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Vacuoles: Main Compartments of Potassium, Magnesium, and Phosphate Ions in *Saccharomyces carlsbergensis* Cells

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The uneven distribution of Mg^{2+}, K^+, and phosphate in *Saccharomyces carlsbergensis* was demonstrated by the differential extraction of ions. Their concentrations were 5, 60, and 1 mM in the cytoplasm and 73, 470, and 110 mM in vacuoles, respectively. The intracellular gradients of these ions were 1:15, 1:8, and 1:110, respectively, across the tonoplast. The determination of free Mg^{2+} (1.35 mM in the cytosol and 20 mM in vacuoles) showed that the ion accumulation in vacuoles could not be explained by the higher degree of ion complexing in these organelles.

The dependence of rates and directions of many metabolic processes on the presence of some inorganic ions suggests that their intracellular concentrations are under strict control, and the controlled changes in ion concentrations provide an important means for metabolic regulation. The level of free ions in a cell was assumed to be controlled by the plasmalemma transport systems as well as by complexing of ions, and in eucaryotic cells, also by the organelle transport systems (16). The participation of organelles in the regulation of ion concentrations in the cytosol could result in the compartmentation of ions. It had been recently demonstrated cytochemically (16, 18) that in yeast cells Mg^{2+} accumulated mainly in vacuoles. The present work shows the predominant accumulation of K^+, Mg^{2+}, and orthophosphate ions in vacuoles and the occurrence of concentration gradients of these ions across the tonoplast.

MATERIALS AND METHODS

Strain and culture media. The yeast *Saccharomyces carlsbergensis*, strain IBPhM-366, was grown at 29°C in shaking 750-ml flasks containing 200 ml of the following medium: 3 g of $(NH_4)_2SO_4$, 0.7 g of $MgSO_4 \cdot 7H_2O$, 0.4 g of $Ca(NO_3)_2$, 20 g of glucose, 2 g of KH_2PO_4, 0.2 g of K_2HPO_4, 2 g of yeast extract, and 1,000 ml of water, pH 5.5. After 18 h, 10 ml of the yeast suspension (optical density = 1.1) was inoculated into 200 ml of a new medium and grown for 5 h (optical density = 0.55). The cells were collected by centrifugation and subjected to analysis after three washes with double-distilled water.

Determination of ion pools. The method of differential extraction of amino acid pools of yeasts (26) was modified in our experiments for the determination of ion pools of *S. carlsbergensis*. One hundred milligrams (0.5 ml) of wet cells was washed with 5 × 2 ml of distilled water, 3 × 1 ml of 0.25 M sodium acetate buffer (pH 4.8), and 3 × 1 ml of 0.7 M sucrose (or 0.7 M mannitol). To make plasmalemma readily permeable to low-molecular-weight compounds and ions, the cells were treated for 9 min with small portions (3 ml) of 1% cytochrome *c* solution in 0.7 M sucrose, pH 7.6, and rewashed with acetate buffer in the presence of 0.7 M sucrose (3 × 1 ml). The cytosol ions were determined in the combined extract. This was followed by the destruction of the vacuoles under an osmotic shock (3 × 1 ml of water). The remaining material was rewashed with acetate buffer (3 × 1 ml). The vacuole ions were determined in the combined extract. The remaining ions were extracted with 0.5 N $HClO_4$ (1 × 1 ml) for 1 min. This resulted primarily in the release of ions from large vacuoles which resisted the osmotic shock, as well as their bound and water-insoluble forms (e.g., ions tightly bound to a cell wall, ribosomes, etc.). All types of extraction were performed at 0 to 4°C with the glass fiber disks (GF/B, Reeve Angel, Whatman) for filtration. The cell debris was washed with concentrated $HClO_4$ at 160 to 180°C. In a number of experiments after the plasmalemma modification by cytochrome *c*, the cells were treated with Triton X-100 in acetate buffer in the presence of 0.7 M sucrose. An increase in the detergent concentration from 0.075 to 0.1% caused the disruption of vacuoles. The number of vacuoles was monitored in a phase-contrast microscope at all stages of determining the ion pools.

Staining and microscopy. The portion of cells rendered permeable by the cytochrome *c* was determined by counting the cells stained with the fluorescent dye primulin (1 μg/200 mg, wet cells) in a luminescent microscope (ML-2, Leningrad, USSR). At the end of the desired incubation time with cytochrome *c*, primulin was added to the suspension, and immediately afterwards the cells were observed in the microscope. The counting was completed in 3 min at room temperature.

Analytical methods. Mg^{2+} and K^+ were determined in an atomic absorption spectrophotometer (Hitachi-207, Tokyo, Japan). To determine Mg^{2+}, the glassware was pretreated with a 1% $La(NO_3)_3$ solution to decrease sorption and elution of Mg^{2+}. Orthophos-

phate was determined as described earlier (15). Free Mg^{2+} ions were determined with isocitrate dehydrogenase (1, 6); arginine was determined by Sakaguchi's method modified by Ramos et al. (19).

