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APPARENT NONSOLVENT WATER AND OSMOTIC BEHAVIOR OF YEAST CELLS

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SUMMARY

1. L-Xylose and dimethylsulfoxide permeate the yeast cell, reaching an intracellular equilibrium concentration significantly lower than the medium concentration. At equilibrium the intracellular dimethylsulfoxide concentration is 70–85%, the L-xylose concentration about 40% of that in the medium. In anaerobically grown yeast, lacking intracellular membranous structures, the same equilibrium concentrations are reached as in normal yeast.

2. Both L-xylose and dimethylsulfoxide are present inside the cells in an osmotically active form.

3. The osmotic response of yeast cells in hypertonic NaCl solutions shows a deviation from ideal osmotic behavior. The deviation could be explained by increase of the number of intracellular particles in hypertonic salt solutions and by elasticity forces in the cell wall, causing some reduction of the cellular volume with a connected increase in the intracellular tonicity.

4. With equilibrium dialysis on disintegrated cells, it could be shown that there exists no bound water or exclusion water with respect to L-xylose and dimethylsulfoxide in the yeast cell.

5. The experimental results make the existence of osmotically inactive compartments in the yeast cell, as suggested in recent literature, highly improbable.

INTRODUCTION

Recently it has been suggested that different water compartments can be distinguished in the yeast cell, with respect to nonmetabolized sugars[1,2]. This assumption was based on the observation that some sugars reach a maximum distribution of only 20–60% of the intracellular water. This would indicate that only 20–60% of the intracellular water fraction is accessible to these sugars. As the amount of intracellular sugar decreased much less than the intracellular water volume in hypertonic NaCl solutions, it was postulated that either these sugars were adsorbed within the cell or that the compartment containing the sugar was osmotically inactive[1].

In a previous paper the transport and phosphorylation of 2-deoxy-D-glucose in yeast was discussed[3]. The experimental results indicated phosphorylation of this sugar, directly associated with the transport mechanism. The only alternate explanation of the experimental results appeared to be the existence of at least two

functionally distinct water compartments inside the yeast cell, in the sense as discussed above[3]. In this connection it appeared to be crucial to investigate the possible existence of such distinct, osmotically inactive compartments directly. Further, it is of importance to determine whether asymmetric solute distribution is restricted to compounds entering the cell *via* facilitated diffusion, *e.g.*, a specific carrier mechanism, or can also be found with other compounds entering the cell by simple diffusion. The results of these investigations are discussed in the present paper.

METHODS

Commercial baker's yeast, obtained from the Gist- en Spiritusfabriek, Delft, was used in these experiments. Anaerobically grown yeast was obtained by culturing under strict anaerobic conditions, at 27° during 48 h, on the following medium: peptone, 0.5%; yeast extract, 0.3%; malt extract, 0.3%; tryptone, 0.1% and glucose, 1%. The yeast was harvested by centrifugation and washed several times with distilled water.

The water fraction of yeast cells was measured by drying at 90° to minimum weight; appropriate corrections were applied for trapped medium and for the specific gravity of the yeast. The mean cellular volume of yeast cells at varying medium tonicities was determined by centrifuging a sample of the suspension for 30 min at 3000 rev./min in an Hamburger-type hematocrit tube. In preliminary experiments, it appeared that this time was sufficient to achieve minimum volume. Intercellular water between cells centrifuged in an Hamburger-type hematocrit tube was measured by adding ^{14}C-labeled inuline (mol. wt. 5100–5200) or ^{14}C-labeled dextran (mol. wt. 60 000–90 000) to the medium. 0.05 ml of packed cells was resuspended in distilled water and spun down again. The supernatant was analyzed for radioactivity.

To measure the tonicity of cell contents, the yeast cells were disintegrated by freezing in liquid air and thawing. Tonicities were measured with a freezing point osmometer (Advanced Instruments), giving a linear relationship between solute concentration and instrument reading. To measure intracellular solute concentrations, a yeast sample was collected on a Millipore filter, washed 3 times with a small volume of ice-cold water and extracted with alcohol. If necessary the extract was diluted with water.

Equilibrium dialysis studies were performed by dialyzing disintegrated yeast in cellophane bags for 48 h at 4° against dimethylsulfoxide or L-xylose solutions of various concentrations. The equilibrium concentration of the solute was measured in the outer phase.

Sodium and potassium were assayed with a flame photometer. L-Xylose was measured with orcinol according to McKAY[4]. ^{14}C-Labeled inuline, dextran and dimethylsulfoxide were measured in a liquid scintillation counter, with the liquid scintillator described by BRAY[5].

