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Effect of plasmolysis upon monovalent cation uptake, 9-aminoacridine binding and the zeta potential of yeast cells

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The ζ potential of yeast cells shows a sudden decrease as the osmotic value of the medium is increased from 1.1 to 1.8 osmol. Above or below these values hardly any change in the ζ potential is observed. This decrease occurs by approximation in the range of osmotic values at which plasmolysis takes place. The surface potential of the yeast probed with 9-aminoacridine on the other hand does not change appreciably over the entire range of osmotic values used. The monovalent cation uptake rate declines gradually as the osmotic value of the medium increases. This seems to be caused by a concomitant rise in cellular K^+ which leads to a drop in the maximal uptake rate of monovalent cations. The K_m of the monovalent cation uptake system is not changed by plasmolysis.

Introduction

Theoretically, the surface potential has a great effect upon ion transport kinetics [1]. This has been experimentally confirmed for monovalent cation uptake in yeast [2,4]. The negative charges on the surface of the cell membrane constituting a negative surface potential lead to high concentrations of cations near to this surface. As a result, the carrier involved in monovalent cation translocation becomes saturated at lower bulk substrate concentrations than would have been the case if the surface potential were zero. As a matter of fact, the K_m for Rb^+ uptake can decrease from 16 mM at zero surface potential to 0.9 mM at a surface potential of about −75 mV [4]. Since the cell wall of yeast also bears a net negative charge [5,6] and because the turgor of the yeast cells causes an intimate contact between the plasmalemma and the cell wall, the charge on the cell wall may also have influence on the accumulation of cations near the cell membrane, and may therefore contribute to the decrease in K_m.

Recently, we showed that for the yeast *Saccharomyces cerevisiae* strain A294 the affinity of Rb^+ for the monovalent cation carrier was not altered by enzymic removal of the cell wall, indicating that the contribution of negative groups on the cell wall to the electrical field near the translocation sites for this strain of yeast is negligible [7]. Unfortunately, *S. cerevisiae* strain Delft II, routinely used in our laboratory for uptake studies is not sensitive to the enzymes normally used for the removal of cell walls [8]. This leaves the question still open whether, in this particular strain of yeast, the cell wall appreciably influences the kinetics of cation uptake. Enzymic removal of the cell wall of *S. cerevisiae* A294 might, in addition, have increased the negative charge of the cell membrane by, for example, the formation of additional negative groups thus compensating the loss of negative charge caused by the removal of the cell wall. We have therefore applied quite a

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different method for studying the possible effect of the cell wall upon Rb^+ uptake kinetics. We plasmolyzed the yeast cells, thus greatly increasing the distance between the cell membrane and the innerside of the cell wall. It is to be expected that an eventual effect of the cell wall upon the Rb^+ uptake will be markedly reduced. We also determined the effect of plasmolysis upon the ζ potential of intact cells and the binding of the surface potential probe 9-aminoacridine to these cells.

Materials and Methods

Preparation of cells, protoplasts and cell walls. Yeast cells, *Saccharomyces cerevisiae,* strain Delft II were starved overnight under aeration. Subsequently, they were washed three times and resuspended in the appropriate buffer or distilled water to the density needed depending on the use. Cells of the non-flocculent brewing yeast *S. cerevisiae* A294 (obtained from Whitbread and Co., Ltd., Luton, U.K.) were grown as described in Ref. 8. After harvesting in the exponential growth phase by centrifugation, the cells were washed three times with distilled water and resuspended in buffer (45 mM Tris-succinate, pH 4.5) to a density of 10% (w/v).

Protoplasts of the strain A294 were prepared according to Ref. 8. They were suspended in 0.82 M sorbitol provided with 1 mM $CaCl_2$ at a density of $0.5 \cdot 10^9$ protoplasts/ml, equivalent to 39 mg dry wt. of intact cells/ml.

Cell walls were isolated according to Ref. 9. The remnants of cell membranes were removed by delipidization in an ethanol/ether (3 : 1) mixture according to Ref. 10. Subsequently, the cell walls were washed ten times in ice-cold distilled water and resuspended in distilled water at a density of 2% (w/v). Per g dry weight of cells 173 mg dry weight of cell walls was obtained.

