182A, Wavetek, San Diego, California). Fast voltage signals were stored by the oscilloscope, the display was plotted on an X-Y plotter (Model ZSK 2, Rohde & Schwarz, München, FRG) and the data processed by a computer program. Time constants of microelectrodes were determined from voltage responses during application of constant square-current pulses.

For TPP^+ uptake experiments, the cells were aerated as a 5% (wt/vol) suspension in distilled water for 4 hr, then centrifuged and resuspended in 45 mM Tris/succinate buffer of appropriate pH. The reaction was started by adding 5 μM ^{3}H-TPP^+. Samples of 0.5 ml were taken at intervals and filtered through Schleicher & Schüll filters (type 602h, Schleicher & Schüll, Dassel, FRG). To prevent absorption of TPP^- in the cell wall (*cf.* Boxman et al., 1982), each sample on the filter was diluted with 5 ml ice-cold $MgCl_2$ solution (20 mM) before a vacuum was applied to suck off the medium. The cells on filters were then washed three times with 2 ml ice-cold water and transferred into vials containing scintillation cocktail (Aqua-luma, Baker, Deventer, Holland). The radioactivity in the cells was counted in a Hewlett-Packard scintillation counter (Model He/18/1980, Zürich, Switzerland). The membrane potential was calculated by substituting the intra- and extracellular concentrations of TPP^+ into the Nernst equation. The intracellular water volume was ascertained by uptake experiments with ^3H_2O and ^{14}C-inulin as described by Rottenberg (1978) and averaged 3.1 ± 0.2 μl/mg dry wt (12 measurements with four different yeast batches ± SEM).

Chemicals

^{3}H-Tetraphenylphosphonium chloride (160 MBq/mol) was from Hoechst AG (Frankfurt, FRG), carbonylcyanide *m*-chlorophenylhydrazone (CCCP) from Boehringer (Mannheim, FRG), and the polyene antibiotic nystatin from Serva (Heidelberg, FRG). All other reagents were of analytical grade from Merck (Darmstadt, FRG). Solutions were made in quartz double-distilled water.

Results

Microelectrode Measurements

Stable and reproducible recordings of membrane potentials with microelectrodes in yeast, under various physiological conditions, were reported by Höfer and Novacky (1986) for *Pichia humboldtii*. However, these results were hampered by the fact that K^+ might have leaked from the microelectrodes (3 M KCl was used to fill them) and hence depolarized the impaled cells. Indeed, membrane potentials recorded with microelectrodes filled with 0.1 M KCl (Fig. 1, upper curve) were in general about 25 mV more negative as compared with earlier values reported by Höfer and Novacky (1986). On the other hand, the reported dependency of $\Delta\psi$ on the concentration of H^+ in the medium (pH_o), and thus on the ΔpH across the plasma membrane, was confirmed by the present results, displaying a maximum at pH_o 6.5. The corresponding $\Delta\psi$ value was −88 ± 9 mV (± SEM, n = 13).

Figure 2 shows a typical recording of a stable microelectrode measurement of $\Delta\psi$ of a *Pichia humboldtii* cell. Applying square-current pulses of 5 pA the specific membrane resistance R_{sp} could be calculated from he recorded voltage response (rec-