Chemicals. Cytochrome *c* and yeast extract were both from Serva Feinbiochemica, Heidelberg, West Germany. Mannitol was purchased from BDH Chemicals Ltd., Poole, Dorset, United Kingdom, or Chemapol, Praha, Czechoslovakia. Isocitrate, isocitrate dehydrogenase, and NADH were from Calbiochem, San Diego, Calif., or Sigma Chemical Co., St. Louis, Mo. All the other reagents were chemically pure.

RESULTS

About 4% of the total cell Mg^{2+}, 23% of the K^+, and 10% of the orthophosphate were released from cells successively treated with water, acetate buffer, and 0.7 M sucrose (extract 1, Table 1). Further release of ions was not achieved by the additional treatment of cells with acetate buffer and sucrose. The same outcome was observed when cells were treated independently with 5 mM EDTA in acetate buffer. All these results in total assume that ions of extract 1 correspond to ions sorbed on a cell wall.

Yeast cells treated isotonically with cytochrome *c* contain vacuoles distinctly visible in a phase-contrast microscope. The fluorescent dye primulin penetrates the cytoplasm of cells treated with the cytochrome *c* but not untreated cells. The cytoplasm of almost all treated cells fluoresced brightly, with vacuoles showing as dark spots against this background.

The isotonic cell treatment with the cytochrome *c* renders the plasmalemma progressively permeable to small molecules, depending on the duration of the treatment. Thus, the extraction of K^+, which is the most mobile of all ions under study, is complete in the first 9 min (Fig. 1). Modification of the plasmalemma with the cytochrome *c* released about 15% of the total K^+, 8% of the total Mg^{2+}, and 2% of inorganic phosphate (Table 1, extract 2). Twelve percent (2.5 μmol/g of cells) of intracellular arginine was found in extract 2. This agrees well with the data on the low content of arginine in the yeast cytoplasm (25, 26). The results show that the isotonic treatment of yeast cells with cytochrome *c* makes permeable only the cytoplasmic membrane and allows the extraction of the cytosol pools of small molecules. Therefore, extract 2 corresponds to the cytosol extract (see, however, Discussion). The major part of remaining ions (36% of the total Mg^{2+}, 41% of the total K^+, and 64% of inorganic phosphate [Table 1, extract 3]) was released from the cytochrome *c*-treated cells subjected to osmotic shock. All the vacuoles except for the largest (about 10%) burst and were no longer visible under the microscope.

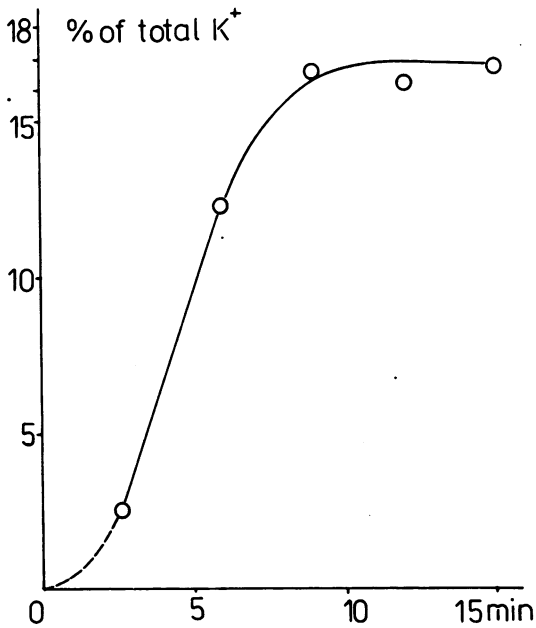

FIG. 1. *Time dependence of the cytochrome c effect on the release of K^+ from cells under isotonic conditions. K^+ in the cytosolic extract is shown as the percentage of the total cellular K^+.*

TABLE 1. *Content of Mg^{2+}, K^+, and orthophosphate ions in various cell compartments*[a]

Extract	Ion concn (μmol/g of wet cells)		
	Mg^{2+}	K^+	Orthophosphate
1 (Cell wall)	1.85 ± 0.2	52.43 ± 0.64	3.1 ± 0.9
2 (Cytosol)	3.08 ± 0.53	35.55 ± 2.3	0.58 ± 0.12
3 (Vacuoles)	14.4 ± 2.25	93.35 ± 6.14	19.35 ± 1.61
4 (Largest vacuoles and bound ions)	12.21 ± 2.67	25.06 ± 0.61	7.29 ± 2.13
Residue[b]	8.72 ± 2.84	21.99 ± 2.22	
Sum	40.26	228.38	30.32

[a] Mean values are calculated from 20 to 26 experiments (means ± standard error). In vacuoles and cytosol the detected ions are the sum of free ions and their water-soluble complexes.

