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The concentration dependence of the depolarization of yeast by monovalent cations

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Monovalent cations decrease the initial rate of uptake of the membrane potential probe 2-(dimethylaminostyryl)-1-ethylpyridinium (DMP) into metabolizing cells, showing that the cells are depolarized. A steep decrease in this rate was found even at low cation concentrations, reaching 62%, 42%, 58%, 40% and 40% at high concentrations of K^+, Rb^+, Cs^+, Na^+ and Li^+, respectively. The corresponding concentrations at which half-maximum decrease was found were 0.22, 0.36, 1.2, 17 and 17 mM. These values are of the same order of magnitude as the half-saturation concentrations for monovalent cation uptake by the yeast.

Introduction

There are several indications that uptake of monovalent cations into yeast causes a depolarization of the cells. Roomans et al. [1] showed that divalent cation uptake in metabolizing yeast cells was impaired by monovalent cations with the same cation selectivity as found for monovalent cation transport in yeast. It was argued that this inhibition was due to depolarization of the yeast cells. Direct evidence for a monovalent cation-selective depolarization of metabolizing yeast cells has been presented by Peña et al. [2]. They showed that K^+ depolarizes the yeast cells more effectively than Na^+. The concentration at which K^+ depolarizes the cells half-maximally appeared to be equal to the K_m for the K^+ uptake system.

We now examined whether monovalent cations other than K^+ also depolarize yeast cells when added at concentrations which gradually saturate the monovalent cation carrier. The effect of the cations was studied at pH 7. At that pH the concentration dependence of both Rb^+ and Cs^+ uptake shows only small deviations from Michaelis-Menten kinetics, whereas at low pH deviations from Michaelis-Menten kinetics are more pronounced [3,4]. On the other hand the deviations found for Na^+ uptake are still considerable at pH 7, if Na^+ is applied at concentrations below 4 mM [3].

We have shown before that DMP is a convenient probe for the membrane potential of yeast, because its use allows a continuous fluorimetric registration of the membrane potential dependent uptake of this probe [5]. In our earlier studies on the kinetics of monovalent cation uptake, initial rates of uptake were obtained from uptake curves determined within about 45 s [3,4]. On prolonged incubation of the yeast with the cations their kinetical parameters for transmembrane transport may change appreciably as has been shown for both Rb^+ and K^+ uptake [6,7]. Therefore kinetics of ion uptake and membrane potential can only be compared properly if both are measured within the same time interval. Since equilibration of DMP between cells and medium proceeds rather slowly and the membrane potential may change appreciably during the time required for probe equilibration [5], we decided to take the initial rate of DMP uptake as a measure for the membrane potential.

Materials and Methods

10% (w/v) *Saccharomyces cerevisiae* (fresh weight of pressed cake of Koningsgist) suspended in distilled water was starved for 24 h by aeration at room temperature. Then the cells were washed twice with distilled water and once with buffer (45 mM Tris brought to pH 7.0 with succinic acid). Subsequently the cells were resuspended in buffer at a cell density of 2.4% (w/v) and kept anaerobically by bubbling N_2 through the suspension.

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Abbreviations: TPP, tetraphenylphosphonium; DMP, 2-(dimethylaminostyryl)-1-ethylpyridinium.

In uptake studies, DMP dissolved in 0.1% (v/v) methanol was added to a final concentration of 1.4 μM. For this purpose 2.5 ml cell suspension was brought into a socalled multi-purpose cuvet [8] to which the appropriate additions were made. The suspension in the cuvet was stirred continuously. The uptake of DMP was studied at a yeast density of 2% (w/v) obtained after adding 5% (w/v) glucose, DMP and when indicated also the various chloride salts. The times at which DMP or the salts were added are indicated in the figures. In the experiments referring to Figs. 4–6 the total added ionic strength was kept constant at 30 mM by means of choline chloride in order to rule out indirect effects of the ionic strength upon DMP uptake into the cells due to changes in the surface potential [9]. As a measure for DMP uptake we determined the fluorescence of the suspension by means of an Aminco SPF 500 spectrofluorimeter at excitation and emission wavelengths of 470 and 565 nm, respectively.

DMP was from Sigma, St. Louis, MO, USA. All chemicals were of analytical grade. The yeast was kindly provided by Gist-Brocades, Delft, Netherlands.

