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Membrane potential in the yeast *Endomyces magnusii* measured by microelectrodes and TPP^+ distribution

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Membrane potentials of the yeast *Endomyces magnusii* were determined with microelectrodes. 0.2 ms after penetration of the cell by the microelectrode the membrane potential (E_m) averaged -124 ± 3 mV (40 cells) at pH 4.5, while at pH 7.1 E_m was -146 ± 5 mV ($n = 24$). As the cell membrane may have been partly depolarized within 0.2 ms, the true (preimpalement) membrane potential was estimated from the time-course of the microelectrode potential resulting in -190 ± 13 mV ($n = 6$) at pH 4.5 and -275 ± 11 mV ($n = 6$) at pH 7.1. Comparison with the accumulation ratio of tetraphenylphosphonium ions showed that E_{TPP^+} cannot be used as a quantitative estimate of the yeast membrane potential.

Estimation of the cell membrane potential from the equilibrium distribution ratio of lipophilic cations between cells and medium has several drawbacks. Extra- and intracellular binding of the probe [1,2], accumulation in mitochondria [3] and/or extrusion from vacuoles [4] may lead to erroneous estimates of the distribution ratio between the cytoplasm and the medium. Moreover the distribution between cytoplasm and medium will not be exclusively determined by the membrane potential when the lipophilic cation is also transported via mechanisms other than simple diffusion [5,6].

Apart from these problems, the attainment of a steady state may take several hours, and may therefore not reflect the original state of the cell. Due to similar pitfalls, the use of fluorescent probes does in general not permit a quantitative evaluation of the membrane potential [7].

The only technique to measure the membrane potential directly is by means of microelectrodes. Derks, W.J.G., Dobbelmann, J. and Barts, P.W.J.A. (unpublished data) found that no stable potentials could be measured with microelectrodes in *Saccharomyces cerevisiae*. Vacata et al. [8] reported stable membrane potentials of -35 mV for the large yeast *E. magnusii*. However, no evidence was given that these potentials reflect the membrane potential of intact cells since the effects of adding protonophore, inhibiting metabolism, or changing medium pH were not tested.

In the present study we evaluated the measurement of the membrane potential with microelectrodes in *E. magnusii* in more detail, and compared estimates for E_m from microelectrodes with the determination of the equilibrium potential for TPP^+ (E_{TPP}).

Cells of *E. magnusii*, CBS 151.30 from Centraal Bureau voor Schimmelcultures at Baarn, were grown overnight in medium A (1% (w/v) Yeast Extract, 2% (w/v) Bacto-Peptone, 2% (w/v) glu-

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Abbreviation: TPP^+, tetraphenylphosphonium ion.

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cose, 0.2% (w/v) $MgCl_2 \cdot 6H_2O$, 3.46% (w/v) KH_2PO_4, 0.04% (w/v) K_2HPO_4, brought to pH 4.5 with HCl) on an orbital shaker at 15 rpm in 250 ml batch cultures at 30°C, harvested, washed three times with distilled water and resuspended to 3% in water. After a small amount of cells was introduced in the perfusion chamber (volume 0.2 ml) and allowed to settle on the bottom, perfusion (2 ml/min) was started. Experiments were begun after a preincubation period of at least 20 min.

Cells were held by suction on micropipettes (pulled from Clark GC120TF15 capillary tubing to tip diameters of 13 to 17 μm) and impaled by glass microelectrodes (pulled from GC120F15 capillaries to resistances of 25 to 75 MΩ when filled with 3 M KCl and measured in 0.3 M KCl). In some experiments the microelectrodes were filled with 0.3 M KCl or 0.5 M K_2SO_4. Tip potentials were less than 15 mV and changed less than 10 mV before and after impalement. Micromanipulation was carried out under microscopical control (Wild microscope, dark field, 120 ×) using two Leitz micromanipulators. The microelectrode was connected via a shielded 0.3 M KCl/Ag/AgCl half cell to a high impedance electrometer (FD223, WP Instruments). A 3 M KCl agar bridge connected to a 0.3 M KCl/Ag/AgCl half cell served as a reference. The bath was grounded via a platinum wire. The resistance and time constant of the microelectrode were measured by injecting a square wave current of 0.5 nA amplitude and 500 Hz frequency into the electrode. The time constant was minimized (without ringing or overshoot) by capacitance compensation via the shield of the microelectrode holder. All measured potentials were continuously displayed on a Tektronix storage oscilloscope and recorded on a W + W pen recorder. Fast transients were recorded (1000 samples/10 ms) with a Datalab DL901 transient recorder and plotted on a pen recorder.