K^+ uptake. Cells of *S. cerevisiae,* Delft II were preincubated anaerobically under N_2 for 60 min at a density of 4% (w/v) in 45 mM Tris-succinate (pH 4.5) provided with 3% (w/v) glucose. They were then mixed 1 : 1 with buffer containing sorbitol at the appropriate concentration and 2 mM KCl. Uptake of K^+ by anaerobic cells was followed by measuring the decrease in K^+ activity in the medium by means of a K^+-sensitive electrode (Philips IS 560 K) connected to a Pye Unicam Model 292 pH meter.

Rb^+ uptake. Cells of *S. cerevisiae* strain Delft II were suspended to a density of 4.7% (w/v) in 45 mM Tris-succinate (pH 4.5) containing 3% (w/v) glucose and anaerobically preincubated for 1 h at 25°C. The suspension was then thoroughly mixed by vortex stirring on a 1 : 1 basis with 45 mM Tris-succinate (pH 4.5) either with or without 4.7 M sorbitol. After 1 min, appropriate amounts of RbCl provided with a fixed amount of $^{86}RbCl$ were added. Nine 1.8-ml samples were taken at 10-s intervals, filtrated according to Ref. 11 and examined for radioactivity.

Cell size determinations. These were carried by means of a Coulter counter Model ZF equipped with a size-distribution analyzer model P64 according to Ref. 12.

Determination of 9-aminoacridine binding. Intact yeast cells were suspended to a density of 10% (w/v), or protoplasts were suspended to an equivalent of 39 mg dry wt. intact cells/ml. 0.5-ml samples were added to 0.5 ml 90 mM Tris-succinate (pH 4.5) in a 1.5 ml Eppendorf tube provided with 2 μM 9-aminoacridine and sorbitol of appropriate concentration. The tubes were centrifuged after thorough mixing and the supernatant was decanted and measured for 9-aminoacridine fluorescence on an Amino SPF 500 fluorimeter at 400 nm excitation and 458 nm emission.

Osmotic value of the medium. The molality of the media was measured using a Roebling osmometer.

Cell electrophoresis. Electrophoretic mobilities of cells were measured at 25°C according to Ref. 13. From the electrophoretic mobility, the ζ potential was calculated by using the Hemholtz-Smoluchowski equation [14].

Determination of the aqueous volume of the cell envelopes. Cells of *S. cerevisiae* strain Delft II were suspended to a density of 30% (w/v). 5.5-ml portions were pelleted after addition in 11-ml tubes by centrifugation. The supernatant was decanted to waste and the tubes wiped dry according to Ref. 15. Wet weights (W) were determined immediately and tubes were capped. Each yeast pellet was suspended with 4 ml 45 mM Tris-succinate (pH 4.5) with the appropriate sorbitol concentra-

tion and containing either [^{14}C]mannitol or [^{14}C]inulin at a total activity of 20000 cpm · ml^{-1}. After 15 min, the tubes were vortexed again. After 1.5 h, to allow for rebound [15], 1 ml supernatant was taken from the top of the tube and measured for radioactivity (A_s) and in addition the osmotic values of the supernatant were determined. The tubes were again wiped dry. Pellets were resuspended in 1% (w/v) SDS in distilled water to a final volume of 2.15 ml and 0.5-ml samples were taken for radioactivity (A_r). Appropriate corrections for quenching by SDS were applied. The aqueous volume of the cell envelope per g wet weight is given by:

$$(4.3 \times A_r/A_s \times W)_{\text{mannitol}} - (4.3 \times A_r/A_s \times W)_{\text{inulin}} \quad (1)$$

Chemicals. [^{14}C]Mannitol, [^{14}C]inulin and ^{86}Rb were purchased from Amersham International, U.K. The other chemicals used were analytical grade and obtained from commercial sources.