RESULTS

The uptake of L-xylose and of dimethylsulfoxide is shown in Fig. 1. Though the intracellular concentration of L-xylose still increases after 6 h, it seems unlikely

that 100% equilibrium will ever be reached. Even after 24 h, the intracellular concentration was only 40% of the sugar concentration in the medium. The dimethylsulfoxide uptake reaches its maximum value after about 100 min; a steady-state equilibrium is reached at an intracellular concentration of 70% of the medium concentration, with dimethylsulfoxide concentrations ranging from 12 to 2400 mM. In different yeast batches, this equilibrium concentration varied from 68 to 84%. As it has been suggested that the hypothetical cellular compartments causing unequal solute distribution may be identical with morphological distinct structures[1], the uptake experiments were repeated with anaerobically grown yeast. An identical asymmetric solute distribution was found in this yeast.

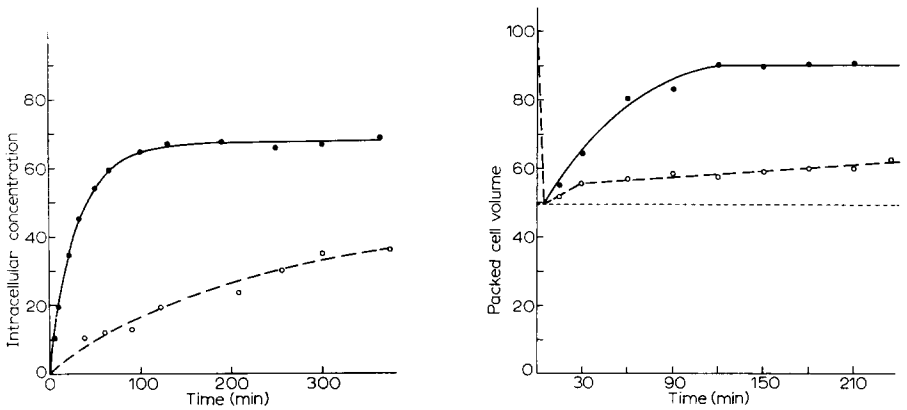


Fig. 1. The uptake of L-xylose and dimethylsulfoxide in yeast cells at 25°, in the course of time. The intracellular concentration is given in percent of the medium concentration. ●—●, 120 mM dimethylsulfoxide; ○---○, 300 mM L-xylose. With other solute concentrations similar results were obtained.

Fig. 2. Packed cell volume in 2.4 osM dimethylsulfoxide (●—●) and in 2.4 osM L-xylose (○---○). The volume is expressed in percent of the volume is distilled water. ---, the volume in 2.4 osM NaCl. The experiments were performed at room temperature.

The osmotic response of yeast cells in a 2.4 osM dimethylsulfoxide and a 2.4 osM L-xylose solution is shown in Fig. 2. As the mean cellular volume increases parallel to the uptake of these solutes, it is obvious that they are present inside the cell in an osmotically active form. The mean cellular volume does not return to its initial value. At equilibrium there is a concentration difference of 0.72 osM dimethylsulfoxide between medium and intracellular water (see Fig. 1). The mean cellular volume in a 0.72 osM NaCl solution appeared to be 89% of the volume in distilled water, in good agreement with the equilibrium volume of 90.5% in dimethylsulfoxide. With L-xylose a similar close relationship between inter- and intracellular concentration difference and mean cellular volume was found.

To study the osmotic behavior of yeast cells, measurements of intercellular water in packed cell columns and of the intracellular water fraction had to be made. The amount of trapped medium was measured at varying NaCl concentrations. The results are shown in Fig. 3. A disturbing influence of dextran adsorption could be excluded by parallel measurements with varying dextran concentrations. Identical results were obtained with dextran concentrations between 0.5 and 3%. The total

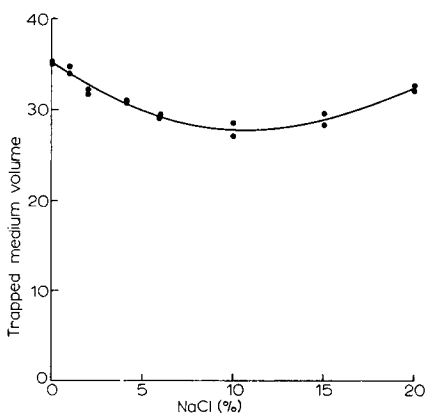
water contents of yeast, spun down from distilled water appeared to be 0.76 ± 0.005 (S.D.) g per g yeast, wet wt. As the specific gravity of this yeast was 1.10, this corresponds to 0.84 ml per ml packed cells of which 0.35 ml is extracellular (see Fig. 3) and 0.49 ml is intracellular water, the remaining 0.16 ml being the solid fraction of the cells. Therefore, the water fraction (W) of the cellular volume must be 0.49/0.65 = 0.75. *Via* a similar calculation, the cellular water fraction of cells, suspended in 5% NaCl appeared to be 0.65.