The standard pH 4.5 solution contained 45 mM Tris titrated with succinate to pH 4.5, 0.1 mM KCl and glucose. In experiments in which the effect of inhibiting metabolism was investigated, a pH 4.5 solution was used in which glucose was replaced by sorbitol and 15 μM antimycin A and 15 mM deoxyglucose were added. The pH 7 solution contained 45 mM Tris-Hepes instead of Tris-succinate.

An experimental recording of a microelectrode penetration into a cell of *E. magnusii* is shown in Fig. 1. When slowly advanced towards the cell the microelectrode registered a potential of about −30 mV upon touching the cell (V_{pre}). Since V_{pre} depended on the ionic strength (and especially the concentration of divalent cations) and the pH of the medium, but not on the presence of uncoupler, while V_{pre} was absent in protoplasts, it appears that V_{pre} reflects the Donnan potential of the cell wall. The mean value of V_{pre} was −25 mV, see Table I. Upon penetrating the cell wall and membrane a fast negative deflection to a peak value (V_{peak}) of about −125 mV was recorded, after which the potential returned to a 'steady' (at least for several milliseconds) level (V_{st}) of about −50 mV. Apparently the leak introduced by the microelectrode discharged the cell membrane within milliseconds. Vst probably reflects a diffusion potential across the leak pathway, while the slow decline may be due to loss of the cell contents. Similar peak transients have been reported by Lassen for Amphiuma red blood cells [9] and by Ince et al. for macrophages [10].

Blatt and Slayman [12] reported that the membrane potential of spherical cells of *Neurospora crassa* depolarized due to efflux of Cl^- from the

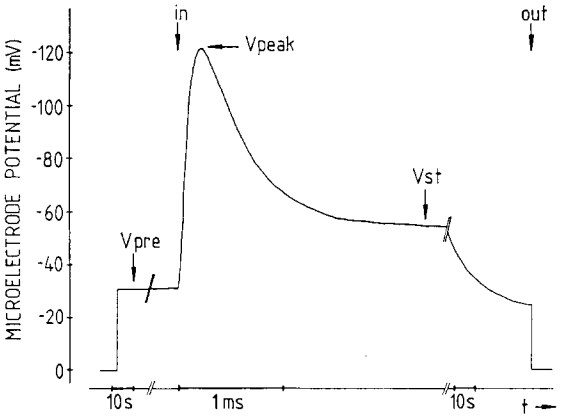

Fig. 1. Recording of the microelectrode potential during impalement of a cell of *E. magnusii*. Note different time scales. Mean values ± S.E. from 40 cells (mV): $V_{pre} = -24 \pm 3$, $V_{peak} = -124 \pm 3$, $V_{st} = -50 \pm 3$. V_{peak} was reached at 0.21 ± 0.02 ms after insertion of the microelectrode. The resistance of the microelectrodes used was 68 ± 5 MΩ, while the time constant was 0.14 ± 0.01 ms.

TABLE I

EFFECT OF ANTIMYCIN A (aA)+DEOXYGLUCOSE (dGlu), pH AND 2,4-DINITROPHENOL (DNP) ON RECORDED POTENTIALS FROM *E. MAGNUSII*

* Significantly different from control at $P < 0.01$. ** Significantly different from control at $P < 0.001$. Concentrations of antimycin A and deoxyglucose were 15 μM and 15 mM, respectively.

	V_{pre} (mV)	V_{peak} (mV)		V_{st} (mV)	n (cells)
Control, pH 4.5	-24 ± 1	-124 ± 3		-50 ± 3	40
aA + dGlu, pH 4.5	-24 ± 1	-60 ± 7	**	-50 ± 3	6
10 μM DNP, pH 4.5	-16 ± 2	-105 ± 7	*	-51 ± 2	7
30 μM DNP, pH 4.5	-21 ± 3	-81 ± 9	**	-48 ± 6	5
100 μM DNP, pH 4.5	-23 ± 3	-60 ± 4	**	-55 ± 3	10
Control, pH 7.1	-25 ± 2	-146 ± 5	**	-40 ± 2	24

cells, which Cl leaked together with K^+ from the microelectrodes. Although the KCl leak from the microelectrode must be negligible during the first 10 ms, it is conceivable that at a longer time scale KCl leak could prevent recovering the cells from the initial depolarisation. Therefore cells were impaled with microelectrodes filled with only 0.3 M KCl or 0.5 M K_2SO_4. However, peak transients similar to the one shown in Fig. 1 were found, and although V_{st} remained stable for several minutes at -50 to -70 mV, no secondary increase of the membrane potential (which would be expected when a seal between the electrode and the membrane developed) was observed. It should be noted that, using the same experimental setup and microelectrodes, impalements of *N. crassa* yielded stable potentials (without an initial peak transient) of about -200 mV (cf. Ref. 13).