[b] The orthophosphate of this extract is mostly a product of high-molecular-weight polyphosphate hydrolysis; therefore, we do not present its content here.

were no longer visible under the microscope.

Extract 3 also contained about 75% (15.6 μmol/g of cells) of the total arginine of *S. carlsbergensis* cells which is localized in vacuoles (25, 26). The appearance of arginine and Mg^{2+} in the extract under osmotic shock observed simultaneously with the disruption of vacuoles, as well as the cytochemical evidence of Mg^{2+} localization in these organelles (16, 18), allows us to identify extract 3 (Table 1) with the vacuolar contents.

The results of the vacuolar lysis induced by Triton X-100 provided additional evidence of the fact that the major quantity of ions is concentrated in these organelles (Fig. 2). After the cell treatment with cytochrome *c*, the vacuoles lysed as the Triton X-100 concentration increased from 0.075 to 0.1%. The vacuole disruption as observed in phase-contrast microscopy was followed by the ion release, resulting in the increase in the ion content of the cytosol extract and its decrease in the vacuolar one (Fig. 2).

About 50% of Mg^{2+} remained in cells subjected to the osmotic shock. The treatment of yeast cells with 0.5 N $HClO_4$ (1 min) resulted in the disappearance of the remaining large vacuoles and additional release of Mg^{2+} (30%) (Table 1, extract 4) and arginine (13%) (3.2 μmol/g of cells). This extract is supposed to originate from the large vacuoles and bound ions. The bulk of intracellular orthophosphate (73%) and K^+ (73%) was in the osmotically free state; about one half of intracellular Mg^{2+} was either bound to high-molecular-weight cell components or occurred in the form of acid-soluble complexes. About 27% of the osmotically free Mg^{2+} was in the form of free Mg^{2+}, whereas the remaining 73% represented water-soluble low-molecular-weight complexes (Table 2).

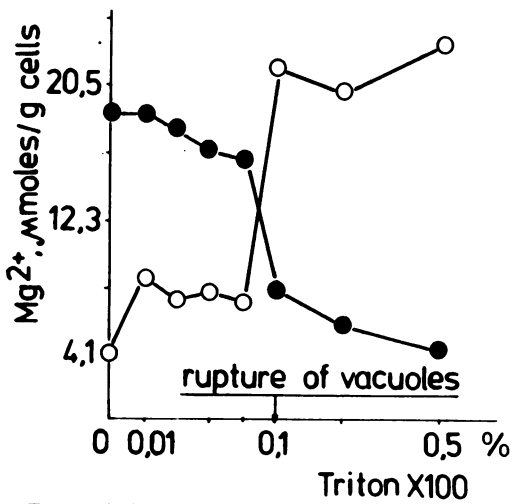

FIG. 2. *Influence of Triton X-100 on the content of Mg^{2+} in vacuolar (●) and cytosolic (○) extracts.*

Of special interest is the unequal distribution of Mg^{2+}, K^+, and orthophosphate between the cytosol and vacuoles, the contents of which in vacuoles was 4.6, 2.6, and 33 times higher, respectively (Table 1). These figures are an underestimate because the largest vacuoles did not burst under the osmotic shock and their ionic content was determined only in 0.5 N $HClO_4$ at 0°C. The Mg^{2+} content in the cytosol was slightly overestimated. Actually, in extracting phospholipids from the plasma membrane, cytochrome *c* evidently extracts a part of its Mg^{2+}. It is noteworthy that the extraction of acid-stable phosphate (310 μg/g, wet cells), not an acid-labile one (40 μg/g, wet cells), occurred predominantly during the cytochrome *c* treatment of cells. The calculation of ion concentrations in the vacuoles and cytoplasm, even disregarding the corrections mentioned, points to the existence of the gradients of Mg^{2+}, K^+, and phosphate concentrations on the tonoplast (Table 2). Inorganic phosphate had the highest concentration gradient. One can think that the phosphatases localized in the vacuoles are able to hydrolize the phosphorus compounds after the osmotic shock, and as a result the orthophosphate content in the vacuoles is overestimated. However, control experiments showed that the determination of phosphate pools performed in presence of 10 mM NaF (an inhibitor of phosphatases) gave the same othophosphate contents (data not shown).

