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The Electrochemical H^+ Gradient in the Yeast *Rhodotorula glutinis*

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Abstract

The electrochemical gradient of protons, $\Delta\tilde{\mu}_{H+}$, was estimated in the obligatory aerobic yeast *Rhodotorula glutinis* in the pH_0 range from 3 to 8.5. The membrane potential, $\Delta\Psi$, was measured by steady-state distribution of the hydrophobic ions, tetraphenylphosphonium (TPP^+) for negative $\Delta\Psi$ above pH_0 4.5, and thiocyanate (SCN^-) for positive $\Delta\Psi$ below pH_0 4.5. The chemical gradient of H^+ was determined by measuring the chemical shift of intracellular P_i by ^{31}P-NMR at given pH_0 values. The values of pH_i increased almost linearly from 7.3 at pH_0 3 to 7.8 at pH_0 8.5. In the physiological pH_0 range from 3.5 to 6, $\Delta\tilde{\mu}_{H+}$ was fairly constant at values between 17–18 KJ mol^{-1}, gradually decreasing at pH_0 above 6. In deenergized cells, the intracellular pH_i decreased to values as low as 6, regardless of whether the cell suspension was buffered at pH_0 4.5 or 7.5. There was no membrane potential detectable in deenergized cells.

Key Words: Electrochemical H^+-gradient; intracellular pH_i; membrane potential; nuclear magnetic resonance; electrogenic transport; transport in yeast, *Rhodotorula glutinis*.

Introduction

Knowledge of the size of the driving force for secondary active transport is essential for evaluation of the energetics of the transport process (see, e.g., Harold, 1976; Kotyk, 1983). Secondary active transport driven by the electrochemical H^+ gradient, $\Delta\tilde{\mu}_{H+}$, has been reviewed recently (Poole, 1978; West, 1980; Eddy, 1982). In *Rhodotorula glutinis*, there is one $\Delta\tilde{\mu}_{H+}$-driven transport system common for electroneutral carbohydrates (Klöppel and

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Höfer, 1976) and their positively charged derivatives, amino sugars (Niemietz *et al.*, 1981), as well as the negatively charged glucuronate (Niemietz and Höfer, 1984). The membrane potential, inside negative, and its dependence on external pH in *Rh. glutinis* has been estimated by means of the hydrophobic cation tetraphenylphosphonium by Hauer and Höfer (1978), Hauer *et al.* (1981), and Höfer *et al.* (1983a), but determination of the intracellular pH for calculation of the actual $\Delta\tilde{\mu}_{H^+}$ across the plasmalemma has been less satisfactory (Höfer and Misra, 1978; Hauer *et al.*, 1981). Reliable values of the intracellular pH can be obtained using ^{31}P-NMR spectra of the intracellular inorganic phosphate. A variety of bioenergetic and metabolism issues in intact cells and tissues have been investigated using this technique (see, e.g., Ugurbil *et al.*, 1979; Shulman *et al.*, 1979; Nicolay *et al.*, 1983a, b). One advantage of pH_i[4] determination by means of ^{31}P-NMR is the ability to measure pH_i in strongly buffered cell suspension. The present communication deals with ΔpH measurements in both energized and deenergized *Rh. glutinis* cells. Using ^{31}P-NMR, the data were used to calculate $\Delta\tilde{\mu}_{H^+}$ in cells under various physiological conditions over a broad range of pH_0. A preliminary report of this work has appeared (Höfer *et al.*, 1983b).

Materials and Methods

The obligatorily aerobic yeast *Rhodotorula glutinis* (*Rhodosporidium toruloides*, ATCC 26194 = CBS 6681) was grown at 28°C and harvested after 24 h, as described previously (Heller and Höfer, 1975).

^{31}P-NMR spectra were collected at 145.8 MHz on a Bruker HX-360 at 20°C. A 3.3-ml portion of cell suspension (0.5 g wet weight per milliter) was put in a 10-mm NMR tube. D_2O was added to a final concentration of 10% and 20 μl of antifoam was added. Aerobic samples were oxygenated continuously during the data collection process. The intracellular pH was determined from the chemical shift position of the intracellular inorganic phosphate resonance, as described by den Hollander *et al.* (1981). The chemical shift position of added glycerophosphorylcholine was used as a reference. For measurements in the anaerobic state, yeast cells were not oxygenated, but N_2 was bubbled through the suspension instead and the NMR tube was sealed.

