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MEMBRANE POTENTIAL AND CATION PERMEABILITY

A STUDY WITH A NYSTATIN-RESISTANT MUTANT OF *RHODOTORULA GRACILIS* (*RHODOSPORIDIUM TORULOIDES*)

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Cells of a nystatin-resistant mutant of the obligatory aerobic yeast *Rhodotorula gracilis* displayed an electrical potential difference, $\Delta\psi$, across the plasma membrane which was, in contrast to the wild-strain cells, virtually independent of the pH of cell suspensions down to 4.5. In addition, the $\Delta\psi$ in mutant cells was insensitive to extracellular K^+ concentrations. The mutant cells failed to cotransport measurable amounts of H^+ by the onset of monosaccharide transport and to take up K^+ in exchange for H^+. Taking into account the lower passive permeability of the mutant plasma membrane for cations, it has been concluded that the pH dependency of $\Delta\psi$ in wild-strain cells is correlated with the electrogenic leak of H^+ back into the cells in course of increasing ΔpH across the plasma membrane.

Introduction

Electrical potential differences across eukaryotic plasma membranes (inside negative) exhibit a distinct pH dependence; becoming smaller as the pH difference across the plasma membrane (inside alkaline) increases [1–5]. The depolarizing effect of increasing external H^+ concentration has not been clear. Hauer and Höfer [5] have suggested, in analogy to bacteria [6], that protons act as permeable cations, thus depolarizing the membrane potential in *Rhodotorula gracilis*. However, such an explanation has been questioned by Miller and Budd [3].

The polyene antibiotic nystatin, which is known to increase unspecifically the ion permeability of sterol-containing membranes [7], effects equilibration of both the pH difference [8] and the electrical potential difference [5] across the plasma membrane of *R. gracilis*. These gradients were found to be insensitive to polyene antibiotics in a nystatin-resistant mutant of this yeast, M 67 [9]. The mutant cells transported monosaccharides actively by the same mechanism as the wild strain [10], i.e., by an H^+ symport [11]. Recently, it has been shown that the plasma membrane of the mutant cells possesses distinctly lower passive permeability for cations as compared with the wild-strain cells [12].

The experimental results presented in this report demonstrate that the membrane potential across the plasma membrane of the mutant cells is virtually independent of the external pH and K^+ concentration. The mutant cells did not show measurable cotransport of H^+ by the onset of monosaccharide transport and did not exchange external K^+ for protons. The results are discussed

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Abbreviation: TTP^+, tetraphenylphosphonium ion.

against the background of plasma membrane permeability for cations.

Material and Methods

The nystatin-resistant mutant, M 67, and the wild strain of the obligatory aerobic yeast *Rhodotorula gracilis (Rhodosporidium toruloides*, mating type a, ATCC 26 194, and CBS 6681) were cultivated as described by Höfer et al. [9] and by Kotyk and Höfer [13]. The growth medium for mutant cells contained 25 μM nystatin (dissolved in 1,3-propanediol), After 24 h (wild strain) or 40 h (M 67) growth at 28°C, the cells were harvested, washed with distilled water and aerated at least 6 h before experiments.

The following transport parameters were measured as described previously: the H^+-K^+ exchange [14], the H^+ cotransport by the onset of monosaccharide transport [10] and the membrane potential [5].

Tritiated tetraphenylphosphonium [^{3}H]TPP^+, 4.33 Ci/mol) was a custom synthesis from Hoechst AG, Frankfurt, F.R.G. All other chemicals were analytical grade purity obtained commercially.

Results

It is a characteristic property of most yeasts that their unbuffered cell suspensions actively adjust the external pH to a value between 4–4.5 (Ref. 14, for a comparative study see Ref. 15). If the steady-state H^+-concentration is shifted, for example, by adding alkali hydroxide, the cells start extruding H^+ until the original pH is attained [14]. The extrusion of H^+ is electrically neutralized by a stoicheiometric (1 : 1) uptake of alkali ions [16]. Such a H^+-K^+ exchange is shown in Fig. 1. Whereas the pH shift of about 1 unit was compensated within 3 min after addition of KOH to wild-strain cells, the mutant cells were incapable of this compensation. Obviously the energy-dependent H^+-K^+ exchange system of the wild-strain cells (cf. Ref. 16) is not operative in the mutant cells. Whether this is due to the missing ergosterol in the vicinity of the exchange system or to an additional mutation of the exchange system or simply to the diminished permeability for one of the two cations (cf. Ref. 12) cannot be decided on