Table I shows the effect of inhibiting metabolism, increasing pH, and addition of uncoupler on the measured potentials. As neither V_{pre} nor V_{st} are affected by either one of these treatments, while V_{peak} depolarized on adding 2,4-dinitrophenol or antimycin A + deoxyglucose and hyperpolarized due to an increase in pH, it may be concluded that not V_{st} but V_{peak} is a reflection of the 'true' membrane potential of *E. magnusii*.

Lassen and Rasmussen [9] already discussed that whenever the membrane potential is changing rapidly, a difference between the potential measured with the microelectrode (V_e) and the membrane potential (E_m) exists due to the finite time constant of the (though capacitance-compensated) electrode. When the microelectrode is considered as a simple RC circuit it can be derived that E_m is given by Eqn. 1 at any time.

$$E_m = V_e + R \cdot C \cdot \frac{dV_e}{dt} = V_e + R \cdot C \cdot V_e' \quad (1)$$

R and C are the resistance and capacity of the microelectrode, respectively. Since $V_e' = 0$ at the very moment of the peak (t_{peak}), it is clear that $E_m = V_{peak}$ at that instant. However, since E_m will be depolarized between $t = 0$ and t_{peak}, V_{peak} only provides a minimum estimate for the preimpalement membrane potential. In order to provide a better estimate for the preimpalement membrane potential, recordings of the time-course of V_e after impaling a cell were analyzed and both V_e and V_e' were determined at 20 μs intervals. With the time constant of the electrode ($\tau_e = R \cdot C$) E_m could be calculated. In this way the time-course of E_m could be derived from V_e. Results from six measurements at both pH 4.5 and pH 7.1 are shown in Fig. 2 and Fig. 3, respectively. The fact that calculated value of E_m reached a maximum only after 80 μs may be due to the time required for the microelectrode to penetrate the cell. Actually the calculated E_m represents the potential at

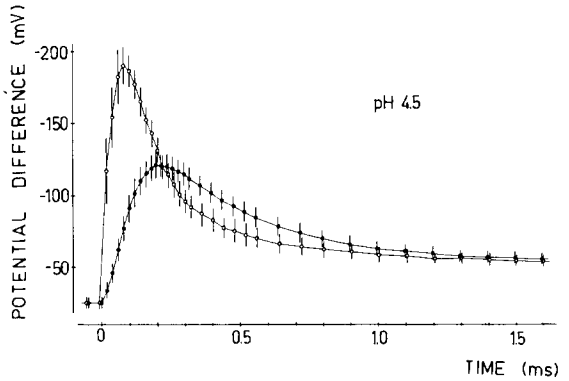

Fig. 2. Time-course of microelectrode potential (filled circles) and membrane potential (open circles) upon impaling *E. magnusii*. Membrane potential was derived from the electrode potential as indicated in the text. Mean $\pm$ S.E. from six cells at pH 4.5. $V_{pre} = -0.25\pm4$ mV, $V_{st} = -55\pm3$ mV, $\tau_e = 158\pm12$ μs, $V_{peak} = -121\pm8$ mV at 220 μs, $E_{m,peak} = -190\pm13$ mV at 80 μs.

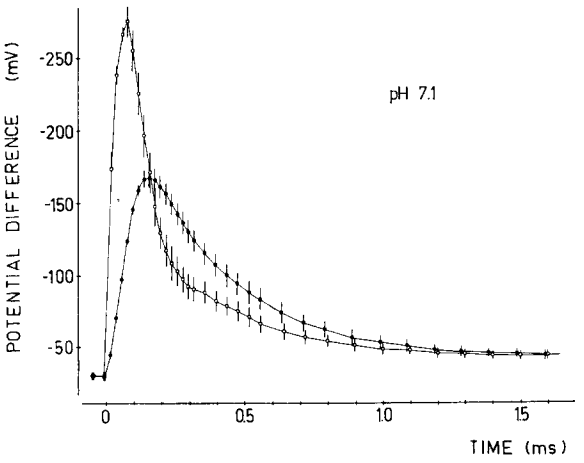

Fig. 3. Time-course of microelectrode potential (filled circles) and membrane potential (open circles) upon impaling *E. magnusii*. Mean ± S.E. from six cells at pH 7.1. $V_{pre} = -29 \pm 3$ mV, $V_{st} = -43 \pm 3$ mV, $\tau_e = 128 \pm 7$ μs, $V_{peak} = -168 \pm 7$ mV at 160 μs, $E_{m,peak} = -275$ at 80 μs.

