Intracellular pH topography: determination by a fluorescent probe

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The distribution of pH inside the yeast *Endomyces magnusii* was measured at 1 μm resolution using different external pH values. In a neutral buffer the pH of the cytoplasm was 6.7–7.2 in the center, decreasing to 6.0 toward the periphery of the cell. A decrease of external pH was followed by a gradual uniform decrease of internal pH. Using a comparison of the 'pH map' with phase-contrast picture of the same cell, the pH of the vacuoles was estimated to be 5.5–5.6.

Intracellular pH topography *Fluorescent probe* *Yeast cell* *Fluorescein*

1. INTRODUCTION

Although intracellular and intraorganellar pH is an important factor controlling many cellular processes, none of present techniques are capable of giving information about the spatial distribution of pH within small cells. Microelectrodes, ^{31}P NMR, spin probes, and permeabilization methods as well as distribution techniques using weak acid or bases and fluorescence quenching methods are supposed to give some kind of the 'mean' intracellular pH value (review [1,2]).

Detailed 'mapping' of pH values inside living cells and organelles is possible using a combination of the fluorescent probe technique [3] with fluorescence microscopy. The spatial resolution is determined by the resolution of the optical microscope. The technique may be promising, particularly in the study of processes in which the determination of the true pH difference across the membrane (protonmotive force component) plays an essential role (e.g., secondary active transport processes).

2. EXPERIMENTAL

The technique [3] is based on the selective excitation of 2 forms of fluorescein, basic (monoanionic) and acidic (dianionic). Each of these forms possesses different fluorescence properties. The proportion of these 2 forms is pH-dependent and may be monitored by the ratio of fluorescence intensities observed following excitation at 435 nm and 490 nm (cf. fig.2 and 3 in [3]). Utilizing this idea, 2 consecutive microscopic pictures of the

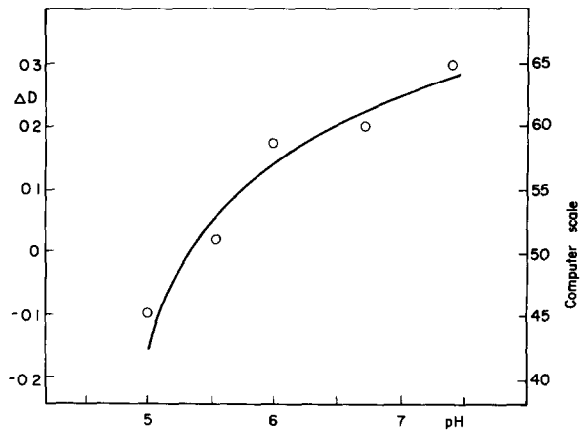

Fig.1. Fluorescence *vs* pH calibration curve. It shows the pH dependence of the difference in optical density ΔD between the 2 negatives, exposed with 425 nm and 466 nm excitation filter, respectively.

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same yeast cell were taken after excitation through 2 different excitation filters – one transmitting around 435 nm, the other around 490 nm. Since the optical density of the film is practically directly proportional to the logarithm of the exposure (i.e., to the logarithm of fluorescence intensity provided the exposure time is kept constant), the difference in optical density within the same domain in the

