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APPARENT SATURATION KINETICS OF DIVALENT CATION UPTAKE IN YEAST CAUSED BY A REDUCTION IN THE SURFACE POTENTIAL

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The concentration dependence of the uptake rate of divalent cations in yeast can be described by a simple diffusion process after accounting for the effect of the surface potential upon the divalent cation concentration near the membrane. It is also necessary to correct for the effect of the cell pH upon the rate of translocation. The apparent saturation kinetics is ascribed to the fact that the quotient of the concentration of the divalent cations near the cell membrane and the bulk aqueous phase concentration is reduced on increasing the divalent cation concentration in the medium. The diffusion process regulated by the surface potential even mimics the saturation kinetics of a two-carrier transport system. The selectivity found between Ca^{2+} and Sr^{2+} uptake can probably be traced to differences in their affinity for the negative groups on the cell membrane determining the surface potential rather than to differences in their affinity for a transport system. The enhancement of divalent cation uptake by loading the cells with phosphate is probably due to the concomitant increase in the net negative charge of the cell membrane.

Introduction

Divalent cation uptake in yeast shows apparent saturation kinetics. In many cases deviations from Michaelis-Menten kinetics are found, which are mostly traced to the involvement of a dual mechanism consisting of two simultaneously operating single-site transport processes with different K_m values [1–5]. However, as pointed out by us in a theoretical study of the effect of the surface potential (ψ) upon ion translocation kinetics [6–8], this type of kinetics may also come to the fore when only one single-site transport mechanism is involved, namely when ψ is reduced on increasing the divalent cation concentration in the medium. Then the quotient of the interfacial substrate concentration near the membrane and the bulk aqueous phase concentration decreases with increasing substrate cation concentration in the medium. This causes the deviations from Michaelis-Menten kinetics. As will be shown in this publication a similar type of kinetics may even be found when a simple diffusion process is involved. Indications that ψ has an effect upon divalent cation uptake in yeast already have been presented earlier [5]. We will now present a more quantitative study of the effects of ψ upon both Ca^{2+} and Sr^{2+} uptake in yeast making use of both the data published earlier [5] and some additional new data.

Methods

Uptake of $^{45}Ca^{2+}$ was studied with metabolizing cells, 2% (w/v), *Saccharomyces cerevisiae,*

Abbreviations: Tris, tris(hydroxymethyl)aminomethane; ψ, surface potential; ζ, zeta potential; pH_i, cell pH; pH_o, medium pH.

Delft 2, in the presence of 3% glucose under anaerobic conditions according to Ref. 5. The medium consisted of 45 mM Tris adjusted to the desired pH with succinic acid. The cells were grown on a phosphate-poor medium. Phosphate-rich cells were prepared according to Ref. 5. Zeta potentials (ζ) were determined by means of microelectrophoresis according to Ref. 9. pH_i was determined according to Ref. 10. Radioactive $^{45}CaCl_2$ was purchased from the Radiochemical Centre, Amersham, U.K. Kinetical constants were computed by means of least-square fitting methods according to the appropriate rate equations. For the $^{89}Sr^{2+}$ uptake experiments, see Ref. 5. Corrections for the effect of changes in pH_i, found on varying pH_o, upon Ca^{2+} or Sr^{2+} uptake were made according to Ref. 5, in which the effect of pH_i at constant pH_o upon divalent cation uptake is determined. The corrected values refer to pH_i = 7.2.

Theory

The concentration dependence for the initial uptake rate of divalent cation translocation by a saturable system with a single binding site is given by:

$$v = \frac{V_{max} s'}{K_m + s'} \tag{1}$$

s' represents the concentration of the cation near the membrane. This concentration is related to the bulk aqueous phase concentration (s) by the Boltzmann equation which on neglecting the differences in activity coefficients between the two phases is given by:

$$s' = s(e^{-F\psi/RT})^2 = sy^2 \tag{2}$$

ψ is the surface potential. F, R and T have their usual meaning. Eliminating s' from Eqn. 1 by means of Eqn. 2 leads to:

$$v = \frac{V_{max} s}{K_m y^{-2} + s} = \frac{V_{max} s}{K_{m,app} + s} \tag{3}$$