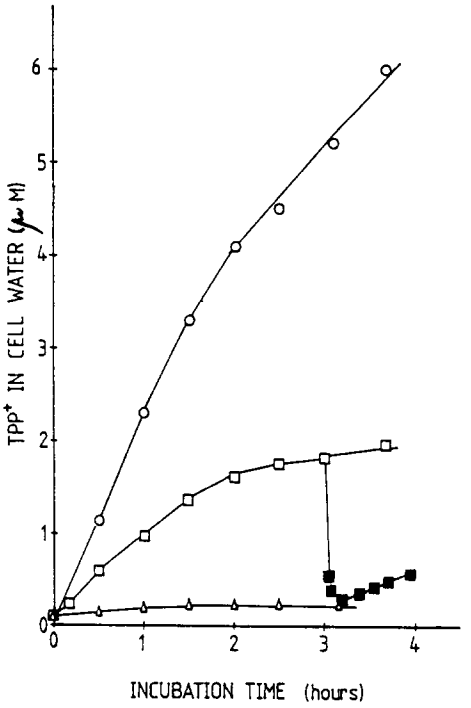

Fig. 4. TPP^+ uptake by cells of *E. magnusii*. ○, pH 7.0; □, pH 4.5; ■, pH 4.5, metabolizing cells 0.1 mM DNP (2,4-dinitrophenol) added at $t = 3$ h; △, pH 4.5, non-metabolizing cells. Initial concentration of TPP^+ in the medium was 0.18 μM. Non-metabolizing cells were obtained by aerating the yeast cells for one night in order to exhaust the cells for internal substrate. Then 15 μM antimycin and 15 mM deoxyglucose were added 10 min before addition of TPP^+.

the tip of the electrode and will only be equal to the membrane potential after the electrode had crossed the cell membrane. The thus obtained values for the membrane potential of *E. magnusii* (-190 ± 13 mV at pH 4.5 and -275 ± 11 mV at pH 7.1) compare well with data reported for *N. crassa* [13].

We studied the uptake of TPP^+ into cells of *E. magnusii* to compare the estimates for the membrane potential from microelectrodes with the TPP^+ equilibrium potential. As is shown in Fig. 4, addition of uncoupler after 3 h led to a rapid loss of TPP^+, while cells incubated in the presence of antimycin A + deoxyglucose showed no considerable accumulation of TPP^+. These observation, together with the increased accumulation of TPP^+ at higher pH, agree well with the effects on V_{peak} (see Table I). As even after 4 h no steady-state distribution of TPP^+ was reached, the TPP^+ distribution ratio was estimated assuming uptake could be described by a single exponential. The calculated E_{TPP} was -65 mV at pH 4.5 and -125 mV at pH 7. Comparing these values with the estimates for E_m (-190 mV at pH 4.5 and -275 mV at pH 7.1) shows that E_{TPP} cannot be used as a quantitative estimate for the membrane potential of *E. magnusii*. As the value for E_{TPP} were not corrected for binding of the probe [1,2], the discrepancy between the real values for E_{TPP} and the membrane potential is probably even larger. The low values for E_{TPP} might be explained by an extrusion mechanism for lipophilic cations as reported by Ritchie [4] and Midgley et al. [6].

Recently Höfer and Novacky [14] reported on measurements of the membrane potentials with microelectrodes in the yeast *Pichia humboldtii*. They showed that stable membrane potentials could be obtained, which were still sensitive to uncouplers. These potentials, however, were much lower than those found by us.

In conclusion: from the effects of pH, uncoupler, and inhibition of metabolism it appears that our microelectrode measurements are a reflection

of the true membrane potential of *E. magnusii*. After correction for the time constant of the microelectrode, values for the preimpalement membrane potential are obtained which are in the range of the membrane potentials reported for *N. crassa* [13]. The disagreement with the values reported by Vacata et al. [8] is probably due to the fact that in that study peak transients were discarded as artefacts. Further technical improvements, especially reduction of the microelectrode capacitance, might provide a means of measuring the membrane potential of even smaller cells such as *S. cerevisiae* in a direct and quantitative way.

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