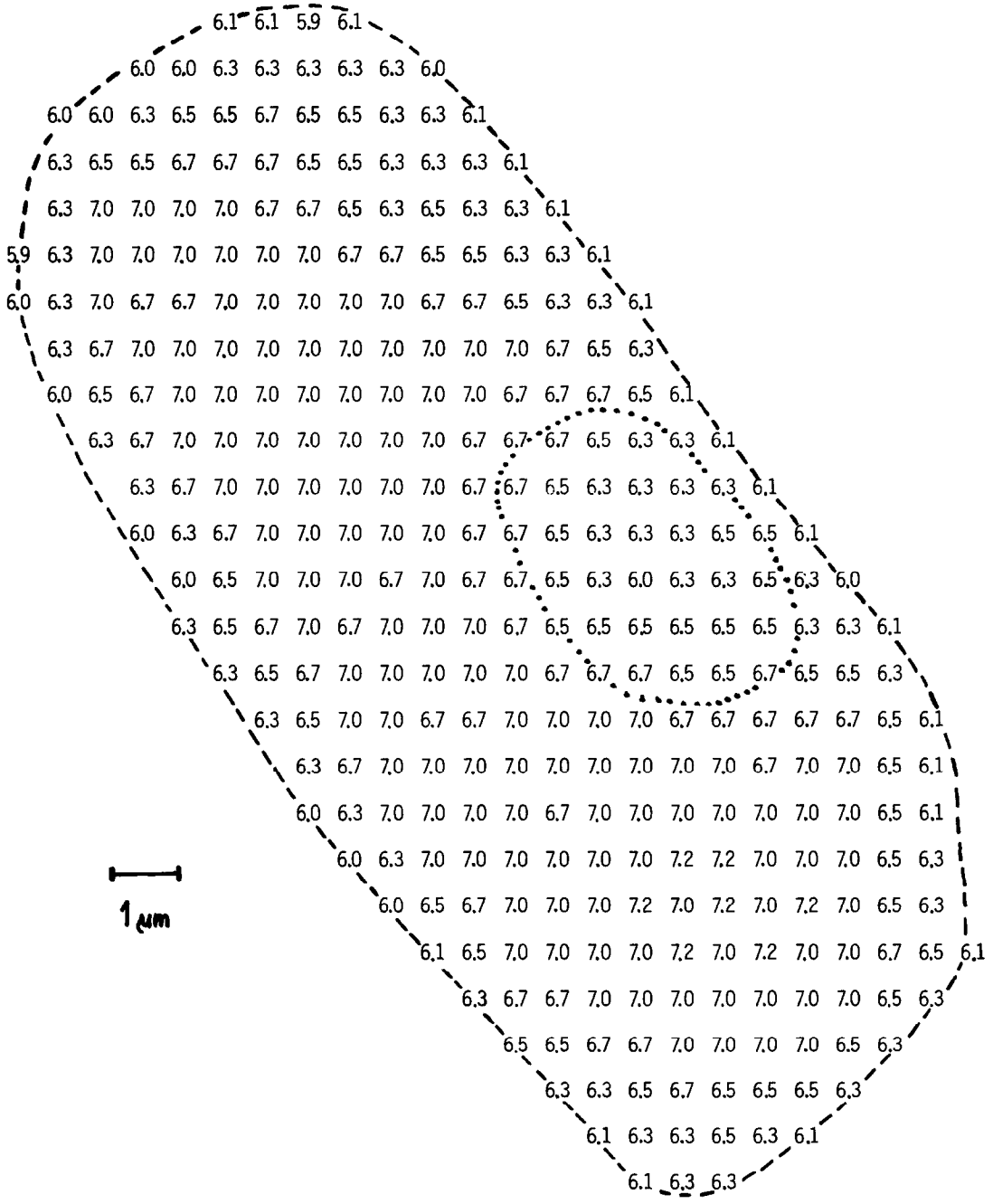

Fig.2. Typical pH distribution inside a young yeast cell at neutral external pH (pH_{out} 7.5). The dotted line demarcates the area, where in the corresponding phase contrast picture a vacuole was observed.

first and in the second picture is directly related to the ratio of fluorescence intensities observed in that domain after a 435 nm and a 490 nm excitation. This ratio determines the pH value for that domain [3]. Thus determined 'pH map' was compared with the phase contrast picture of the same cell.

The pictures were taken using the FLUOVAL I fluorescence microscope equipped with a high pressure 200 W mercury lamp, thermal filter, UV absorption filter, high quality interference filters with maximum transmission at 425 nm and 466 nm, respectively, and a yellow-green cut-off filter type OG 4 (all from Carl Zeiss, Jena, GDR). Because of its favourable densitometric properties, a special film (Agfapan-Vario XL, Agfa, FRG) was used. The subtraction of optical densities was made digitally using a TAS picture analysing system (Texture Analyse System, Leitz, FRG).

The calibration curve was prepared as follows. The specimen in the microscope was replaced with a small shallow dish filled consecutively with fluorescein solutions in different buffers. In each case, 2 pictures (one after excitation with the 425 nm interference filter and another after excitation with the 466 nm filter) were taken and the optical densities of the film field were evaluated. The differences between these 2 densities were plotted against the pH of the buffer (fig.1). The exposure times were kept constant during the whole calibration procedure and in all subsequent experiments. The fluorescence fading was negligible.

The yeast *Endomyces magnusii* (chosen for its relatively large size) was grown, harvested, and incubated with 50–100 μM fluorescein diacetate as in [3].

3. RESULTS AND DISCUSSION

3.1. *Technique*

After incubation with fluorescein diacetate, all cellular compartments are likely to contain a certain amount of free fluorescein sufficient for pH measurement (cf. [3]). It appears that the observed fluorescence of fluorescein is determined solely by ambient pH. The effects of buffer composition, ionic strength, and viscosity of the medium are <0.1–0.2 pH unit, as revealed by the detailed calibration procedure [3]. However, some errors are introduced by background fluorescence, im-