Results

We have at first determined the osmotic values of the medium at which plasmolysis starts and at which plasmolysis is completed. The first value is derived from the dependence of the aqueous volume of the cell envelopes upon the osmotic value of the medium, see Fig. 1, which shows that this volume greatly increases above 0.9 osmolal. Apparently, the cells become plasmolyzed above 0.9 osmolal, a value slightly below 1.1 osmolal found by Arnold and Lazy [15] for incipient plasmolysis of *S. cerevisiae.*

A good estimate of the osmotic value at which plasmolysis is completed can be obtained from the dependence of the cell size on the osmotic value of the medium, see Fig. 2. The occurrence of nonrestricted osmotic behavior, meaning the linear relationship between cell size and the reciprocal of the osmotic value of the medium (π^{-1}), indicates complete plasmolysis. This linear relationship is found at osmotic values of 1.8 osmolal or higher. Below 1.8 osmolal the relationship between the cell size and the reciprocal osmotic value is nonlinear.

The maximal rate of Rb^+ uptake decreases dramatically on plasmolyzing the yeast cells by increasing the osmotic value of the medium to 2.9

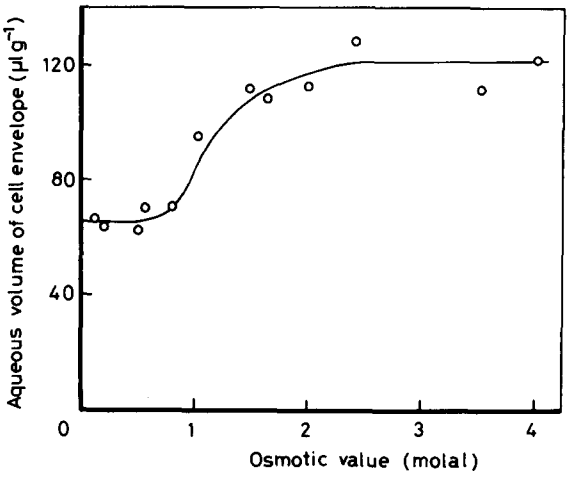

Fig. 1. Dependence of the aqueous volume of the cell envelope of *S. cerevisiae*, Delft II upon the osmotic value of the medium. The osmotic value of the medium (45 mM Tris-succinate of pH 4.5) was varied by adding appropriate amounts of sorbitol. The aqueous volume of the cell envelope is the volume in 1 g wet weight of packed cells which is accessible to mannitol but not to inulin. Each point is determined at least in duplicate. The volume of the cell envelope did not increase significantly on increasing the osmotic value from 0.14 to 0.58 osmolal. The difference in volume was 0.2 ± 2.5 µl · g^{-1} (mean of four experiments, each being carried out in duplicate ± standard error of the mean).

osmolal (18.7 ± 1.5 mmol kg^{-1} · min^{-1} at 0.075 osmolal and 7.0 ± 0.6 mmol kg^{-1} · min^{-1} at 2.9 osmolal). The apparent K_m of Rb^+ uptake how-

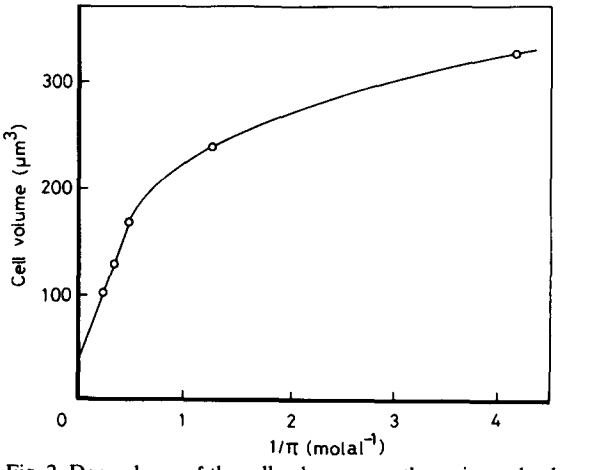

Fig. 2. Dependence of the cell volume upon the reciprocal value of the osmotic value of the medium. The osmotic value (π) was varied by adding appropriate amounts of sorbitol to the 0.9% (w/v) NaCl solution in which the cells were suspended.