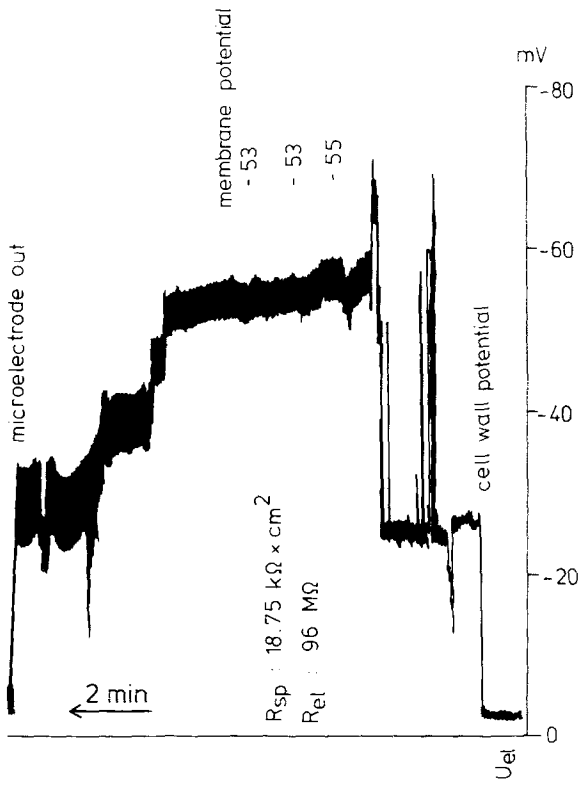

Fig. 2. Typical recording of $\Delta\psi$ during insertion of a microelectrode into a *P. humboldtii* cell. Constant current pulses of 5 pA were applied on the microelectrode to allow calculation of specific membrane resistance R_{sp}, R_{el} electrode tip resistance, U_{el} electrode tip potential. Experimental conditions as described in Materials and Methods; pH_o was 5.2. The later dissipation of $\Delta\psi$ may be caused by mechanical injury of the cell, e.g. due to a decline of suction pressure

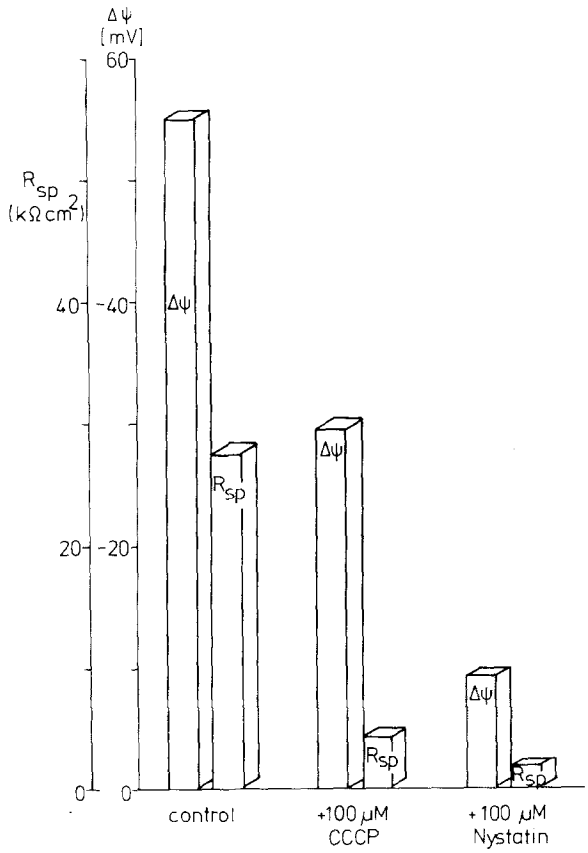

Fig. 3. Membrane depolarization by an uncoupler (CCCP 100 μM) and by the polyene antibiotic nystatin (100 μM) measured with microelectrodes. R_{sp} specific membrane resistance. Experimental conditions as in Fig. 1, pH_o was 5.2

tangular deviations on the trace). The pattern of how specific membrane resistance changes with variation of the membrane potential (effected by variation of pH_o) is depicted in Fig. 1 (lower curve, squares). The R_{sp} curve exhibits equal but opposite deflection to $\Delta\psi$, displaying a minimal specific membrane resistance of 10 kΩ · cm² at pH_o 6.5 (when $\Delta\psi$ was at its maximum).

The measured membrane potential was energized by cell metabolism. Applying an uncoupler (100 μM CCCP) or increasing the plasma membrane permeability by adding polyene antibiotic (100 μM nystatin), both led to cell depolarization down to values between −7 and −35 mV with a concomitant decrease of the specific membrane resistance (Fig. 3).