The extracellular pH was determined with a combined glass electrode connected to a Radiometer PHM 26 pH-meter (Copenhagen, Denmark). When cell suspensions were buffered, 125 mM Tris citrate was used. The pH_0 was adjusted to the desired value by small additions of either solid citric acid or Tris base.

[4]Abbreviations: pH_i, internal pH; pH_0, external pH; pmf, protonmotive force.

Measurements of membrane potential, positive inside, was carried out by means of the steady-state distribution of the hydrophobic anion thiocyanate ($S^{14}CN^-$). The uptake of $S^{14}CN^-$ into the cells was determined by membrane filtration (Heller and Höfer, 1975). The filters with cells were dissolved directly in 10 ml of Packard Emulsifier Scintillator 299®, and the ratioactivity was counted in a Packard scintillation Spectrometer B 400. All experiments were carried out with 8 mg dry weight per milliliter cell suspensions in 20 mM Tris citrate buffer at 25°C. The membrane potential, $\Delta\Psi$, was calculated by means of the Nernst equation for diffusion potentials. The free enthalpy of the electrochemical proton gradient, $\Delta\tilde{\mu}_{H+}$, was calculated as

$$\Delta\tilde{\mu}_{H+} = (\Delta\Psi - 0.059\ \Delta pH)F \qquad (25°C)$$

where $\Delta\Psi$ is the membrane potential and F the Faraday constant.

^{14}C-Thiocyanate (potassium salt, 2.1 TBq mol^{-1}) was from Amersham Buchler, Braunschweig, Germany. All other reagents were from Merck, Darmstadt, Germany.

Results

The ^{31}P-NMR spectra collected in cell suspension buffered over a broad range of pH_0 values demonstrated that the intracellular pH was maintained fairly constant (Fig. 1). The pH_i changed almost linearly from a value of 7.8 at $pH_0 = 9$ to a value of 7.2 at $pH_0 = 3$. At pH 7.75 the extracellular pH equalled the intracellular pH; $\Delta pH = 0$.

The electrical component of $\Delta\tilde{\mu}_{H+}$, the membrane potential, $\Delta\Psi$, displays a distinctly different pH_0 dependence. Due to increasing back-diffusion of cations, the membrane potential becomes depolarized with decreasing pH_0 so that at pH_0 4.5 no negative $\Delta\Psi$ was detectable (Hauer *et al.*, 1981). Accumulation of the hydrophobic anion thiocyanate, SCN^-, at pH_0 values below 4.5 (and not above) demonstrated a change of polarization of $\Delta\Psi$ at pH_0 4.5 (Table I).

Using the pH_i and $\Delta\Psi$ data, we calculated the free enthalpy of the overall electrochemical gradient of H^+ across the plasmalemma of *Rh. glutinis* (Table 1). In the table, the negative $\Delta\Psi$ values for cell suspension at $pH_0 \geqslant 4.5$ were taken from Hauer *et al.* (1981). The data demonstrate that the $\Delta\tilde{\mu}_{H+}$ in *Rh. glutinis* is maintained fairly constant over the range of physiological pH_0 values between 3.5 and 6. At pH_0 above 6, $\Delta\tilde{\mu}_{H+}$ decreases gradually.

The response of pH_i upon deenergizing the cells either by introducing anaerobic conditions or by adding uncouplers is summarized in Table II. In deenergized cells the intracellular pH fell rapidly to values around 6.2 regardless of whether the cell suspension was buffered or not. If unbuffered cell

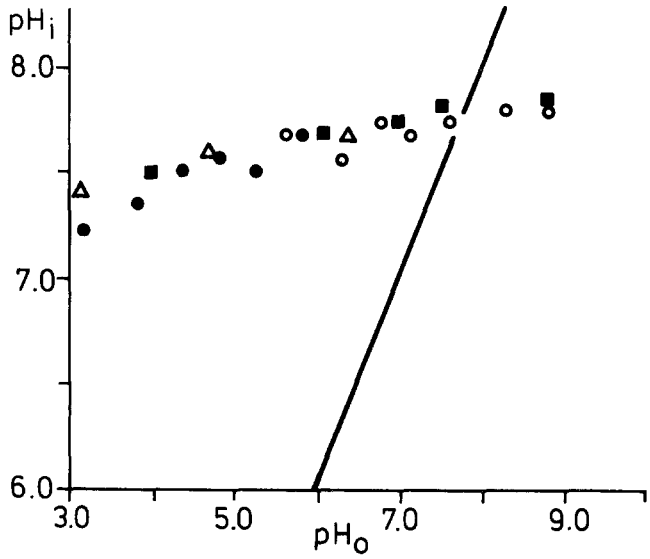

Fig. 1. Intracellular pH of *Rh. glutinis* cells suspended in 125 mM Tris citrate buffer of given pH values. The different symbols correspond to measurements with different batches grown independently. The straight line indicates $pH_i = pH_0$.