Results

We first determined the most suitable conditions for studying the effects of the monovalent cations upon the initial rate of DMP uptake. Fig. 1 shows the time course of DMP uptake in the yeast when glucose and DMP were added together at zero time. As has been shown earlier [5], under these conditions there is an initial rapid influx of DMP and equilibration of DMP between cells and medium is already reached within 4 min. Thereafter a slow decrease in the cellular DMP concentration was found pointing to a gradual depolarization of the cells. Fig. 1. shows that at 25–35 min the cellular DMP uptake reached a minimum, whereafter it increased again gradually up to about 90 min after the addition of glucose at which time a second peak in the time course of DMP uptake was found. Prolonged incubation provoked again a gradual release of DMP from the cells. When N_2 was passed through the suspension and the fluorescence was measured at equal intervals, first during 10 min each 30 s and after that time each 2 min, the second peak appeared to be less pronounced then when the suspension was stirred mechanically. The first peak was not affected by N_2.

Apparently the membrane potential of the yeast is not constant but varies with time. We have chosen the incubation period of 25–30 min after the addition of glucose for studying the effects of the monovalent cations upon the uptake of DMP, because in this time interval the membrane potential is fairly constant. Furthermore, during this time interval the release of K^+ from the cells is also minimal, whereas at the peaks K^+

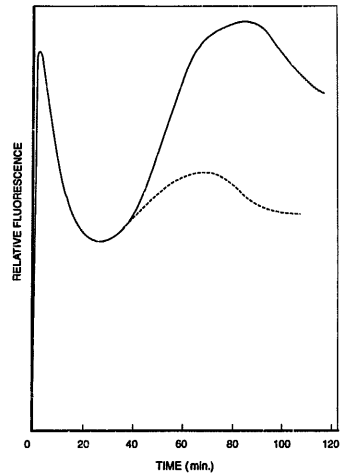

Fig. 1. Time course of DMP uptake measured as the intensity of fluorescence on adding 1.4 μM DMP together with 5% (w/v) glucose to a 2% (w/v) yeast suspension (final concentrations). Full drawn line: trace recorded continuously of a mechanically stirred yeast suspension. Dashed line: DMP fluorescence measured discontinuously of the suspension that was bubbled with N_2 between the measurements.

release is maximal [5] which could interfere with interpreting effects of cations on the membrane potential. In addition, at 25–30 min no effect of stirring the suspension or passing N_2 through the suspension on DMP uptake is observed. As will be outlined in the discussion, the larger DMP uptake observed in mechanically stirred suspensions is probably due to accumulation of CO_2 in the suspension. In order to minimalize this accumulation, the cell suspension was gassed during the first 25 min with N_2, whereafter the cells were kept in suspension by stirring which allowed continuous registration of the fluorescence intensity of the suspension.

When cells are depolarized by adding K^+, one would expect that DMP is released from cells which have been loaded with DMP in the presence of glucose. This appeared to be true, and is shown in Fig. 2. Addition of KCl at 25 min almost immediately decreased the fluorescence of the suspension, which decrease appeared to be partly transient. Probably the initial transient decrease in fluorescence was due to a disturbance of the yeast suspension caused by injecting the small

volume of solution in which the KCL was dissolved, since a similar dip in fluorescence is found when an aliquot of distilled water is injected. 10 and 100 mM KCl enlarged this initial decrease in fluorescence. This additional decrease in fluorescence might be ascribed to a reduction in the small amount of DMP bound to the outerside of the yeast cells and constituents released by the cells into the medium, which binding was suppressed by increasing the ionic strength of the medium (data not shown). The fast initial decreases in the fluorescence of the suspension were followed by a slower reduction of the fluorescence that was caused by efflux of the dye from the cells. The maximal slopes of the efflux curves were 3.6 ± 0.1, 3.6 ± 0.3 and 4.0 ± 0.2 at 1, 10 and 100 mM KCl, respectively, taking the rate of DMP efflux at zero KCl as 1.