Steady-State TPP+ Distribution

Our earlier attempt to compare the size of $\Delta\psi$ measured directly by using microelectrodes with values calculated from the steady-state distribution of the lipophilic cation tetraphenylphosphonium (Höfer & Novacky, 1986) failed because of the high cation exchange capacity of the cell wall of *P. humboldtii* cells (Lammert, Prasad & Höfer, 1987). In the present work this problem was overcome by washing off the TPP+ absorbed within the cell wall with 20 mM $MgCl_2$ (Boxman et al., 1982). Using this procedure we obtained smooth uptake kinetics of TPP+ reaching a steady-state within 60 min (Fig. 4). As expected, agents known to depolarize yeast cells (Hauer & Höfer, 1978; Höfer & Künemund, 1984; Prasad & Höfer, 1986) such as CCCP, nystatin or HCl (due to increase of extracellular H^+ concentration), all caused a rapid efflux of previously accumulated TPP+. However, unlike another yeast, *Rhodotorula glutinis* (Hauer & Höfer, 1978), some TPP+ still remained in *P. humboldtii* cells, corresponding to potentials of about −25 and −15 mV, when cells were de-energized or acidified, respectively. These values are coincident with those obtained with microelectrodes under the same experimental conditions (*cf.* Fig. 3).

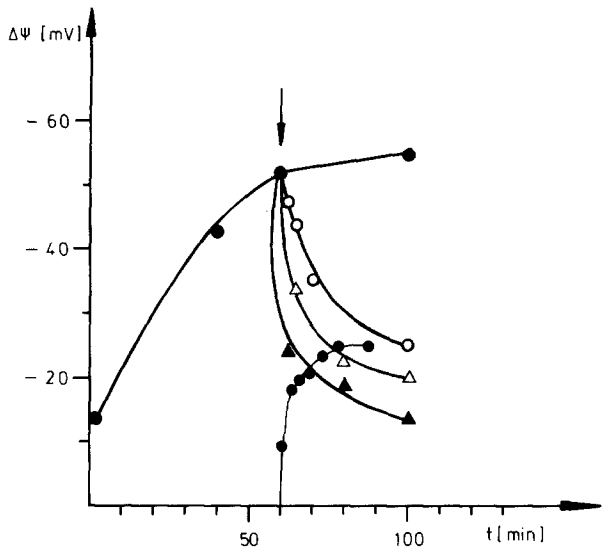

Fig. 4. Δψ calculated from intracellular TPP^+ accumulation in *P. humboldtii* cells. When steady state of TPP^+ uptake was reached (arrow), cells were transferred into flasks containing either 100 μM nystatin (△), 100 μM CCCP (○) or HCl to give pH_o 3.0 (▲). Experimental conditions as described in Materials and Methods; pH_o 5.5. TPP^+ accumulation in cells preincubated for 5 min with 100 μM CCCP is represented by the lowest curve (●), TPP^+ added at t = 60 min

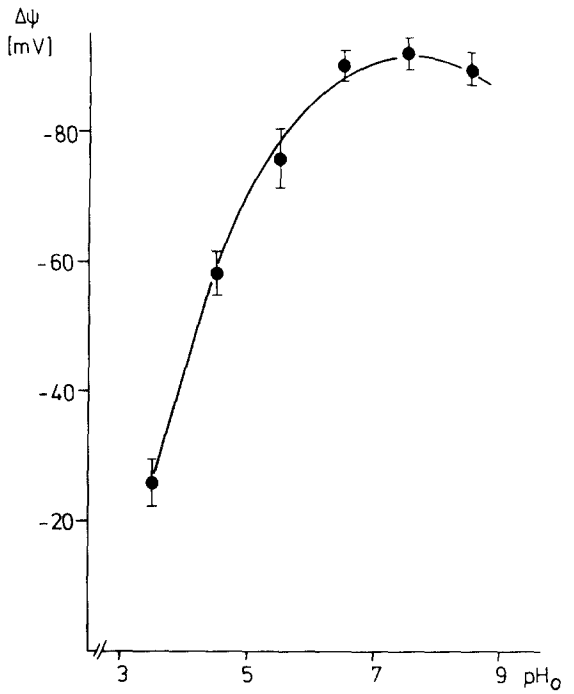

Fig. 5. Calculated values of Δψ from steady-state TPP^+ distribution at various pH_o values. The experimental conditions were as in Fig. 4. Data given are arithmetic means ± SEM (n = 5)

In order to prove that TPP^+ accumulation in de-energized cells is not the result of a Δψ-independent TPP^+ absorption to cellular structures, uptake kinetics under de-energized conditions (as shown in Fig. 4) were determined. The time-dependent uptake of TPP^+ into cells preincubated for 5 min with 100 μM CCCP led to the same steady-state accumulation of TPP^+ as was achieved by the outflow of TPP^+ from preloaded cells induced by the uncoupler. Thus, de-energized cells of *P. humboldtii* maintain a residual membrane potential, as already indicated by direct microelectrode measurements, which we ascribe to the Donnan potential.