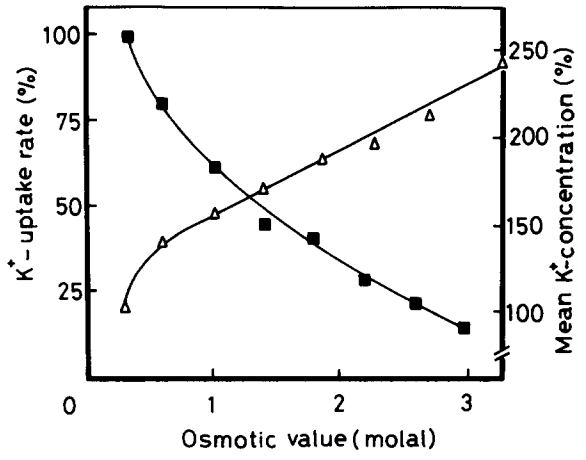

Fig. 3. Dependence of the initial rate of net K^+ uptake at 1 mM K^+ and the initial mean cellular K^+ concentration of *S. cerevisiae*, Delft II upon the osmotic value of the medium. The mean cellular K^+ concentration and the initial uptake rate of K^+ are expressed as percentages of their values determined in the buffer without sorbitol (246 mM K^+ and 16.8 mmol K^+ per kg dry weight per min, respectively). Appropriate corrections for non-solvent cell volume obtained from cell-size determination at varying osmotic value were applied in calculations of the cellular K^+ concentration. ■, Initial rate of K^+ uptake; △, mean cellular K^+ concentration.

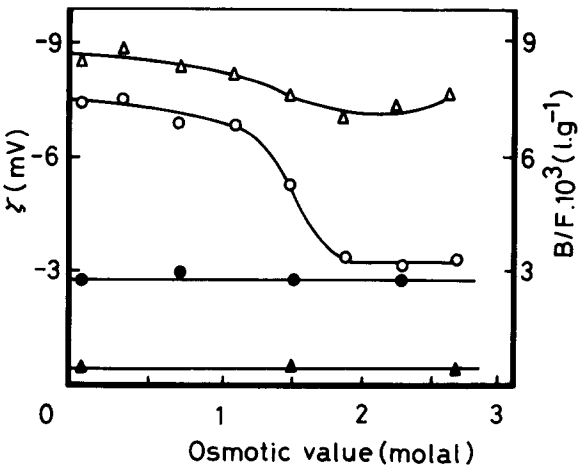

Fig. 4. Binding of 9-aminoacridine to intact yeast cells of *S. cerevisiae*, Delft II, and cell walls. Effect of the osmotic value of the medium on this binding. Comparison with ζ potential of intact cells and cell walls. ○, ●, ζ potential; △, ▲, 9-aminoacridine binding. ○, △, intact cells; ●, ▲, delipidized cell walls. *B*, the amount of dye bound per g dry weight of intact cells, is expressed in $\mu mol \cdot g^{-1}$. *F* is expressed in μM and is the remaining dye concentration in supernatants of the suspension (see Ref. 5).

ever, remains virtually constant (1.34 ± 0.1 and 1.32 ± 0.1 mM at the lower and higher osmolality, respectively).

In order to ascertain whether this change in the maximal rate of monovalent cation uptake is related to the plasmolysis point, we have determined the rate of K^+ uptake. This cation is translocated via the same carrier as Rb^+, under conditions of almost complete saturation of the carrier [11], namely at 1 mM.

Fig. 3 shows that the rate of K^+ uptake decreases gradually as the osmotic value of the medium increases and comparison with Fig. 1 learns that the decrease in the maximal rate of K^+ uptake is not directly related to the increase in periplasmic space. Fig. 3 also shows the change in intracellular mean K^+ concentration resulting from the withdrawal of cell water caused by increasing the osmotic value of the medium.

