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INTRACELLULAR pH DISTRIBUTION AND TRANSMEMBRANE pH PROFILE OF YEAST CELLS

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The pH-dependent fluorescence excitation of fluorescein located intracellularly and in the vicinity of cells of the yeast *Saccharomyces cerevisiae* and *Endomyces magnusii* was used to obtain local pH values at a linear resolution 0.2 μm. Cells suspended in water or in a diluted (5 mM) acidic buffer had a relatively alkaline interior (about 7.0–7.5) with pH decreasing gradually toward the periphery and further out through the cell wall to the value of the bulk solution. In slightly alkaline weak buffers the cells also showed an alkaline center and a slightly acidic ring-shaped area, but the peripheral region close to the membrane was again alkaline with pH increasing toward the bulk solution. The heterogeneity of intracellular pH was reduced or nearly abolished in starved or antimycin-treated cell. Suspension of cells in strong (200 mM) buffer resulted within 15–20 min in a nearly homogeneous pH pattern throughout the cell, attaining pH values of 5.5–7.5, depending on the pH of the buffer. Addition of glucose with concomitant pH decrease of the extracellular medium did not change appreciably the intracellular pattern for 20–30 min, except with diethylstilbestrol (inhibitor of proton-extruding ATPase) when the cell became more acidic. It appears that the ΔpH measurements between the cell as a whole and the bulk solution (as are used for the calculation of the electrochemical potential of protons in proton-driven transports) are not substantiated, the probable pH difference across the plasma membrane being substantially smaller than previously supposed.

Introduction

The recently described technique of cell pH topography estimation using pH-sensitive fluorescent dyes [1,2] made it possible to estimate the pH of intracellular areas as small as 0.05 μm^2 so that a heterogeneity of intracellular pH could be clearly established. At the same time it provided a tool for a more precise determination of the pH difference across membranes that are known to mediate proton-driven transports, such as the yeast plasma membrane [3].

In the present paper estimates of the pH gradient across the plasma membrane of two facultative anaerobic species under different incubation conditions are presented and shown to be much less than those obtained from macroscopic measurements.

Methods

Yeast

Two yeast species, similar in their metabolic pattern but of very different size and shape, were used, viz. *Saccharomyces cerevisiae* (strain K, CCY 21-4-60) and *Endomyces magnusii* (CCY 42-1-2) employed routinely in this laboratory [4]. They

Abbreviation: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

were maintained on wort agar slopes and propagated in a semi-synthetic medium at 30 °C on a reciprocal shaker [4]. Cells were harvested after 20 h when they have reached the early stationary phase. Unless otherwise stated, they were then aerated for 1 h at room temperature and resuspended in water or triethanolamine-phthalate buffer which covers the entire physiological range from pH 2.5–8.5.

pH measurement

Prior to the measurement the cells were incubated for 20 min in 100 μM fluorescein diacetate to reach an equilibrium concentration of fluorescein. The fluorescein molecules liberated in situ were used as a pH-sensitive fluorescent indicator [1]. The pH-dependent representation of the two fluorescent forms of the dye was followed using selective excitation. Two fluorescence microphotographs of the same cell were taken together with a phase-contrast picture as reference. Computerized densitometry of the fluorescence pictures yielded the differences in densities in different places, point by point, convertible through a calibration curve to the local pH values [2].

The pH maps are based on a two-dimensional vertical projection of a three-dimensional object. In those instances where peripheral cytoplasm is more acidic than the cell center, the apparent pH of the central part is in fact distorted by the overlying and underlying peripheral regions, so that the true cytoplasmic pH in the cell center is actually higher (perhaps by 0.3 pH units in Fig. 1). Similarly, the pH in vacuolated regions is distorted because of the acidic intravacuolar pH [2]. Surprisingly, the nucleus does not appear in the fluorescence pictures and in the resulting pH map. The cell wall does not bind fluorescein (the septum in budding cells was completely dark in the fluorescence picture). When measuring pH profiles, the addition of fluorescein to the medium was so small that the fluorescence of the medium above and under the cell during photography was negligible compared to the fluorescence intensity of the cell interior.

All necessary corrections required by the imperfection of the film (such as the nonlinearity of the relationship between log exposure and density) were taken. Bleaching of fluorescein caused by illumination was negligible as short exposures and narrow-band excitation were used; when not, an appropriate correction was made. However, in spite of careful controls the absolute pH values presented here may be shifted as a whole by up to 0.3–0.5 pH unit in either direction due to the uncertainty inherent in the densitometric (photometric) procedures. In the present arrangement the method is capable of resolution to 0.1 pH unit in the pH range between 5 and 7, the linear resolution being limited by the laws of optics to about 200 nm.