The osmotic behavior of yeast cells was studied in NaCl concentrations of 0–20%. The relationship between medium tonicity and mean cellular volume is shown in Figs. 4 and 5. At low cell concentrations this relationship is given by the equation[6,7]:

$$\frac{V}{V_0} = WR\left(\frac{1}{C}-1\right)+1 \qquad (1)$$

where V = mean cellular volume, V_0 = mean cellular volume when $C = 1$, W = the water fraction of the cells at $C = 1$, C = tonicity (osmolarity of the medium in percent of the osmolarity of an isotonic solution) and R = an empirical constant. For a perfect osmometer R is equal to one. Calculation of R over the straight part of the curve in Fig. 5 revealed a value of $R = 0.72$, indicating deviation from ideal osmotic behavior.

To explore the background of this deviation, direct measurements of medium and intracellular tonicities were made. Approx. 6 g of yeast were suspended in 40 ml of an NaCl solution of varying concentrations. After 30 min the cells were spun down and disintegrated by freezing and thawing. The osmolarity of supernatant and disintegrated cells was measured. The osmotic value of the cell contents had to be corrected for trapped medium (dextran space), having the same tonicity as the supernatant. The results are given in Table I. In control experiments, it appeared that the experimental error in the instrument reading increased from 1% at 0.1–0.3 osM to about 4% at 2.5 osM. Measurements at higher tonicities were impossible. Further, small errors in the measured dextran space and cellular water fraction will have a



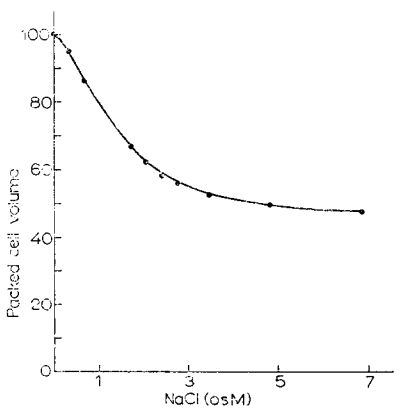


Fig. 3. Trapped medium volume of yeast after centrifugation (in percent of the total packed cell volume) at varying NaCl concentrations, measured with [^{14}C]dextran. With [^{14}C]inuline similar results were obtained.

Fig. 4. Packed cell volume of yeast cells (expressed in percent of the value in distilled water) in varying NaCl concentrations.

pronounced influence on the correction factor, used to calculate the real intracellular tonicity. Notwithstanding these errors, it is beyond any doubt that, even at a medium tonicity of 2.2 osM, the cell contents were hypertonic with respect to the medium.

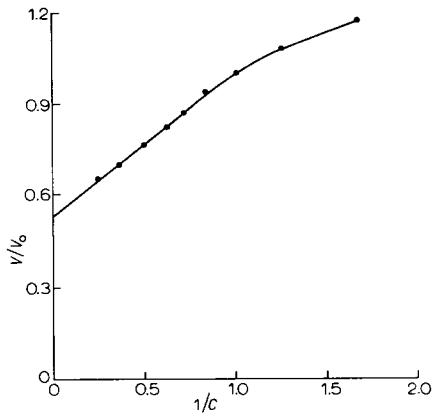


Fig. 5. The relationship between V/V_0 and $1/C$. V was calculated from the packed cell volume, corrected for the dextran space. As unit of tonicity ($C = 1$) 5 % NaCl was chosen. The experimental points are average values of at least 5 determinations. An R value of 0.72 was calculated over the straight part of the curve, by the method of least squares.

A systematic error introduced by the high solid fraction of the disintegrated cell sample could be excluded; similar measurements on disintegrated red blood cells with an even higher solid fraction always indicated equal tonicities of medium and cell contents, within the experimental error.