Electrophysiological measurements showed a characteristic dependence of Δψ on the external H^+ concentration (Fig. 1). Figure 5 depicts Δψ values calculated from the steady-state distribution of TPP^+ as influenced by variation of external pH_o. Between pH_o 3.5 and 6.5 the calculated Δψ closely follows the pH_o-dependent Δψ pattern measured directly with microelectrodes (Fig. 1). However, at pH_o above 6.5 the TPP^+ accumulation did not decrease, as was expected from the microelectrode data. Presumably at $pH_o > 6.5$, TPP^+ was also accumulated in intracellular organelles which are not directly affected by external H^+ concentration, probably mitochondria.

In order to test this assumption, the effects of the uncoupler CCCP and of the polyene antibiotic nystatin were determined. If TPP^+ was accumulated to a significant extent into mitochondria at $pH_o > 6.5$, it was expected that nystatin, interacting only with the plasma membrane and not with mitochondria (*see* Hedenström & Höfer, 1979), would not cause a complete efflux of TPP^+ from preloaded cells at alkaline pH_o values. CCCP, on the other hand, should uncouple both types of membranes independent of pH_o. The results shown in Fig. 6 correspond well with the predicted effects of the two agents. At pH_o 7.5 a considerable amount of TPP^+ was still kept in *P. humboldtii* cells in the presence of nystatin, whereas at pH_o 4.5 nystatin caused a complete outflow of preaccumulated TPP^+. Addition of CCCP resulted in a complete outflow of TPP^+ at both pH_o values. Control experiments performed under the same conditions proved that both nystatin and CCCP induced a complete outflow of preaccumulated 2-deoxy-D-glucose from the cells. 2-Deoxy-D-glucose is distributed only in the cytosol (*data not shown*). Intracellular pH_i was estimated by the steady-state distribution of ^{14}C-propionic acid (Seaston, Carr & Eddy, 1976); it was 8.2 and 7.0 at pH_o 7.5 and 4.5, respectively.

Fast Transient Δψ Signals

Fast transient Δψ peaks (a few msec long) were detected when signals of the Δψ-sensing microelectrode (V_{el}) were recorded during its insertion into

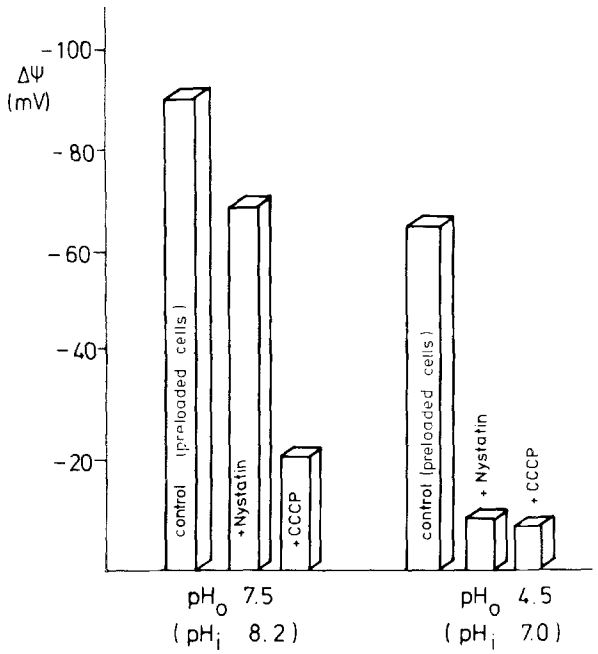

Fig. 6. Effect of nystatin and of CCCP on $\Delta\psi$ calculated from steady-state TPP^+ distribution at pH_o 4.5 and 7.5. Experimental conditions as in Fig. 4. After 60 min cells were divided into flasks containing either nystatin (100 μM) or CCCP (100 μM) and incubated for a further 30 min. The columns give the steady-state membrane potentials representative of three repeated experiments