$K_{m,app}$ still depends upon s. We assume that ψ is negative, then $y > 1$ and $K_{m,app} < K_m$. $K_{m,app}$ will increase on increasing s, since ψ is reduced with increasing divalent cation concentrations [7]. This will give rise to deviations from Michaelis-Menten relations. If $K_{m,app} \gg s$ Eqn. 3 will approximate to:

$$v = (V_{max}/K_m) y^2 s = Dy^2 s = Ds' \tag{4}$$

If the influx of the substrate into the cells proceeds by a simple diffusion a similar equation for the influx equation would be found with D as the diffusion constant, and s' as the substrate concentration. Since y decreases with increasing substrate cation concentration no simple linear relation between v and s will be found, but v will increase less than proportionally with increasing s.

According to Eqn. 4 a plot of $\ln(v/s)$ against ψ will give rise to a straight line according to Eqn. 5:

$$\ln(v/s) = \ln D - 2F\psi/RT \tag{5}$$

As shown recently, for yeast, ψ is linearly related to ζ [9]. Therefore $\ln(v/s)$ will also be linearly related to ζ:

$$\ln(v/s) = \ln D - Q\zeta \tag{6}$$

From Eqns. 5 and 6 it follows that ψ is related to ζ by:

$$\psi = (QRT/2F)\zeta \tag{7}$$

From the effect of divalent cations upon the kinetical constants of Rb^+ uptake in yeast found earlier [11] it was derived that:

$$1/y = (1/y_0) + a\sqrt{s} \tag{8}$$

y_0 is the value of y in the absence of added divalent cations. Eliminating y from Eqn. 4 by means of Eqn. 8 leads after rearranging the equation to:

$$\sqrt{(s/v)} = (1/\sqrt{D})\left((1/y_0) + a\sqrt{s}\right) = A + B\sqrt{s} \tag{9}$$

This relation can be applied in order to linearize the rate equation involved.

Provided that D is constant, A is a measure for y_0 thus also indirectly for ψ determined in the absence of added divalent cations. B is a measure for the effectivity by which the divalent cations reduce ψ. If y_0 is known, a can be calculated from

the quotient B/A. The factor a will increase with increasing affinity of the divalent cations for the negative groups located at the medium side of the cell membrane. D can be calculated from:

$$D = (Ay_0)^{-2} \tag{10}$$

Results

The rate of divalent cation uptake depends upon pH_i [5]. As pH_i changes concomitantly with pH_o, for a proper evaluation of the effect of pH_o upon the uptake rates a correction for the effect of pH_i upon the uptake rates should be made, see under Methods.

Fig. 1 shows the concentration dependence of Ca^{2+} uptake represented according to Hofstee [11]. For each pH_o applied two curves are given; one referring to the actual rates of uptake and one after correcting them for the effect of pH_i. If the concentration dependence is described by a single Michaelis-Menten equation straight lines would be found in the Hofstee plot. However, concave curves are found, which can be described formally by the sum of two Michaelis-Menten equations: one with a low K_m of about 0.1 mM and one with a high K_m of the order of magnitude of 2–8 mM (see Table I).

In Fig. 2 we compare the data of Fig. 1 with some data on Sr^{2+} uptake published earlier [5]. The values of v/s are proportional to the rate of uptake of the radioactive isotope added together with non-radioactive Ca^{2+} or Sr^{2+}. These rates