suspensions the pH_i was equilibrated with pH_0. However, even in suspensions buffered at pH_0 4.5 pH_i never decreased below 6. This demonstrates a very high buffering capacity of the cytosol, around pH_i of 6.2. The pH_i value decreased to below 7 also in deenergized cells buffered at pH_0 7.5.

Table I. Dependence of Free Enthalpy of the Electrochemical Gradient of H^+, $\Delta\tilde{\mu}_{H+}$, across the Plasmalemma of *Rh. glutinis* on the External pH[a]

pH_0	ΔpH (units)	ΔΨ (mV)	$\Delta\tilde{\mu}_{H+}$ (kJ mol^{-1})	pmf[b] (mV)
3	4.3	+ 42.5	−20.4	−211
3.5	3.9	+ 34	−18.9	−196
4	3.4	+ 15	−17.8	−185
4.5	3.0	− 5[c]	−17.7	−182
5.5	2.0	− 65	−17.7	−183
6	1.6	− 89	−16.9	−175
6.5	1.1	− 95	−15.5	−160
7	0.7	− 89	−12.5	−130
7.5	0.2	−105	−11.3	−117
8.5	−0.7	−155	−10.9	−113

[a]For experimental conditions and calculation of $\Delta\tilde{\mu}_{H+}$ see Materials and Methods.
[b]pmf = protonmotive force ($\Delta\Psi - 0.059\ \Delta pH$, volts, at 25°C).
[c]The values of negative ΔΨ were taken from Hauer *et al.* (1981).

Table II. Intracellular pH of *Rh. glutinis* Cells under Various Physiological Conditions[a]

Experimental conditions[b]	pH_0 before–after	pH_i before–(4-min value)–after
Air ⟶ N_2 (unbuffered)	5.7 ⟶ 6.0	7.8 ⟶ 6.1
(Tris citrate)	4.5	7.8 — (6.4) → 6.2 (5.85, after 120 min)
(Tris citrate) + 100 μM CCCP	7.5	7.75 — (7.0) → 6.7
(Tris citrate)	4.5	7.75 ⟶ 6.35
(Tris citrate) + 25 μM SF6847	7.46 ⟶ 7.34	7.75 ⟶ 6.88
(Tris citrate)	4.5	7.75 ⟶ 6.38

[a]The cells were suspended at 0.5 g fresh weight per milliliter either in distilled water (unbuffered) or in 125 mM Tris citrate buffer of given pH.
[b]Treatment period: 10 min for uncoupler, 20 min anaerobiosis.

The ^{31}P-NMR spectra collected under different physiological conditions are shown in Fig. 2. It is striking that, in deenergized cells, the P_i peak increased whereas the peak of polyphosphates decreased. This may be indicative of a breakdown of polyphosphates to orthophosphates under deenergized conditions. Consequently, the enhanced concentration of inorganic phosphate within the cells may be responsible for the high buffering capacity of the cytosol around pH_i 6.2.

Discussion

Determination of the intracellular pH in *Rh. glutinis* by means of the ^{31}P-NMR spectra of intracellular inorganic phosphate led to somewhat higher pH_i values than estimated earlier either on the basis of weak acid distribution data (Höfer and Misra, 1978) or on the basis of nystatin effect on weakly buffered cell suspensions (Hauer *et al.*, 1981). Consequently, the calculated free energy of the electrochemical H^+ gradient increased, becoming sufficient to energize a 1000-fold accumulation of a transport substrate ($G^0 = +17.16\,kJ\,mol^{-1}$) as observed for D-Xylose by Kotyk and Höfer (1965). Intracellular pH around 7.5 fits also better with the pH optima of the most cytoplasmic enzymes. Finally, such a pH_i is in favor of a higher efficiency of the plasma membrane-bound carrier(s). The monosaccharide carrier, having a very broad specificity for most carbohydrates and their derivatives, displays a pK value of about 6.5 (Höfer and Misra, 1978; this was confirmed recently by measurements in deenergized cells, unpublished results). At pH_i of 7.5 about 90% of the carrier population take part in transport catalysis.