the cell by means of a storage oscilloscope. Regarding the microelectrode as a simple *RC* circuit, where R_{el} and C_{el} are the electrode resistance and capacitance, respectively, the voltage across the membrane (V_m) is given at any time (t) by the equation (Lassen & Rasmussen, 1978; Bakker et al., 1986):

$$V_m(t) = V_{el}(t) + R_{el} \cdot C_{el} \cdot dV_{el}(t)/dt.$$

V_{el} describes the actual recorded potential, R_{el} and C_{el} were ascertained for each microelectrode, $dV_{el}(t)/dt$ was numerically determined from the recorded data.

Using this equation, values as high as -160 mV were calculated for plasma membrane potentials of *P. humboldtii* at pH_o 6.5. A computerized outprint of these fast transient $\Delta\psi$ signals is depicted in Fig. 7, giving both the reprinted oscilloscope signal (V_{el}) and the calculated membrane potential (V_m). The inset to Fig. 7 reflects the dependence of the calculated $\Delta\psi$ peak values on the extracellular H^+ concentration. The shape of the curve corresponds to that of Fig. 1 for the stable $\Delta\psi$ recordings; however, the calculated values are in general around 70 mV more negative. It should be emphasized that these fast transient $\Delta\psi$ peaks detected during the process of microelectrode impalement are superimposed on the stable $\Delta\psi$ measurements recorded for several minutes after the microelectrode penetrated the plasma membrane. Such transient $\Delta\psi$ peaks were not observed in de-energized cells (either by CCCP or by nystatin).

Discussion

Measurements of electrical potential differences ($\Delta\psi$) across the plasma membrane of the yeast *Pichia humboldtii* by two independent methods produced equal results. The agreement of $\Delta\psi$ values determined under various physiological conditions by either direct measurements with microelectrodes or by calculation from the steady-state distribution of the lipophilic cation tetraphenylphosphonium (TPP^+) leads to the conclusion that the measured values reflect true plasma membrane potential.

The above statements are valid for acidic media up to pH_o 6.5. At neutral and slightly alkaline pH_o, $\Delta\psi$ values calculated from the steady-state distribution of TPP^+ were higher than those measured directly with microelectrodes. The effect of the polyene antibiotic nystatin on TPP^+ accumulation compared with that of an uncoupler, indicated that at slightly alkaline pH_o, TPP^+ was also accumulated in subcellular compartments insensitive to nystatin, very likely mitochondria. Nystatin, which interacts with plasma membrane sterols, caused only a partial efflux of TPP^+ from preloaded cells at pH_o 7.5. The uncoupler CCCP, on the other hand, depolarizing both the plasma membrane and the inner mitochondrial membrane, induced a complete outflow of TPP^+ under the same experimental conditions. The accumulation of TPP^+ in mitochondria at pH_o 7.5 might be due to the change of intracellular pH_i which was 8.2 at pH_o 7.5 and 7.0 at pH_o 4.5. At pH_i 8.2 $\Delta\psi$ across the inner mitochondrial membrane is likely to be higher than at pH_i 7.0, thus leading to the observed extra accumulation of TPP^+ into cells at $pH_o > 6.5$.

The membrane potential in *P. humboldtii*, as measured with microelectrodes, decreased gradually from neutral to slightly alkaline pH_o of the cell suspensions. The pH_o-dependent depolarization of the plasma membrane coincided with concomitant reduction of cell respiration (*results not shown*). Since *P. humboldtii* is an obligatory aerobic yeast, reduced cell respiration causes necessarily a lower energy state of the cells which, in turn, decreases plasma membrane potential.