All results shown are typical results observed in the majority of cells, but there were individual differences between cells in the population.

Results

Cells in water

When suspended in water (final pH between 5 and 6) both yeast species displayed heterogeneous pH values in different parts of the cell. The usual pattern was a neutral pH (6.8–7.5) in the central part of the cell which gradually decreased toward the cell periphery (down to pH 5.5–6.0), (Fig. 1).

The pH difference between the central and the peripheral part diminished in cells after prolonged

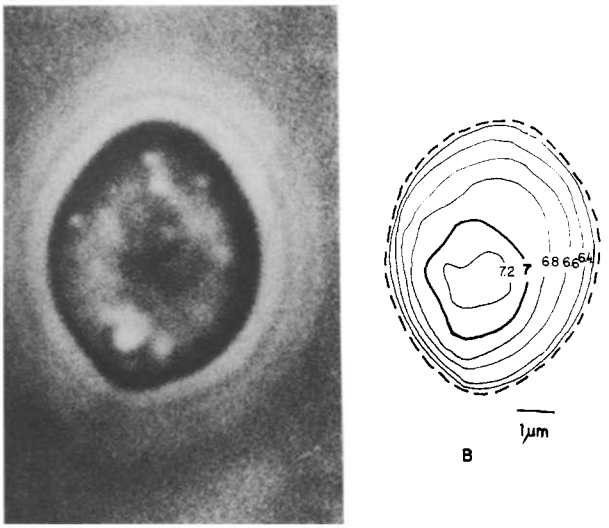

Fig. 1. Comparison of a phase-contrast photograph (A) of a *S. cerevisiae* cell suspended in water (final pH 5.5) with the pH map of the same cell (B) using pH isolines spaced at 0.2 pH units.

(up to 3 days) starvation and in antimycin-treated cells, the intracellular pH being 6.0–6.5 throughout the cell. Some cells after excessive starvation display monotonous alkaline pH values throughout the whole cell, possibly due to NH_4^+ ions liberated during autolysis [5].

A comparison of pH maps of *E. magnusii* (typically 10×30 μm) and *S. cerevisiae* (6×8 μm) cells shows that in larger cells the central neutral region represents a relatively larger part of the cell than in smaller cells, but the region where pH increases centripetally from the plasma membrane is of about the same thickness (1–2 μm).

Cells in buffers

Yeast cells suspended in a buffer (which produces intracellular pH shifts; Fig. 2A) were seen to change their pH map substantially, the intracellular pH becoming practically constant throughout the cell (Fig. 2B).

However, the process was relatively slow, the equilibrium was reached after 15–20 min, as shown in Fig. 3, illustrating pH maps of a cell at different

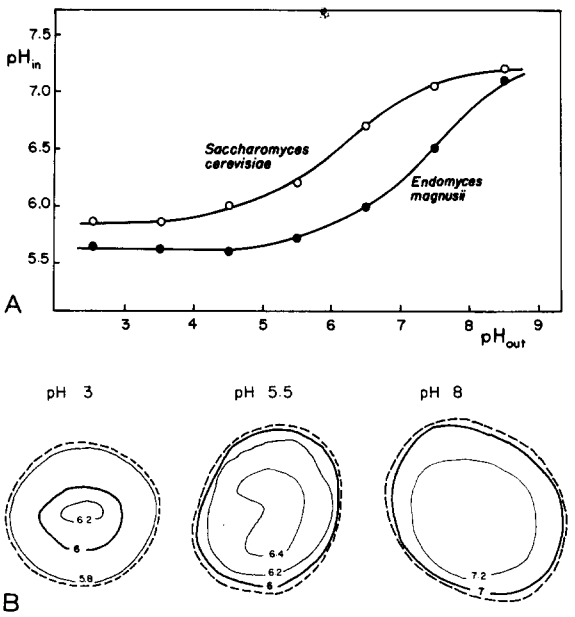

Fig. 2. (A) Dependence of the overall intracellular pH on pH_{out} for *S. cerevisiae* and *E. magnusii*. (B) pH maps of a typical *S. cerevisiae* cell suspended in buffers of pH 3, 5.5 and 8. All data refer to equilibrium values attained after about 20 min in 0.2 M buffer.