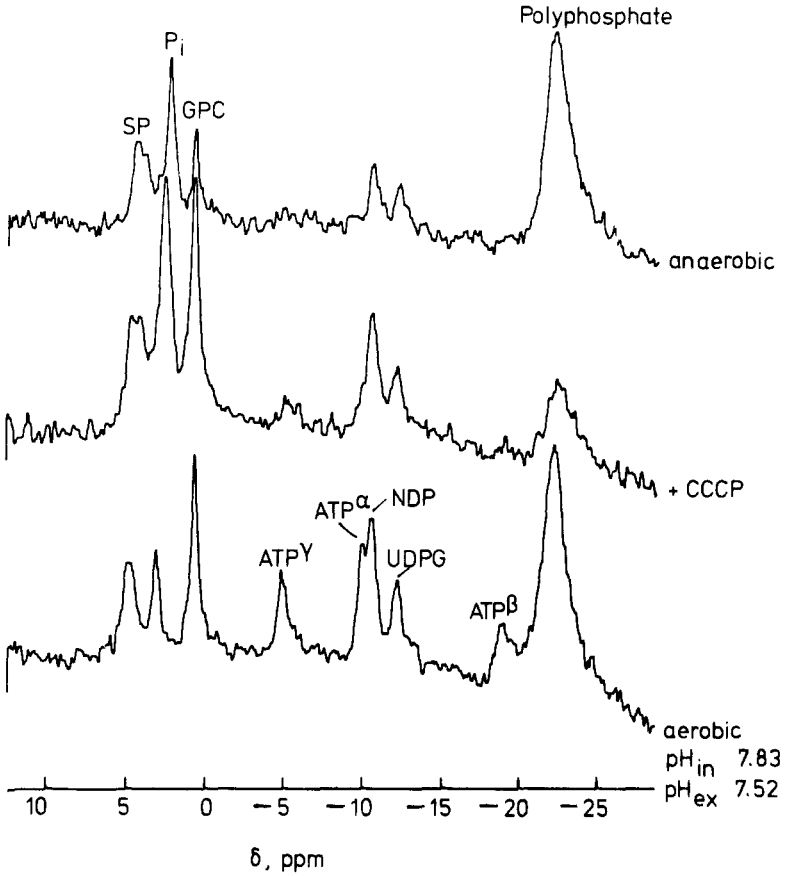

Fig. 2. ^{31}P-NMR spectra of *Rh. glutinis* cells under different physiological conditions (aerobic, anaerobic, and in the presence of 100 μM carbonylcyanide-*m*-chlorophenylhydrazone, CCCP). pH_{in} = intracellular pH; pH_{ex} = extracellular pH; SP = sugar phosphate; GPC = glycerophosphorylcholine (0.49 ppm, internal chemical shift reference); NDP = nicotinamide-dinucleotides; UDPG = uridine-diphosphoglucose.

The data of Table I demonstrate a fair constancy of the electrochemical H^+ gradient in *Rh. glutinis* in the range of physiological pH_0 values. In this respect *Rh. glutinis* behaves similarly to *Kluyveromyces fragilis*, in which the protonmotive force was considerably lower, around 100 mV (van den Broek *et al.*, 1982). A monotonous decrease of $\Delta\tilde{\mu}_{H^+}$ was reported for *Saccharomyces cerevisiae* by de la Peña *et al.* (1982) and for the maize scutellum by Humphreys (1983). Although the protonmotive force, pmf, in yeast amounted to 100–200 mV, pmf in maize scutellum exceeded the value

of 300 mV. The capability of maintaining $\Delta\tilde{\mu}_{H^+}$ constant over a certain pH_0 range appears to be a property specific to cells of an individual organism.

The intracellular pH decreased in deenergized cells, but never below the value of 6. Hence, in cell suspensions buffered at pH_0 4.5, a pH gradient of 1.5 pH units remained. Under these conditions there is another, opposite, 100-fold gradient of K^+ across the plasmalemma of *Rh. glutinis* (Künemund and Höfer, 1983). At present, we have no explanation as to what forces are responsible for maintaining the two oppositely directed ion gradients. Under deenergized conditions, no electrical potential difference across the plasmalemma, negative (Hauer and Höfer, 1978) or positive (unpublished results with thiocyanate anion), could be measured.

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