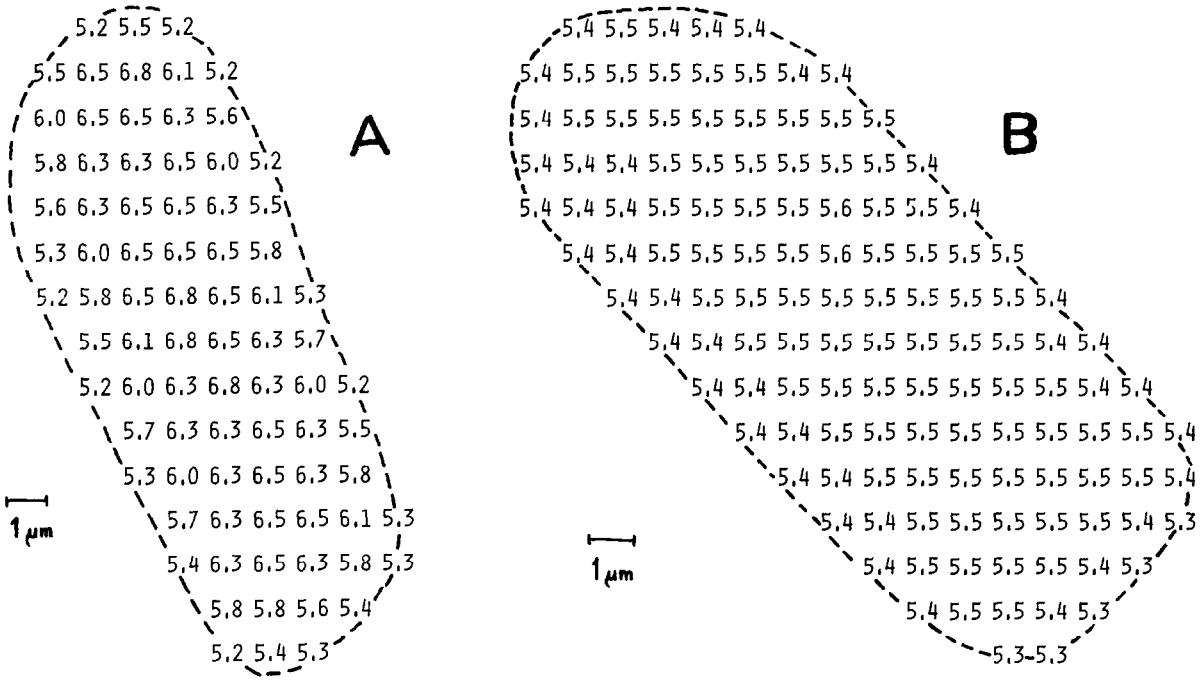

Fig.3. The distribution of pH inside the cell (A) 3 min and (B) 15 min after external pH was changed from 8 to 3 (0.2 M triethanolamine–phthalate buffer).

perfections of the linearity of the densitometric curve of the film, and of the evaluation of densities by the TAS system.

The resolving power may be increased up to the limits of the optical microscope (i.e., ≤ 200 nm). Moreover the spatial resolution may, in principle, be supplemented with the time resolution (movie camera) thereby allowing both space- and time-resolved measurements of intracellular pH.

3.2. *Yeast cells*

The cells of *Endomyces magnusii* in neutral buffer show a fairly uniform gradient of pH from 7.0–7.2 near the center to 5.9–6.3 at the periphery (fig.2). The older cells containing many vacuoles have a generally lower pH area in vacuolated regions (the pH inside vacuoles was estimated to be 5.5–5.6) while higher pH values are retained in the extravacuolar cytoplasm (the effect of a small vacuole is shown in fig.2). This is in agreement with ^{31}P NMR spectroscopy measurements pointing to an existence of a compartment with pH more acidic than that of the cytoplasm, which was also ascribed to vacuoles [4,5]. Therefore changes in the average cell pH, as monitored by other methods [1,2] need not reflect changes in the cytoplasm, but they may also be due to changes in the size of organelles.

The effect of external pH, as described in [3], was also studied in the space-resolved arrangement. The process of gradual acidification of the cell interior after transferring cells from an alkaline to an acidic medium is illustrated in fig.3. The pH of the cytoplasm is seen to decrease from the initial value of 7.2–7.3 to 5.5–5.6. The results fully confirm the data obtained in the 'average' cell pH before (cf. fig.5 in [3]).

Work is now under way to determine pH changes in cells exposed to different physiological situations (e.g., oxidation, fermentation, active transport).

REFERENCES

[1] Roos, A. and Boron, W.F. (1981) Physiol. Rev. 61, 296–434.
[2] Iles, R. (1981) Biosci. Rep. 1, 687–699.
[3] Slavík, J. (1982) FEBS Lett. 140, 22–26.
[4] Navon, G., Shulman, R.G., Yamane, T., Eccleshall, T.R., Lam, K., Baronofsky, J.J. and Marmur, J. (1979) Biochemistry 18, 4487–4499.
[5] Borst-Pauwels, G.W.F.H. (1981) Biochim. Biophys. Acta 650, 88–127.