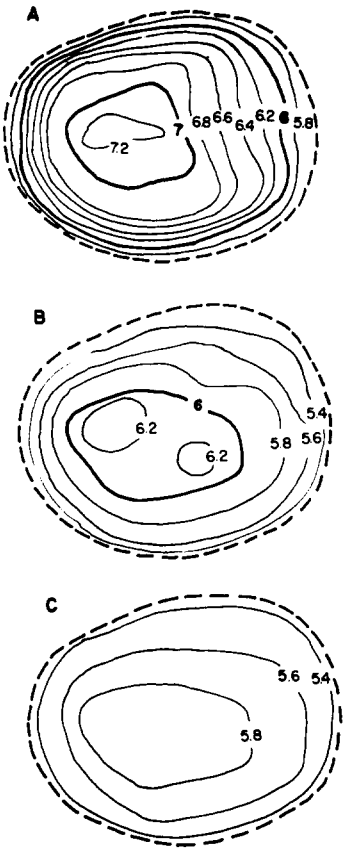

Fig. 3. pH maps of *S. cerevisiae* cell transferred from water to a 0.2 M buffer of pH 3. (A) After 2 min; (B) after 5 min; (C) after 20 min.

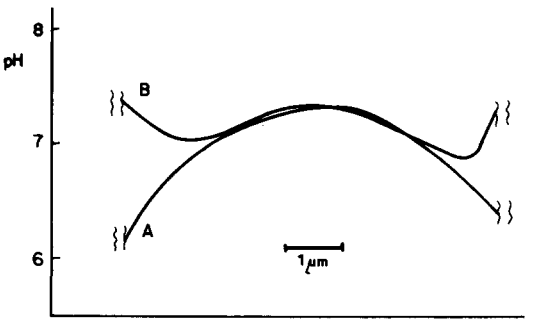

Fig. 4. Typical transcellular pH profiles of a *S. cerevisiae* cell suspended in water (A) and in 5 mM buffer of pH 8 (B). The wavy lines represent the position of the cell wall.

times after transfer from water to 0.2 M buffer of pH 3. Here again, the values tally with the overall pH change as shown in Fig. 5 of Ref. 1.

In cells transferred from water to weaker (5 mM) buffers, some pH differences between the central and the peripheral part persisted. Cells in a weak alkaline buffer showed an alkaline center, a slightly acidic ring-shaped area and an alkaline periphery (Fig. 4). In weak acidic buffers the acidic peripheral area was more pronounced.

The pH maps of *E. magnusii* cells were similar to those of *S. cerevisiae*, and therefore are not shown here.

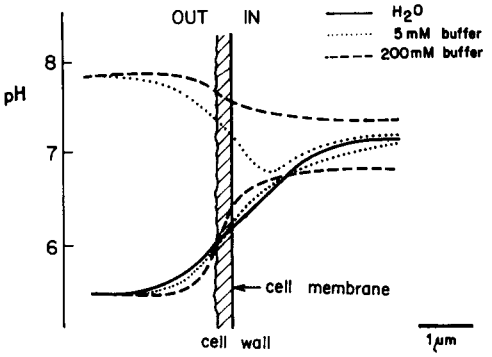

Fig. 5. pH profiles in the vicinity of the plasma membrane of a *S. cerevisiae* cell suspended in water or buffers at pH 5 or 8.

Glucose addition

Addition of glucose, which is known to induce an intense acidification of the medium [6,7] did not change the pH map substantially. The pH maps of cells taken from shaken suspensions to which 1% glucose was added, displayed after 10, 20 or 30 min of incubation a similar pH pattern as cells incubated in pure water. Longer incubation seemed to acidify the cell interior a little.

However, a sequence of microphotographs of the same single cell squeezed between two microscopic slides, taken during a 20 min period following glucose addition, revealed gradual acidification of the cell interior, resulting in a homogeneous equilibration of intracellular pH to about 5.7 (similar to Fig. 2B; $pH_{out} = 3$). As anaerobiosis does not play an important role in the external acidification by this yeast [7], the likely explanation is that the situation approaches that of a cell suspended in a strong buffer which results from a powerful extrusion of acidity plus buffering substances after glucose addition [8]. The observed intracellular pH value of 5.7 corresponds to an external pH of 3 or less (cf. Fig. 2A) as often found in the medium after glucose addition.