Fig. 3 shows the time course of uptake of DMP when DMP was added 25 min after the addition of glucose. The rate of influx was about 9-times lower than 3 min after the addition of glucose at which time the rate of DMP uptake was maximal. Addition of 10 mM KCl together with the DMP led to a considerable reduction in the rate of DMP uptake. A small part of this reduction could be ascribed to the increase in ionic strength, since an equimolar amount of choline also led to a decrease in the rate of DMP uptake. On comparing the effects of equal concentrations of KCl and choline chloride on the fluorescence increase it is seen that the relative effect of KCl was maximal near zero time. Furthermore, when DMP was added 5 min after KCl, the initial rate of DMP uptake was increased again, showing that the inhibition of DMP uptake by K^+ was partly transient. This supports our view that

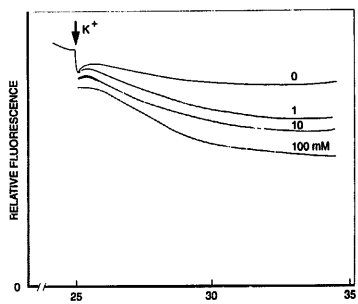

Fig. 2. Effect of varying amounts of KCl added at 25 min after the addition of glucose and 1.4 μM DMP upon the fluorescence of DMP. The final concentrations of KCl added were 0, 1, 10 and 100 mM.

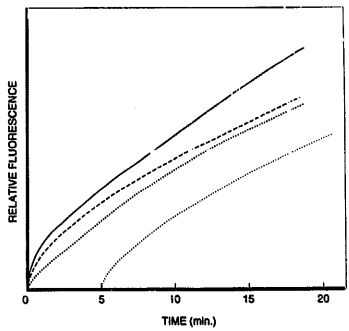

Fig. 3. Time course of the uptake of DMP found on adding DMP at 25 min after the addition of 5% (w/v) glucose to 2% (w/v) yeast suspension. Effect of 10 mM choline chloride and 10 mM KCl added at 25 min. Fulldrawn line, control without addition, dashed line 10 mM choline and dotted line 10 mM KCl. In a parallel experiment the DMP was added to the suspension 5 min after the addition of 10 mM KCl.

for a proper comparison of ion uptake kinetics and membrane potential it is necessary to study initial uptake rather than the final equilibrium partition of DMP between cells and medium.

We decided to examine the effects of monovalent cations upon DMP uptake by adding both DMP and the cations at 30 min after the addition of glucose. Furthermore in all experiments the change in ionic strength was kept constant at 30 mM by means of choline chloride. Under these conditions 5 mM K^+ led to a reduction in the initial uptake rate of DMP amounting to $51 \pm 5\%$ of the control value. When the K^+ and the choline were added 5 min prior to the DMP, the uptake rate was reduced to only $74 \pm 10\%$, confirming that the depolarization by K^+ was partly transient.

As shown in Fig. 4 the DMP influx within the short period of 2 min was still rather complex. First a very fast process, which was completed within 10 s or less and which was independent of the presence of glucose, occurred. This process consisted for the greater part of binding of DMP to cellular constituents released from the cells during the 30-min preincubation period. Almost the same increase in fluorescence was namely found on adding DMP to supernatants of cell suspensions that were incubated in the presence of glucose for 30 min. Furthermore, binding of DMP to the outerside of the yeast cell may also contribute to the immediate increase in fluorescence. The rapid increase in fluorescence was not affected by adding K^+ up to 5

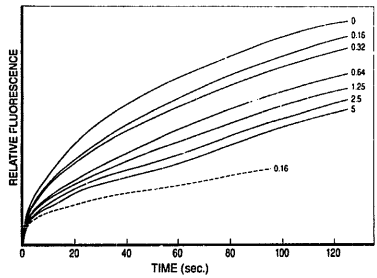

Fig. 4. Effect of varying amounts of K^+ added 30 min after the addition of glucose together with 1.4 μM DMP upon DMP uptake. Effect of omittance of glucose. The final concentrations of K^+ added expressed in mM are indicated. Dashed line, uptake of DMP at 0.16 mM K^+ in the absence of glucose. The increase in ionic strength caused by the addition of the salt is kept constant at 30 mM by means of choline chloride added together with K^+ and DMP.

mM. On the other hand the increase in fluorescence found after the initial rapid increase appeared to depend upon both the presence of glucose and the concentration of added K^+. Similar results were obtained for the effect of Rb^+, Cs^+, Na^+ and Li^+ (data not shown). The effect of Rb^+ was slightly smaller than that of K^+, whereas for Na^+ and Li^+ far more higher concentrations were required to provoke a detectable decrease in DMP uptake. The effect of Cs^+ was intermediate to that of Na^+ and Rb^+.

The concentration dependence of the decrease in DMP influx by K^+ and the other cations is shown in Fig. 5. We took the tangents to the DMP uptake curves at 10 s after the addition of DMP as a measure for the rate of DMP uptake. Then the rapid initial increase in fluorescence, which was independent of metabolism and cation concentration, was almost completed.