Information about the dependence of the surface potential of the yeast cells upon the osmotic value of the medium is obtained from studies of the binding of 9-aminoacridine to the yeast cells [5]. Fig. 4 shows that this binding is only slightly decreased by increasing the osmotic value of the medium. Binding of the dye to isolated delipidized cell walls is small and is in addition independent of the osmotic value of the medium. The latter is also true for the ζ potential of the cell walls. The ζ

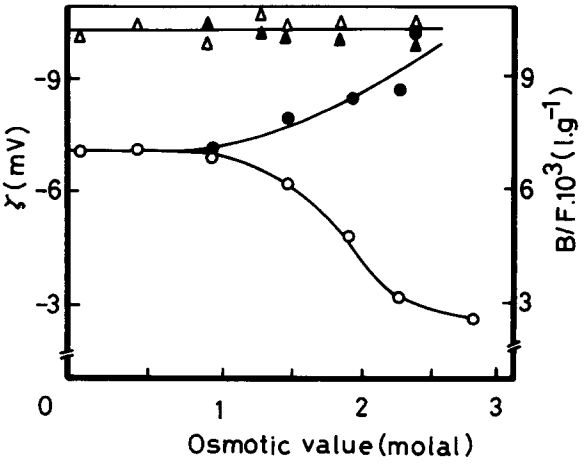

Fig. 5. Effect of osmotic value of the medium on 9-aminoacridine binding and ζ potential of intact cells and protoplasts of *S. cerevisiae* A294. △, ▲, 9-aminoacridine binding; ○, ●, ζ potential. △, ○, intact cells; ▲, ●, protoplasts. See also legend to Fig. 4.

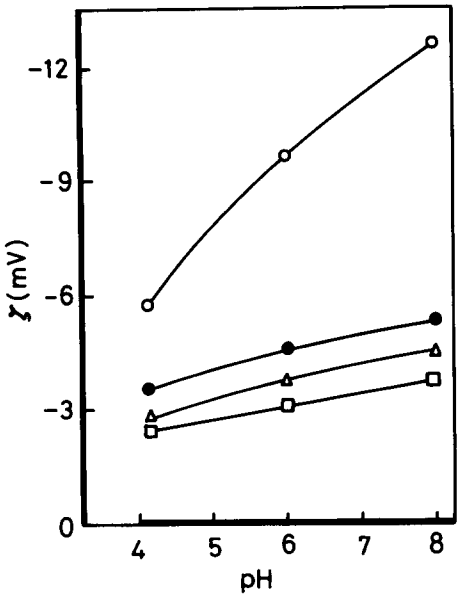

Fig. 6. pH dependence of the ζ potential of intact cells of *S. cerevisiae*, Delft II, and their cell walls at two extreme osmotic values. (○) Intact cells at 0.075 osmolal and (●) at 2.5 osmolal by which the cells are rendered plasmolyzed; (△) cell walls at 0.075 osmolal and (□) at 2.5 osmolal. The buffer consisted of 45 mM Tris brought to the appropriate pH with succinic acid.

potential of intact cells, however, shows a drastic decrease occurring precisely in the range of osmotic values coinciding with the begin and end of plasmolysis, see Figs. 1 and 2.

Fig. 5 shows that the ζ potential of protoplasts made from the yeast strain A294 increases with increasing osmotic value of the medium, whereas the binding of 9-aminoacridine to both intact cells and protoplasts remains constant having the same value for both. Also with intact cells of this strain we see a decrease in the ζ potential over the range of osmotic values of the medium at which plasmolysis occurs. These experiments are carried out with a strain of yeast, different to the one commonly used in our laboratory, the Delft II strain, because, from the latter no protoplasts can be obtained.

Non-plasmolyzed yeast cells show a much greater pH dependence of the ζ potential than plasmolyzed cells. The latter is more similar to that of isolated cell walls as shown in Fig. 6. In accordance with Fig. 4 the effect of osmotic pressure changes upon the ζ potential of isolated cell walls is very small.