Addition of diethylstilbestrol (final concentration, 4 μg/ml), which is supposed to inhibit the proton-extruding ATPase [9], together with 1% glucose, resulted in an overall acidification of the cell interior below pH 5.5.

pH profiles

As in water-suspended cells the pH gradually rose centripetally from the periphery, it was examined whether an analogous gradual pH change took place outward from the plasma membrane. For this purpose, fluorescein together with fluorescein diacetate was added to the medium, so that the fluorescence arrived from fluorescein molecules both inside and outside the cell.

It was found in water-suspended cells that the outside pH changed gradually outward from the cell, making the pH transition diffuse, extending to 1–2 μm on both sides with no clear ΔpH discernible across the membrane itself. In buffers the 'unstirred layer' outside the cell was somewhat narrower, especially in strong buffers (Fig. 5). Addition of 10 μM CCCP (which is believed to dissipate both the membrane potential and the proton gradient) brought only insignificant changes in the profile.

Discussion

Intracellular pH, total and local

A variety of techniques [1,10–16] have now established the average pH of yeast cells (both *S. cerevisiae* and *E. magnusii*) and its dependence on the external pH (cf. also Fig. 2A). Values computed from the present pH maps are in fine agreement with the above averages.

Intracellular pH heterogeneity was suggested to explain broad [^{31}P]NMR lines seen in living systems (for a review, see Ref. 17). Experiments with pH-sensitive microelectrodes (as reviewed e.g. in Ref. 18) done in large muscle fibers indicated that the intracellular pH near the plasma membrane was more acidic than in the central part [18,19] but no convincing evidence was given.

The novel finding described here is the observation that under physiological conditions (when no strong buffer is added to the medium) there is a pH gradient from the cell center toward its periphery which persists even when glucose is metabolized (and H^+ ions are actively extruded) and, more significantly, also when the outside pH is higher than that of the cell periphery (cf. Fig. 4). A similar intracellular pH pattern was observed in *Penicillium cyclopium* protoplasts (Slavík, J. and Roos, W., unpublished data). One can only speculate about the nature of pH-modifying processes within the cell, the active character of which is documented by their disappearance when the cell is starved, not viable or when its metabolism is impaired by inhibitors.

Somewhat surprising is the 'homogenization' of intracellular pH in a more concentrated buffer (Fig. 2B). Apparently the forces maintaining the intracellular pH gradient are overcome by the H^+ or OH^- ions entering the cell although the buffering capacity of the cell interior is still such that the overall (and fairly homogeneous) pH of the cell interior may be up to nearly 3 pH units higher than the extracellular one (say, when $pH_{out} = 2.5$).

Transmembrane pH profile

The situation observed at the intracellular face of the plasma membrane is paralleled by that on the outside of the cell. There is a gradual transition of the pH from the value observed at the plasma membrane toward the bulk solution, the cell wall representing no hindrance in this respect. Hence, typically, in water or in a weak acidic buffer there is a pH gradient from the cell center at pH around 7–7.5 passing smoothly through the plasma membrane (at about 6) to the bulk solution around 5.

The gradual nature of the change of pH toward the membrane from the bulk solution both inside and outside the cell is in keeping with what is known about unstirred layers existing at the membrane, their effect being pronounced if the substance in question is transported across the membrane relatively rapidly in comparison with its diffusion in the adjacent aqueous medium (cf. [20]). In stronger buffers the profile is steeper, as corresponds to a lower unstirred layer effect at more efficient supply of H^+ ions. This is understandable if one realizes that H^+ (or OH^-) ions are transported across the membrane by a saturable process while they only diffuse in the solution around the cell, so that at a speedier supply the transmembrane movement plays a relatively smaller role. However, within the limits of resolution, there is no break at all in the pH profile at the membrane barrier, the apparent ΔpH across the membrane being as small as 0.01–0.1 pH unit. Even if some blurring of the basically optical measurements underlying the pH estimation is possible, the ΔpH would be less than 0.5 pH unit. This is much smaller than the values used under similar conditions for computations of the protonmotive force (which presumably drives proton symports [21]): using bulk values of pH_{in} when $pH_{out} = 3$, the contribution of ΔpH to the protonmotive force would be roughly 150 mV, using the corrected values reported here it might be as low as 6 mV.

Since ΔpH is an established component of the force driving many transmembrane movements both in yeast and other cells, one is compelled to assume that the ΔpH which the transport systems 'see' is highly localized within the membrane, probably near the principal producer of extracellular acidity, the H^+-expelling ATPase, and that it does not contribute in a measurable way to the overall ΔpH across the yeast plasma membrane.

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