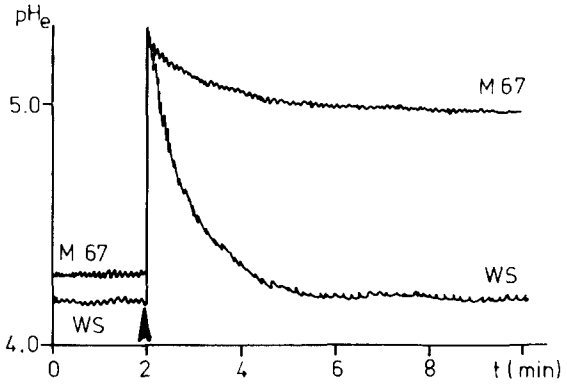

Fig. 1. Proton extrusion in unbuffered cell suspensions of the wild strain (WS) and of the mutant (M 67) after addition of potassium hydroxide. 5% (wet wt./volume) aqueous suspensions of 24 h aerated yeast cells were incubated with 1 mM $CaCl_2$ (to increase the ionic strength, cf. Ref. 10) at 32°C. At the arrow, KOH was added to give a concentration of 100 μM.

the basis of the present results. In the above experiments, only the extracellular pH was recorded. However, since in the wild strain the uptake of K^+ was stoicheiometrically coupled to an efflux of H^+ [16,17], as were the passive fluxes in the mutant [12], the lack of a pH change is indicative also of a missing K^+ movement.

Similar inability of the mutant cells to effect any measurable H^+ flow through the plasma mem-

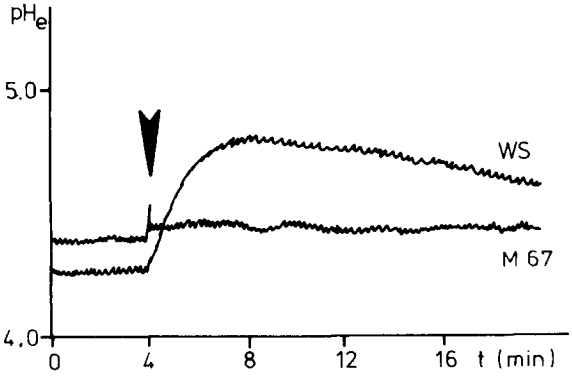

Fig. 2. H^+ cotransport by the onset of monsaccharide uptake in unbuffered cell suspensions of the wild strain (WS) and of the mutant (M 67). Experimental conditions were as in Fig. 1, except that the cells were aerated only for 8 h and finally suspended as a 2.5% (wet wt./vol.) aqueous suspension. At the arrow, D-xylose was added to give a concentration of 10 mM.

brane, i.e., a flow of protons compensated by an opposite flow of K^+, showed in experiments on H^+/monosaccharide symport (Fig. 2). In wild-strain cells the onset of sugar transport is accompanied by a distinct (transient) alkalinization of unbuffered cell suspensions due to H^+ cotransport [10], which is electrically compensated by stoicheiometric efflux of K^+ [17]. Again, no proton uptake by the onset of D-xylose transport could be observed in suspensions of the mutant cells, although monosaccharides are accumulated in them by an H^+ symport [9,11].

An electrical potential difference (inside negative) across the plasma membrane of *R. gracilis* cells can be demonstrated by intracellular accumulation of lipid-soluble cations such as tetraphenylphosphonium (TPP^+) [5]. The membrane potential

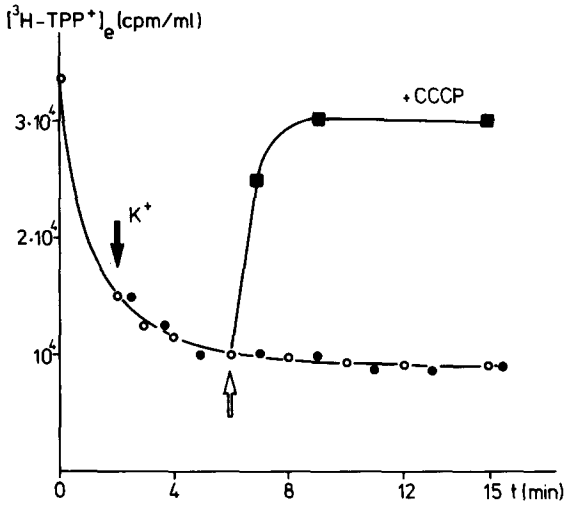

Fig. 3. Lack of effect of K^+ on the intracellular accumulation of tetraphenylphosphonium ions (TPP^+) as indicator of the plasma-membrane potential in the mutant cells. 4% (wet wt./v) cell suspension in Tris-citrate buffer (pH 5.0) was incubated at 32°C. At $t = 0$, 20 μM [^{3}H]TPP^+ chloride (0.09 μCi/ml, open circles) and to a portion of the cell suspension, at the full arrow, 100 mM KOH (closed circles) were added. To another portion of the cell suspension, 10 μM carbonylcyanide *m*-chlorophenylhydrazone (CCCP) was added at the open arrow in order to demonstrate the membrane-potential-dependent accumulation of TPP^+ (close squares). Samples were taken at intervals and centrifuged, and the radioactivity of the supernatant was measured (cf. Ref. 5). The intracellular TPP^+ accumulation was calculated from the decrease of its extracellular concentration, using the value of 2.0 μl intracellular water volume per mg yeast dry wt. (cf. Ref. 10).