Discussion

Our results strongly indicate that the negative groups of the cell wall do not contribute appreciably to the electrical field near the binding sites of the monovalent cation carrier. Increasing the distance between the plasmalemma and the cell wall by plasmolysis does not have any detectable effect upon the apparent affinity of Rb^+ to the carrier. This means that accumulation of Rb^+ in the region near the plasmalemma is not appreciably influenced by the Donnan potential of the cell wall. Plasmolysis has also very little influence on the binding of the positively charged 9-aminoacridine. Since this cation mainly binds to the plasmalemma and binding to the cell wall is very small it can be concluded that the binding of this cation to the plasmalemma is not affected by plasmolysis. It follows also that the accumulation of 9-aminoacridine in the region near the plasmalemma is not reduced on increasing the distance between the cell wall and the plasmalemma and that the surface potential is not changed. Our results now support our previous findings obtained with the strain A294 which showed that enzymatic removal of the cell wall affected neither the kinetics of Rb^+ uptake nor the binding of the dye [7] and indicates that removal of the cell wall does not create additional negative groups.

Our observation that the zeta potential of intact cells is much more negative than the zeta potential of isolated cell walls is in apparent contradiction with earlier findings [16], which showed that the ζ potentials of intact cells and cell walls do not differ much. It should be emphasized, however, that the cell walls used by us have been delipidized, whereas the results previously reported were obtained with non-delipidized cell walls. In the latter case the cell walls may still have plasmamembrane remnants adhering to them.

The ζ potential, supposedly, measures the potential that exists at the hydrodynamic plane of shear [17], also referred to as the slipping plane [18]. The exact location of this plane, however, has not yet been determined and remains uncertain. Our present results with yeast cells suggest that this slipping plane is situated in the region of or passing through the cell wall, as will be argued below.

Because of turgor, the plasmamembrane makes intimate contact with the cell wall. As we have already shown, it is the plasmamembrane which carries the negative charges which determine the surface potential and as long as the plasmamembrane is close to the cell wall the negative charges of the membrane will contribute to the ζ potential of the cell. As the osmotic value of the medium is increased the intimate contact of the plasmamembrane and the cell wall becomes less. The drop in ζ potential begins at 1.1 osmol. This value is only slightly higher than the value at which plasmolysis commences and reaches a value nearly equal to that of the cell wall at 1.8 osmol, the osmotic value at which plasmolysis is complete. This could mean that because the plasmamembrane is now placed at a greater distance from the cell wall, the negative charges on the plasmamembrane no longer contribute to the potential at the slipping plane, which we assume to be situated in the cell wall. In this way, we can account for the drop in the ζ potential which takes place between 1.1 and 1.8 osmol. This view is supported by the fact that the pH dependence of the ζ potential of the plasmolyzed cells markedly differs from that of non-plasmolyzed cells, this being almost the same as that of cell walls. The large pH dependence of the ζ potential of intact cells indicates that a relatively large contribution to it is given by weakly acidic groups, probably carboxylic groups on the cell membrane [5]. The small pH dependence, however, of the cell walls and plasmolyzed cells indicates that in these cases mainly strongly acidic groups are involved, presumably the phosphate groups of the phosphomannans located in the cell wall [19].

The increase in the ζ potentials of protoplasts of the A294 strain found on increasing the osmotic value of the medium points to an increase in negative charge density of the cells caused by shrinkage of the protoplasts. The finding that the binding of 9-aminoacridine is unaltered by increases in the osmotic value of the medium, on the other hand, suggests that the net charge density remains more or less unchanged. This apparent discrepancy can be understood by assuming that the charges are discretely distributed over the plasmamembrane, in accordance with previous findings [5].

The gradual reduction of the K^+ uptake resulting from increasing the osmotic value of the medium can be explained by a drop in the maximal uptake rate probably resulting from the increase in the internal monovalent cation concentration as a consequence of the shrinkage of the cells. This rise in cellular K^+ seems to reduce the maximal uptake rate of K^+ by some feedback system [20].

On summing up, increasing osmotic stress on yeast cells brings a decrease in the maximal uptake rate of monovalent cations, but leaves the affinity of monovalent cations for the uptake sites unaffected. The surface potential of the plasmamembrane as measured by the binding of 9-aminoacridine remains unchanged. This seems to be a reliable measure for the surface potential also during osmotic stress. However, the application of the ζ potential measured via free-flow cell electrophoresis as a diagnostic tool in studies on changes in the surface potential of yeast cells [4] should be restricted to non-plasmolyzed cells.

Acknowledgments

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