If the disintegrated cell fraction, prepared from cells that had been suspended in distilled water, is diluted with water, the measured tonicity decreased linearly with the total concentration. Apparently the osmotic coefficient[8–10] of the cellular proteins is not concentration dependent.

Determination of the intracellular K^+ and Na^+ concentrations revealed that the total K^+ contents per cell was constant over the whole range 0–2.7 osM: 137 mequiv K^+ per kg yeast (original weight in distilled water, corresponding to 10^{13}

TABLE I

THE RELATIONSHIP BETWEEN MEDIUM AND INTRACELLULAR TONICITY

V_d = relative dextran space; V_w = relative intracellular water space; C_m = tonicity of the medium (mosM); $C_{d.c.}$ = tonicity of disintegrated cells (mosM); C_i = intracellular tonicity, calculated from the equation:

$$C_i = \frac{C_{d.c.}(V_d + V_w) - V_d.C_m}{V_w}$$

Expt. No.	V_d	V_w	C_m	$C_{d.c.}$	C_i	$C_i - C_m$	$C_i \cdot V_w$
1	0.35	0.50	0	472	802	802	401
2	0.33	0.46	258	588	825	567	380
3	0.30	0.40	530	791	987	457	395
4	0.26	0.34	893	1050	1170	277	398
5	0.24	0.30	1064	1214	1334	270	400
6	0.21	0.27	1412	1570	1693	281	457
7	0.19	0.24	1692	1855	1984	292	476
8	0.16	0.20	2224	2360	2469	245	494

cells). The intracellular sodium contents increased, however, from 3 mequiv/kg yeast in distilled water to 8.6 mequiv at a medium tonicity of 1.7 osM NaCl and 15.5 mequiv/kg yeast at a medium tonicity of 2.7 osM NaCl.

The volume fraction of solvent water for L-xylose and dimethylsulfoxide in yeast, disintegrated by freezing and thawing, was measured by equilibrium dialysis. 15 g of disintegrated yeast were dialyzed against 4 ml of a L-xylose or dimethylsulfoxide solution. The results indicated a volume of distribution of 100.3 $\pm$ 1.6% of the total cell water volume for L-xylose (at L-xylose concentrations of 2 and 4%) and of 101.4 $\pm$ 2.1% for dimethylsulfoxide (at dimethylsulfoxide concentrations of 2 and 4%).

DISCUSSION

The asymmetric L-xylose distribution, corresponding to only 40% of the intracellular water contents, agrees with the observations of KOTYK[1] and KOTYK AND HAŠKOVEC[2]. Apparently an asymmetric distribution is not restricted to non-metabolized sugars, entering the cell *via* a more or less specific transport system. Dimethylsulfoxide, entering the cell by simple diffusion[11], shows a similar asymmetric equilibrium distribution. The possibility of asymmetric distribution of substances entering the cell *via* a carrier mechanism has been deduced theoretically among others by ROSENBERG AND WILBRANDT[12], starting from a conceivable asymmetry of the carrier system itself. These considerations cannot explain, however, an asymmetric distribution of substances entering the cell by simple diffusion. In this context, the observed asymmetric distribution of urea in growing and nitrogen-deficient yeast cells as described by KOTYK AND KLEINZELLER[13], and of dimethylsulfoxide as described in the present communication, is of theoretical significance.

Anaerobically grown yeast is much less structured, as compared to normal yeast[14–16]. The promitochondria in anaerobically grown yeast are much smaller than the normal mitochondria. If morphological distinct structures, inaccessible to certain solutes, would be responsible for unequal solute distribution, differences in this respect between normal and anaerobically grown yeast would have been likely. As shown, such differences were not observed, however.

Apparently L-xylose and dimethylsulfoxide taken up by yeast are present inside the cell in an osmotically active form. From the experimental results it may be concluded that no substantial adsorption inside the cell takes place. As a consequence of the unequal distribution of these substances between medium and intracellular water, there is a permanent change in the ratio of intracellular and medium tonicity, resulting in a persistent decrease in the mean cellular volume at relatively high medium concentrations of these solutes. The osmotic response of the cells to dimethylsulfoxide and L-xylose, as shown in Fig. 2, makes it very improbable that these substances would be taken up in an osmotically inactive cell compartment. The hypothesis that unequally distributed substances would be taken up in a compartment, showing no osmotic response in hypertonic NaCl solutions, seems inconsistent with the fact that the cell does react osmotically to sugar or dimethylsulfoxide uptake into this compartment, as demonstrated experimentally.