DISCUSSION

The method of the differential extraction by cytochrome *c* treatment was first applied to determine amino acid pools of the yeast *Candida utilis* (26). In the present work this modified method was employed for the successive extraction of ion pools of the yeast *S. carlsbergensis*. The modification (high concentration of

TABLE 2. *K^+, Mg^{2+}, and inorganic phosphate in S. carlsbergensis cytosol and vacuoles*[a]

Ion	Concn (mM) in:		Gradients (cytosol/vacuoles)
	Cytosol	Vacuoles	
K^+	60	470	1:8
Osmotically free Mg^{2+}	5	73	1:15
Free Mg^{2+}	1.35	20	1:15
Orthophosphate	1	110	1:110

[a] The concentrations of ions were calculated on the basis that 80% of the wet yeast cell is water (determination of the dry weight of yeast) and the vacuolar volume is 25% of the protoplast volume.

the cytochrome *c* and alkaline pH during the treatment with cytochrome *c*) was due to the resistance of cells to the lytic action of the cytochrome *c* under the standard conditions of extraction of amino acid pools of *C. utilis*. Evidently, the sensitivity of various yeast species and even strains to cytochrome *c* is different.

The results obtained as well as the cytochemical data (16, 18) provide evidence in favor of the applicability of the method, although with some limitations, for determination of various ion pools of *S. carlsbergensis*. Ion pools described as vacuolar comprise ions of vacuoles and vesicles sensitive to osmotic shock. Some mitochondrial and nuclear ions are probably released as a result of osmotic shock. The content of vacuolar ions may be therefore overestimated. However, such overestimation is evidently not high and does not change the principal picture of ion distribution between the cytoplasm and vacuoles. We failed to find marked concentration of Mg^{2+} in cell organelles other than vacuoles (16, 18). A high concentration of Mg^{2+} could not be expected in mitochondria because their ATPase is inhibited when the molar proportion of Mg^{2+}/ATP is higher than 0.5 (4). Increased concentrations of Mg^{2+} result also in the incorrect transcription (27), so that a high Mg^{2+} concentration in the nuclei is unlikely. As for inorganic phosphate, the ratio of acid-labile and inorganic phosphorus was found to be 2.0 to 2.5 in both the vacuolar extract and isolated vacuoles (data not shown).

The conditions under which cells were pretreated and the phase of their growth are of great importance when determining ion pools, at least that of K^+. For instance, in experiments by Roomans and Seveus (21), yeast cells taken from the stationary growth phase were aerated for 1 day in distilled water and then incubated for 8 h in presence of glucose and CsCl or RbCl. *S. carlsbergensis* cells lose much of their vacuolar K^+ after 1 h of incubation with 100 mM glucose (Lichko, Okorokov, and Kulaev, submitted for publication). Moreover, the K^+ content in yeast cells is minimal during lag and stationary growth phases and maximal during the logarithmic stage of growth (14). These are probably reasons why other authors (8, 21) have not found that K^+ is concentrated in the yeast vacuoles. Differentiation between cytosol and vacuolar pools of Mg^{2+}, K^+, and inorganic phosphate allows the comparison between *S. carlsbergensis* and other species. For example, the concentration of osmotically free Mg^{2+} in the *S. carlsbergensis* cytosol is 5 mM, i.e., practically the same as in *Escherichia coli* (4 mM) and *Bacillus cereus* (6 mM) (12, 22). Also, compare our data on K^+ or inorganic phosphate with data from other organisms (8, 11, 20, 23). The concentration of the enzymatically estimated free Mg^{2+} is no more than 1.35 mM and almost the same as that of *E. coli* (0.5 to 2.0 mM) (7) and animal cells (0.55 to 1.34 mM) (24). The concentration of osmotically free Mg^{2+} in *S. carlsbergensis* vacuoles is about 2.5 times as much as that found in mature plant tissue vacuoles (30 mM) (11). There are several consequences of the compartmentation of ions in the yeast cells. First, the true concentration of cytoplasmic ions is lower than believed earlier, when the compartmentation was ignored. However, the comparison between K^+, Mg^{2+}, and phosphate concentrations in the cytoplasm and K_m and K_i of different enzymes suggests that key enzymes and main metabolic pathways can operate under the optimal conditions (5, 13). The second consequence is the intracellular gradients of the ion concentration across the tonoplast. The difference between concentrations of osmotically free ions in the cytosol and vacuoles could be explained by the higher ion complexing inside the vacuoles. In this case, concentrations of free ions are expected to be the same on both sides of the tonoplast. However, the degrees of the Mg^{2+} complexing in the two compartments are equal (Table 2), and the concentration gradient of free magnesium ions across the tonoplast remains unchanged, i.e., 1:15. It would be logical to assume the concentration gradient of K^+, Mg^{2+}, and phosphate ions to be created by special transport systems localized in the tonoplast.

The problems of the functional role of the postulated tonoplast transport systems, as well as the ability of yeast and fungal vacuoles to accumulate ions, is in need of further investigations. It might be good to point out in this connection that there are data available on the Fe^{3+} and Ca^{2+} accumulation in vacuoles of *Penicillium chrysogenum* (17) and *Physarum* (3), respectively.

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