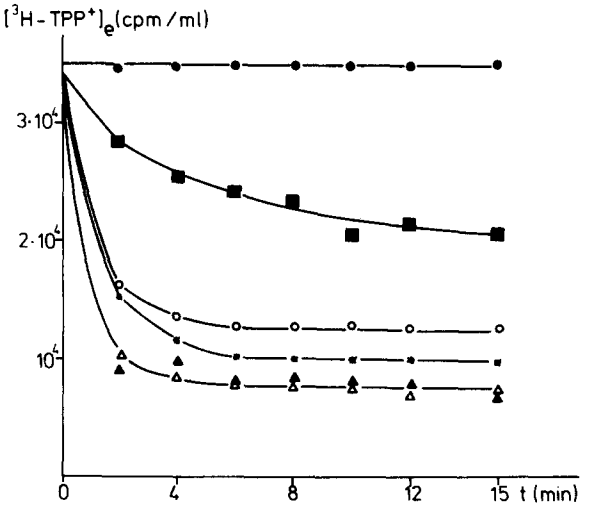

Fig. 4. Uptake of tetraphenylphosphonium ions (TPP^+) in suspensions of mutant cells with increasing external H^+ concentrations. Experimental conditions were as in Fig. 3, except that the cells were suspended in 150 mM potassium phosphate buffer of given pH; ●, 2.5; ■, 3.5; ○, 4.5; ★, 5.5; △, 6.5; ▲, 7.5.

in wild-strain cells was depolarized by a high extracellular K^+ concentration as visualized by a rapid outflow of TPP^+ from preloaded cells [5]. In

TABLE I

CALCULATED PLASMA MEMBRANE POTENTIALS ($\Delta\psi$) OF THE MUTANT CELLS AS COMPARED WITH THE WILD-STRAIN CELLS

The values were calculated from the data of Fig. 4 according to the Nernst equation

$$\Delta\psi = \frac{2.3\,RT}{F} \log \frac{[TPP^+]_e}{[TPP^+]_i}$$

$[TPP^+]_i$ was calculated from the decrease of $[TPP^+]_e$ according to Fig. 3; pH_e = extracellular pH. n.m., not measured; n.a., no accumulation of TPP^+.

pH_e	$\Delta\psi$ (mV)	
	M 67	wild strain [a]
8.0	n.m.	−155
7.5	−156	−111
6.5	−156	−95
5.5	−146	−70
4.5	−135	> −10
3.5	−110	n.a.
2.5	n.a.	n.a.

[a] Values from Hauer et al. [16].

contrast, the plasma membrane potential of the mutant cells proved to be independent of any enhancement of K^+ concentration outside, yet sensitive to depolarization by uncouplers (Fig. 3).

On the basis of these experiments, and taking into account data on the passive permeability [12], it has been concluded that the plasma membrane permeability for both H^+ and K^+ is in general considerably lower in the mutant cells as compared with the wild strain. Consequently, if the plasma membrane potential is short-circuited by a diffusion potential of H^+ at low external pH values in the wild strain of *R. gracilis*, it should be much less sensitive to external pH in the mutant cells. Indeed, Fig. 4 demonstrates that the accumulation of TPP^+ in the mutant cells is virtually independent of the pH down to a value of 4.5. The calculated values of the membrane potentials both in mutant cells and in wild-strain cells are summarized in Table I.

Discussion

It is difficult to determine actual ion permeabilities in microorganisms. Passive cation permeabilities have been measured in the obligatory aerobic yeast *R. gracilis* after introducing anaerobic conditions and in the presence of uncouplers [12]. The results presented in this report extend the data by measurements in energized cells. It is clear from the results that the pH dependency of the plasma membrane potential is correlated with the plasma membrane permeability for H^+ and K^+.

Whereas the membrane potential was short-circuited by a diffusion potential of both H^+ (at pH values below 5) and K^+ (in the presence of 100 mM K^+) in the wild-strain cells (cf. Ref. 5), it was independent of external pH and K^+ concentration in the mutant cells.

The lack of a measurable H^+ cotransport by the onset of D-xylose transport cannot be taken as indicative of the missing H^+ symport in the mutant cells (Fig. 2). It is, rather, the consequence of the missing compensating efflux of K^+ from mutant cells [12]. H^+/D-xylose symport was evidenced by measurements of other parameters, e.g., sugar-transport-dependent depolarization of the membrane potential [11]. Thus, the mass H^+/D-xylose cotransport can be electrically compensated only by electrogenic H^+ efflux through the plasma membrane ATPase (cf. Ref. 17); consequently, no net H^+ uptake could be observed. This also explains the lower rate of D-xylose uptake in mutant cells, since the compensation by the ATPase is obviously slower than that by rapid K^+ efflux.

The resistance to nystatin in *R. gracilis* correlates well with the ergosterol content of the cells. The phenotype nystatin-resistance is the physiological expression of the defected pathway of ergosterol synthesis in the mutant [9]. Hence, the conclusion is justified that ergosterol plays an important role in the cation (H^+ and K^+) permeability of the plasma membrane of *R. gracilis*.

Acknowledgement

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