Both the direct measurement with microelectrodes and the steady-state distribution of TPP^+ indicated membrane potentials between -35 and -20 mV under de-energized conditions, in the presence of either CCCP or nystatin. This residual $\Delta\psi$ was

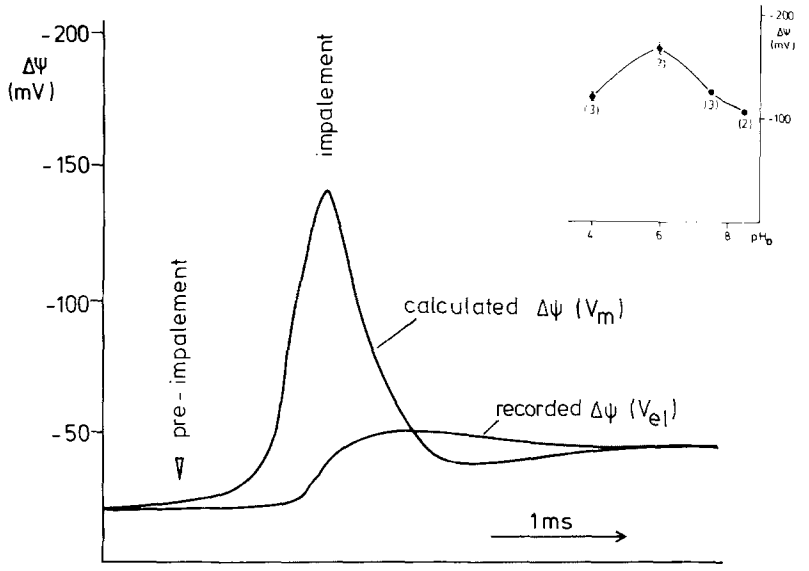

Fig. 7. Representative computerized outprint of a $\Delta\psi$ signal (V_{el}) during microelectrode impalement (pH_o = 5.2) and the corresponding course of $\Delta\psi$ (V_m) calculated according to the equation on page 259. *Inset:* Dependence of the calculated $\Delta\psi$ values ($V_{m,max}$) on extracellular H^+ concentration, pH_o. Experimental conditions as in Fig. 1. For details *see* Materials and Methods

independent of metabolic energy and reflected obviously Donnan diffusion potentials. It is worth noting that corresponding Donnan potentials seem to be absent in another yeast, *Rhodotorula glutinis* (Hauer & Höfer, 1978). On the other hand, Donnan diffusion potentials are commonly measured in plant cells (Higinbotham, 1973; Novacky, Karr & Sambeck, 1976). The peculiar feature of *R. glutinis* plasma membrane potential was the change of its polarity at pH_o 4.5. Increasing H^+ concentration of the cell suspensions depolarized gradually the plasma membrane so that at pH_o 4.5 $\Delta\psi$ was zero. At pH_o below 4.5 thiocyanide accumulation indicated positive plasma membrane potentials (Höfer & Künemund, 1984). Whether the manifestation of Donnan diffusion potentials in *P. humboldtii* correlates with the high cation exchange capacity of its cell wall, which is not existent in *R. glutinis* (Lammert et al., 1987), remains to be proved in further experiments.

Recently, Bakker and co-workers (1986) also measured electrical potential differences across the plasma membrane of the yeast *Endomyces magnusii* with microelectrodes. They reported very short $\Delta\psi$ pulses in the range of msec, immediately following microelectrode insertion, after which the cell became depolarized. According to their explanation, the fast decay of $\Delta\psi$ was due to a fatal cell injury by the penetrating microelectrode. The authors calculated $\Delta\psi$ from the decay curves monitored by an oscilloscope and presented values as high as −275 mV (at pH_o 7.5) as true preimpalement membrane potentials. TPP^+ distribution in *E. magnusii* determined by the same authors corresponded to much lower $\Delta\psi$, about −75 mV. The authors concluded that TPP^+ is not suitable as $\Delta\psi$ indicator.