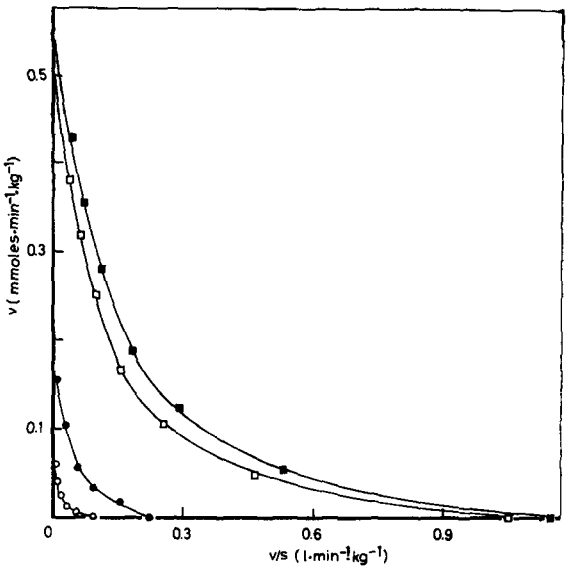

Fig. 1. Concentration dependence of Ca^{2+} uptake at pH_o 4.5 and 6.5. Plot of v against v/s. ●, ○, pH_o 4.5; ■, □, pH_o 6.5. Closed symbols no pH_i correction, open symbols with pH_i correction according to Ref. 5. The uptake rate at pH_i 7.1 is taken arbitrarily as 1.

decrease with increasing non-radioactive ions. Apparently the decrease in the $^{89}Sr^{2+}$ uptake rate with increasing Sr^{2+} concentrations is less than the decrease in $^{45}Ca^{2+}$ uptake rate with increasing non-radioactive Ca^{2+} concentration found at pH_o 6.5. Preloading the cells with orthophosphate leads to an increase in the rate of Sr^{2+} uptake.

There is a striking similarity between the dependence of $\ln(v/s)$ upon the divalent cation con-

TABLE I

KINETICAL PARAMETERS OF DIVALENT CATION UPTAKE

The K_m and V_{max} values are calculated by fitting the data of Fig. 2 to the rate equation for a dual transport mechanism according to Ref. 11. A and B are calculated by fitting the data to Eqn. 9. y_0 is the value of y calculated by means by Eqn. 2 when no divalent cations are present. The coefficient a equals the slope of the straight line obtained on plotting $1/y$ against $\sqrt{s}$ (data not shown). According to Eqn. 9 a should equal B/Ay_0. K_m is expressed in mM; V_{max} in $mmol \cdot min^{-1} \cdot kg^{-1}$ dry weight; A in $kg^{0.5} \cdot min^{0.5} \cdot l^{-0.5}$; B in $kg^{0.5} \cdot min^{0.5} \cdot mmol^{-0.5}$; y_0 is dimensionless; a is expressed in $mM^{0.5}$ and D in $l\ kg^{-1} \cdot min^{-1}$. P−, means no phosphate loading; P+, means cells loaded with phosphate.

P	Ion	pH_o	$K_{m,1}$	$K_{m,2}$	$V_{max,1}$	$V_{max,2}$	A	B	y_0	a	B/Ay_0	10^6D
−	Ca^{2+}	4.5	0.29	8.5	0.02	0.071	3.32	3.17	24	0.028	0.040	158
−	Ca^{2+}	6.5	0.071	2.5	0.055	0.398	1.03	1.36	81	0.0162	0.0163	155
−	Sr^{2+}	6.5	0.20	5.8	0.11	0.77	1.12	0.97	81	0.0092	0.0107	122
+	Sr^{2+}	6.5	0.17	2.9	0.39	2.54	0.50	0.48	245	0.0035	0.0039	67

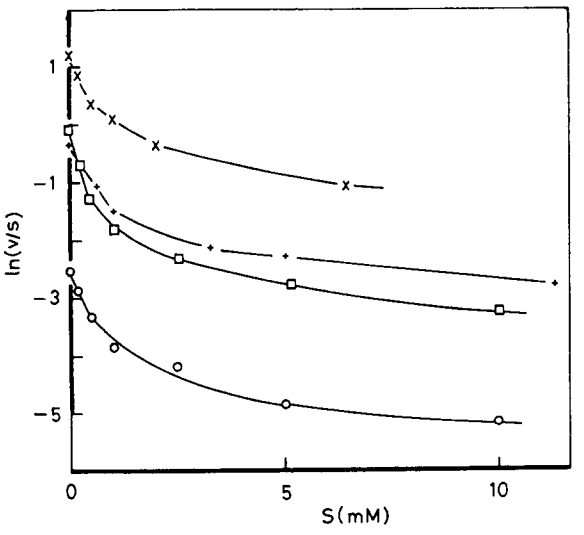

Fig. 2. Dependence of the rate of radioactive divalent cation uptake upon the concentration of non-radioactive ions, at varying pH_o and the effect of preloading the cells with phosphate. The uptake rates are corrected for the effect of varying pH_i upon the uptake rates, see also subscript to Fig. 1. ○, Ca^{2+} uptake at pH_o 4.5; □, Ca^{2+} uptake at pH_o 6.5; +, Sr^{2+} uptake at pH_o 6.5 and ×, Sr^{2+} uptake at pH 6.5 in phosphate-loaded cells.