The intercellular space of packed cells measured with [^{14}C]inuline and [^{14}C]-dextran appeared to be about 35% of the packed cell volume in distilled water. This

figure is significantly higher than the value of 23%, published by CONWAY AND DOWNEY[17]. This may be attributable to differences between different yeast strains. As discussed above, an experimental error introduced by dextran adsorption to the cell surface could be excluded. The direct measurement of the trapped medium space (as opposed to the usual dilution method) gave good reproducible results and could be used at high salt concentrations. A small but significant fluctuation of the trapped medium volume occurred at increasing NaCl concentrations. The initial value of about 35% in distilled water drops to 28% at 10–12% NaCl, increasing again gradually to 33% in 20% NaCl. Probably these fluctuations are connected with the decreasing cellular volume and with a possible shape change of the cells in hypertonic NaCl solutions.

As the yeast cell has an intracellular tonicity of about 0.8 osM when suspended in distilled water, a considerable pressure must be exerted on the cell wall. Cell shrinking is observed already at medium tonicities, much lower than the intracellular tonicity, apparently evoked by elasticity forces in the cell wall. It might be expected that the yeast cell would display ideal osmotic volume changes at relatively high tonicity, when these elasticity forces would have disappeared. Eqn. 1 was originally derived, to described the osmotic behavior of red blood cells[6,18]. R is a constant, introduced to account for deviations from ideal osmotic behavior. The R value of 0.72 could indicate an osmotically inactive compartment of the yeast cell of 28% of the cellular water fraction in 5% NaCl. Considering the shift of the total intracellular water fraction from 0.27 to 0.50, when the medium tonicity is changed from 5% NaCl to zero (see Table I), this corresponds to 15% of the total cellular water fraction of cells suspended in distilled water.

The alternative explanations for R values lower than unity are[18]: (1) binding of water of solvation to intracellular proteins; (2) a concentration dependence of the osmotic coefficient of intracellular proteins[8,10,18]; (3) changes in the total number of intracellular particles, *e.g.* by transmembrane salt fluxes; (4) a rigidity of the cell membrane or cell wall, resisting volume changes; (5) concentration-dependent changes in the net charge of intracellular proteins[19].

The experiments on equilibrium dialysis demonstrate the absence of protein-bound water, or exclusion water[19,20] with respect to L-xylose and dimethylsulfoxide. This rules out the existence of a functional water compartment, inaccessible to these solutes. Further, as discussed above, the osmotic coefficient of the cellular proteins appeared to be concentration independent, within the experimental error. As shown in Table I, however, the product $C_i \cdot V_w$ increases at high medium tonicities, indicating an increase in the number of osmotically active particles inside the cell. Na^+ uptake accounts for 20–25% of this increase. It is very well possible that a concentration-dependent change in the net charge of intracellular proteins, as demonstrated by GARY BOBO AND SOLOMON[19] for hemoglobin, will explain the further increase. Investigation of this possibility was beyond the scope of the present study. Moreover, even in 2.2 osM NaCl the tonicity of the cell contents exceeds the medium tonicity (see Table I). Apparently even in these shrunken cells elasticity forces in the cell wall are still present. These facts will cause R values less than unity. After correction for the increased number of intracellular particles and for the intracellular over-pressure according to TEORELL[21], the remaining R value appeared to be 0.92–1.04, in different series of experiments. Despite the relatively high experimental error, it seems very

probable that the deviation from ideal osmotic behavior can be explained completely along these lines. But even if the lowest remaining R value (0.92) would be accepted, a hypothetical osmotically inactive compartment in the cell would include less than 5% of the total intracellular water fraction of cells, suspended in distilled water.

The discussed experimental results indicate that the asymmetric solute distribution cannot be ascribed to morphological or functional intracellular water compartments, inaccessible to the solutes. Two alternative explanations remain. The lower intracellular solute concentration may be explained by assuming that the solutes are less soluble in intracellular water than in the surrounding medium water. This hypothesis was postulated among others by TROSHIN[22,23], based on the assumption that water, present in protoplasm, is in an organized ("bound") form. Although the results with disintegrated cells failed to reveal the presence of bound water, these experiments do not definitely exclude the possibility of organized water in the intact cell. However, the analysis of the osmotic behavior of the yeast cells seems to contradict the assumption of organized intracellular water. Finally, the unequal solute distribution may be ascribed to a property of the cellular membrane, causing asymmetric solute influx and outflux. At present this seems to be the most probable explanation. Further experiments to elucidate this problem are in progress and will be discussed in a forthcoming paper.

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