Very short $\Delta\psi$ peaks superimposed on stable membrane potentials were also observed in *P. humboldtii* cells when voltage signals of the microelectrodes during impalement were registered with an oscilloscope. Computing $\Delta\psi$ (V_m) from V_{el}, R_{el}, C_{el} and $dV_{el}(t)/dt$ (*see* Results, p. 259), maximum values as high as −160 mV at pH_o 6.5 were obtained. However, these high $\Delta\psi$ values appear to represent transient hyperpolarizations of the plasma membrane since stable membrane potentials (about 70 mV less negative) were reproducibly observed afterwards. It seems impossible that in *P. humboldtii* microelectrodes injured cells reproducibly such that each time membranes were "partially depolarized" to the same degree. Moreover, $\Delta\psi$ of these "partially depolarized" membranes would have to agree coincidentally with values calculated from the steady-state TPP^+ distribution. On the other hand, since the initial peaks occurred only in energized cells they cannot reflect an artificial transient electronmotive force associated with microelectrode penetration. Rather, they may indicate a transient plasma membrane hyperpolarization due to the mechanical stress on the yeast cell during microelectrode impalement. Plasma membrane hyperpolarization in cells subjected to mechanical pressure, although to a much lower extent, were demonstrated in *Valonia utricularis* by Zimmermann and Steudle (1974) and in cell protoplasts of *Commelina communis* L. by Pantoja and Willmer (1986). Similar effects induced by osmotic pressure have been described for *R. glutinis* (Höfer & Künemund, 1984) and recently for broadbean mesocarp cells (Li

& Delroit, 1987). On the basis of our results we conclude that not the initial peaks but the stable $\Delta\psi$ recordings reflect true membrane potentials of intact yeast cells. The nature of the $\Delta\psi$ peak potentials still remains to be clarified.

The electrophysiological experiments were carried out in the Abteilung für Cytologie des Botanischen Instituts der Universität Bonn. The generous support of Drs. A. Sievers and H. Lühring is greatly appreciated. The authors are obliged to J. Ludwig for writing the computer program and for helpful discussions during our work. This work was supported by the Deutsche Forschungsgemeinschaft (grant no. Ho. 555).

References

Bakker, R., Borst-Pauwels, G.W.F.H., Dobbelmann, J. 1986. Membrane potential in the yeast *Endomyces magnusii* measured by microelectrodes and TPP^+ distribution. *Biochim. Biophys. Acta* **861**:205–209

Bentrup, F.W. 1981. Botanische Elektrophysiologie. *Naturwissenschaften* **72**:169–179

Bihler, I., Crane, R.K. 1962. Studies on the mechanism of intestinal absorption of sugars. V. The influence of several cations and anions on the active transport of sugars, in vitro, by various preparations of hamster small intestine. *Biochim. Biophys. Acta* **59**:78–93

Boxman, A.W., Barts, P.W.J.A., Borst-Pauwels, G.W.F.H. 1982. Some characteristics of tetraphenylphosphonium uptake into *Saccharomyces cerevisae*. *Biochim. Biophys. Acta* **686**:13–18

Eraso, P., Mazon, M.J., Gancedo, J.M. 1984. Pitfalls in the measurements of membrane potential in yeast cells using tetraphenylphosphonium. *Biochim. Biophys. Acta* **778**:516–526

Etherton, B., Keifer, D.W., Spanswick, R.M. 1977. Comparison of three methods for measuring electrical resistances in plant cell membrane. *Plant Physiol.* **60**:864–688

Felle, H., Porter, J.S., Slayman, C.L., Kaback, H.R. 1980. Quantitative measurements of membrane potential in *Escherichia coli*. *Biochemistry* **19**:3585–3590

Gimmler, H., Greenway, H. 1983. Tetraphenylphosphonium (TPP^+) is not suitable for the assessment of electrical potential in *Chlorella emersonii*. *Plant Cell Environ.* **6**:739–744

Hauer, R., Höfer, M. 1978. Evidence for interactions between the energy-dependent transport of sugars and the membrane potential in the yeast *Rhodotorula gracilis*. (*Rhodosporidium toruloides*). *J. Membrane Biol.* **43**:335–349

Hedenström, M. von, Höfer, M. 1979. The effect of nystatin on active transport in *Rhodotorula glutinis* (*gracilis*) is restricted to the plasma membrane. *Biochim. Biophys. Acta* **555**:169–174

Higinbotham, N. 1973. Electropotentials of plant cells. *Annu. Rev. Plant Physiol.* **24**:25–46

Höfer, M., Künemund, A. 1984. Tetraphenylphosphonium is a true indicator of negative plasma-membrane potential in the yeast *Rhodotorula glutinis*. *Biochem. J.* **225**:815–819