As shown in Fig. 5A the influx rate of DMP was strongly decreased at already very low K^+ concentrations. 0.12 mM K^+ already caused a reduction of 16%, whereas a maximal reduction in DMP influx was already found at 1 mM K^+. The effect of Rb^+ upon the DMP influx was somewhat smaller than that of K^+. In Fig. 5B we compared the effects of Cs^+, Na^+ and Li^+ upon DMP uptake. The effectivity of monovalent cations in reducing the rate of DMP uptake was for Cs^+ much larger than for Na^+ and Li^+.

In all cases examined the effect of the various cations upon DMP uptake showed saturation kinetics. So we tried to fit the data to a Michaelis-Menten equation. Fig. 6 shows plots of the decrease in DMP influx against the quotient of this decrease and the concentration of the cation applied. According to this way of plotting a linear relationship will be found if a Michaelis-Menten equation applies [10]:

$$DIF = DIF_m \cdot S/(S_{1/2} + S) = DIF_m - S_{1/2} \cdot DIF/S \qquad (1)$$

DIF is the difference in DMP uptake rate found in the absence of added cation and that found in the pres-

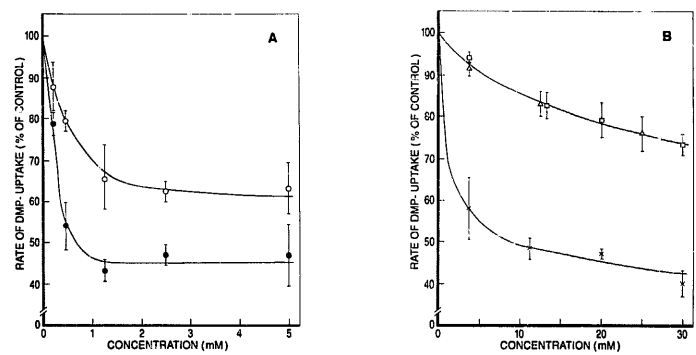

Fig. 5. Dependence of the rate of uptake of DMP added at 30 min after the addition of glucose upon the concentration of added cations. The rates of DMP uptake were obtained by taking the slope at 10 s after addition of the salts and DMP to the yeast suspension. Same conditions as in Fig. 4. (A) Effect of K^+ (●) and Rb^+ (○). (B) Effect of Cs^+ (×), Na^+ (△) and Li^+ (□). The bars indicate the standard errors of the mean.

TABLE I

Comparison of half-value concentrations ($S_{1/2}$) for the effect of the monovalent cations upon DMP uptake with half-value concentrations for cation uptake (K_m)

The half-value concentrations are expressed in mM.

	DIF_m	$S_{1/2}$	K_m [a]	K_m [b]	K_m [c]
K^+	62	0.22	0.09	0.5	–
Rb^+	42	0.36	0.33	1.0	–
Cs^+	58	1.2	2.3	7.0	–
Na^+	40	17	18	16	–
Li^+	40	17	36	27	33

[a] These values are obtained from Ref. 11. They represent the sum of the values of the dissociation constants for the activation site and the substrate site. This equals the slope of the straight line in a Hofstee plot, and is therefore directly comparable with the $S_{1/2}$ values obtained for DMP uptake studies. The values have been determined under approximately similar conditions as applied in the DMP uptake experiments except that no choline was present and the time of preincubation of the cells in the presence of glucose was 60 min instead of 30 min. For K^+ and Li^+ no uptake studies have been carried out by us, therefore we took the calculated dissociation constants obtained from competitive inhibitor studies of Rb^+ uptake by K^+ or Li^+. [b] See Ref. 12. [c] See Ref. 13.

ence of added cation expressed as the percentage of DMP influx without added cations. DIF_m is the maximal decrease in DMP influx also expressed in percents of DMP influx in the absence of added cations and $S_{1/2}$ represents the concentration of the monovalent cation concerned at which the decrease in DMP influx is half-maximum. Fig. 6 shows that the effects of the various cations upon the DMP influx can be described by a Michaelis-Menten equation within the experimental error. The rates of DMP uptake found in the presence of Li^+ and Na^+ did not differ significantly. Therefore a single straight line was drawn through the data points for Li^+ and Na^+ in Fig. 6B. The maximal decrease in DMP uptake (DIF_m) appeared to be the largest for K^+ followed by Cs^+ and Rb^+ and was the smallest for Li^+ and Na^+. K^+ had the lowest half-maximum concentration followed by Rb^+, whereas Cs^+ has a still higher $S_{1/2}$. The highest values were found for Li^+ and Na^+, see Table I in which we compared $S_{1/2}$ with K_m values for cation uptake reported in literature.