Höfer, M., Novacky, A. 1986. Measurements of plasma membrane potentials of yeast cells with microelectrodes. *Biochim. Biophys. Acta* **862**:372–378

Komor, E., Tanner, W. 1974. The hexose proton symport system of *Chlorella vulgaris*. Specificity, stoichiometry, energetics of sugar induced proton uptake. *Eur. J. Biochem.* **70**:197–204

Kovac, L., Varecka, L. 1981. Membrane potentials in respiring and respiration-deficient yeasts monitored by a fluorescent dye. *Biochim. Biophys. Acta* **637**:209–216

Lammert, T., Prasad, R., Höfer, M. 1987. Relevance of cation exchange capacity of cell wall in detecting H^+-symport in yeasts. *Biochem. Int.* **15**:753–759

Lassen, U.V., Rasmussen, B.E. 1978. Use of microelectrodes for measurement of membrane potentials. *In:* Membrane Transport in Biology: Concepts and Models. D.C. Tosteson, editor. pp. 169–203. Springer, Berlin

Li, Z.S., Delroit, S. 1987. Osmotic dependence of the transmembrane potential difference of broadbean mesocarp cells. *Plant Physiol.* **84**:895–899

Novacky, A., Karr, A.L., Sambeck, J.W., van 1976. Using electrophysiology to study plant disease development. *BioScience* **26**:499–504

Pantoja, O., Willmer, C.M. 1986. Pressure effects on membrane potentials of mesophyll protoplasts and epidermal cell protoplasts of *Commelina communis* L. *J. Exp. Bot.* **37**:315–320

Pena, A., Uribe, S., Pardo, J.P., Barbolia, M. 1984. The use of a cyanine dye in measuring membrane potential in yeast. *Arch. Biochem. Biophys.* **231**:217–225

Prasad, R., Höfer, M. 1986. Tetraphenylphosphonium is an indicator of negative membrane potential in *Candida albicans*. *Biochim. Biophys. Acta* **861**:377–380

Robinson, J.D., Flashner, M.S. 1979. The (Na^+ + K^+)-activated ATPase. Enzymatic and transport properties. *Biochim. Biophys. Acta* **549**:145–179

Rottenberg, H. 1978. The measurement of membrane potential and pH in cells, organelles and vesicles. *Methods Enzymol.* **55**:547–586

Schmidt, R.F. 1971. Neurophysiologie. Springer, Berlin, Heidelberg, New York

Seaston, A., Carr, G., Eddy, A. 1976. The concentration of glycine by preparations of the yeast *Saccharomyces carlsbergensis* depleted of adenosin triphosphate. *Biochem. J.* **154**:669–676

Slayman, C.L. 1965. Electrical properties of *Neurospora crassa:* Effects of external cations on the intracellular potential. *J. Gen. Physiol.* **49**:69–92

Slayman, C.L., Gradmann, D. 1975. Electrogenic proton transport in the plasma membrane of *Neurospora crassa*. *Biophys. J.* **15**:968–971

Slayman, C.L., Slayman, C.W. 1974. Depolarization of the plasmamembrane of *Neurospora crassa* during active transport of glucose: Evidence for a proton dependent cotransport system. *Proc. Natl. Acad. Sci. USA* **71**:1935–1939

Vacata, V., Kotyk, A., Sigler, K. 1981. Membrane potentials in yeast cells measured by direct and indirect methods. *Biochim. Biophys. Acta* **643**:265–268

West, I.C., Mitchell, P. 1973. Stoichiometry of lactose proton symport across the plasma membrane of *Escherichia coli*. *J. Bioenerg.* **3**:445–462

Wolk, U., Höfer, M. 1987. Interactions between cyanine dyes and yeast cells. Do cyanine dyes act as membrane potential sensitive probes? *Biochem. Int.* **14**:501–509

Zimmermann, U., Steudle, E. 1974. The pressure-dependence of the hydraulic conductivity, the membrane resistance and membrane potential during turgor pressure regulation in *Valonia utricularis*. *J. Membrane Biol.* **16**:331–352

Received 12 November 1987; revised 17 February 1988