Discussion

In a earlier study we demonstrated that the rate of uptake of the lipophilic cation TPP by yeast is transiently increased shortly after the addition of glucose to the cell suspension at pH 7 [5]. This is also true for the influx of DMP (data not shown). Furthermore the cellular contents of both TPP and DMP show a peak at about 4 min, whereafter they decrease again indicating that the transient increase in probe influx is at least partly due to transients in the membrane potential. However, additional changes in the permeability of the cell membrane or in the negative surface potential can still not be ruled out.

The reduction in the peak of DMP uptake by passing N_2 through the yeast suspension instead of stirring the suspension may be due to removal of CO_2 accumulated into the medium. Accumulation of CO_2 in the medium causes a decrease in cell pH [14]. By this the plasma membrane ATPase may be stimulated giving rise to an increase in the rate of H^+ extrusion and concomittant hyperpolarization of the cells [15,16].

Taking the rate of influx of DMP into the cells as a measure for the membrane potential, as we have done, is only allowed if the cations added do neither affect the permeability of the cells for DMP nor their surface

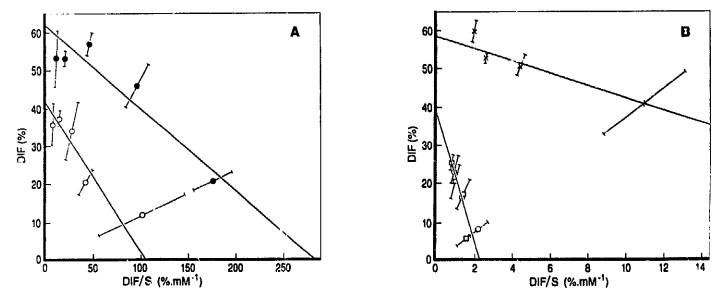

Fig. 6. Hofstee plots of the difference in the percentage of initial rate of DMP uptake and the quotient of this difference and the monovalent cation concentration. See further legend to Fig. 5.

potential. The latter has been assured by keeping the total added cation concentration at 30 mM. The increased DMP efflux rate observed at 1 to 100 mM K^+ shows that the cells are indeed depolarized by K^+. Since uptake of Rb^+ is driven by the membrane potential [17], the depolarization caused by the cations will be primarily due to the influx of the positively charged ions. The decrease in the 'initial' rate of DMP uptake determined by us may mainly reflect this effect. On prolonged incubation of the cells with K^+ additional changes in the membrane potential may occur. Initially the cell pH will increase [14,18,19] and the ATP level of the cytosol will decrease [20], which may give rise to a decrease in H^+ extrusion causing a further depolarization. However, there are no indications that within the time interval of 10–30 s after the addition of K^+ the relative decreased in DMP uptake rate is changed (data not shown). On the other hand on incubating the cells with K^+ for 5 min the reduction in DMP uptake is considerably lowered, pointing to a hyperpolarization of the cells following the initial depolarization. This may be due to the fact that loading the cells with K^+ is accompanied by a decrease in K^+ influx [6,7]. Also acidification of the cells following the initial alkalinization accompanying K^+ uptake may contribute to this effect [14]. The situation will be even more complex if also changes in H^+ permeability are involved [21].

We applied the cations in a concentration range at which their uptake shows no appreciable deviations from Michaelis-Menten kinetics [11]. The values of the apparent K_m values for monovalent cation uptake given in Table I refer to the same range of cation concentrations. Addition of 30 mM choline reduces the negative surface potential of the yeast cells which increases the K_m for Rb^+ uptake just like Tris does [22]. For K^+, Rb^+ and Cs^+ uptake the K_m may increase about 60%. For Na^+ and Li^+ uptake this increase will be somewhat smaller. Apparently there is a rather good correlation between the K_m values and the half-saturation concentrations for reduction of DMP uptake, not only for K^+, as has been already shown by Peña et al. [2], but also for Rb^+, Cs^+, Na^+ and Li^+.

Cation uptake affects the membrane potential on the one hand, but on the other hand the membrane potential also influences cation uptake [23,24]. Calahorra et al. [17] have provided direct evidence that uptake of Rb^+ into plasma membrane vesicles of yeast is driven by the membrane potential. Therefore a depolarization of the yeast by cat ion uptake, showing similar kinetics as cation uptake is quite in line with expectations.

Acknowledgements

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