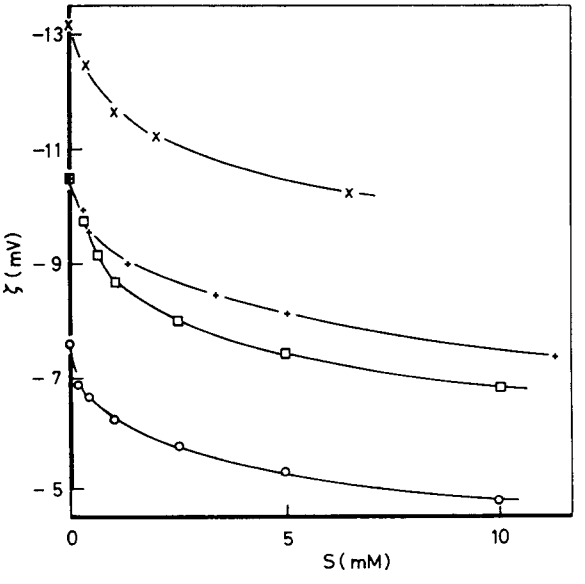

Fig. 3. Dependence of the zeta potential upon the divalent cation concentration, pH_o and upon preloading the cells with phosphate. For symbols see Fig. 2.

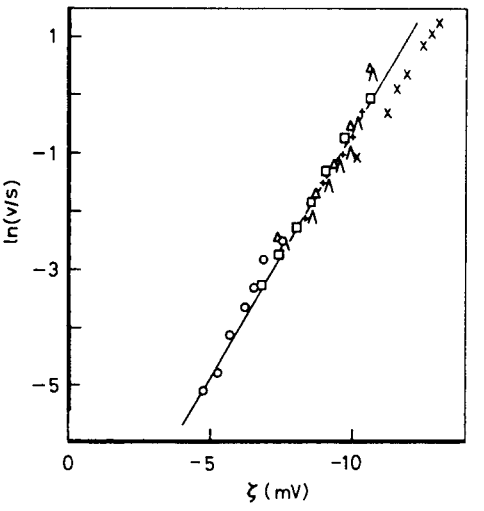

Fig. 4. Dependence of the uptake rate of divalent cations upon the zeta potential at varying divalent cation concentration, varying pH_o and the effect of preloading the cells with phosphate. Λ, carrier-free Sr^{2+} uptake at varying pH_o. For the meaning of the other symbols, see legend to Fig. 2. The rates of Sr^{2+} and Ca^{2+} uptake are corrected for the effect of pH_i.

centration and the dependence of ζ upon this concentration, see Fig. 3. The reduction in ζ found on increasing the divalent cation concentration in the medium is less with Sr^{2+} than with Ca^{2+}. Preloading the cells with phosphate leads to an increase in the value of ζ. In addition ζ is reduced considerably on decreasing pH_o from 6.5 to 4.5.

Fig. 4 shows that a linear relationship exists between $\ln(v/s)$ and ζ for both Ca^{2+} and Sr^{2+} uptake independent of the way by which v/s is varied namely either by increasing the divalent cation concentration or by varying pH_o. The data points referring to Sr^{2+} uptake by phosphate-loaded cells lie below the straight line drawn through the points obtained for cells with a low phosphate content. The slope of the straight line drawn is 0.84. According to Eqn. 7 $\psi = 10.8\zeta$; with Eqn. 2 we get: $y = \exp(-0.42\zeta)$.

Fig. 5 shows that $\sqrt{(v/s)}$ is linearly related to $\sqrt{s}$ according to Eqn. 9. Values of the coefficients a, see Eqn. 9, are calculated from the quotient B/A and values of y_0. The values of a calculated are compared with values of a obtained from plots of $1/y$ agaisnt $\sqrt{s}$ which plots yield straight lines (data not shown) according to Eqn. 8. It is seen

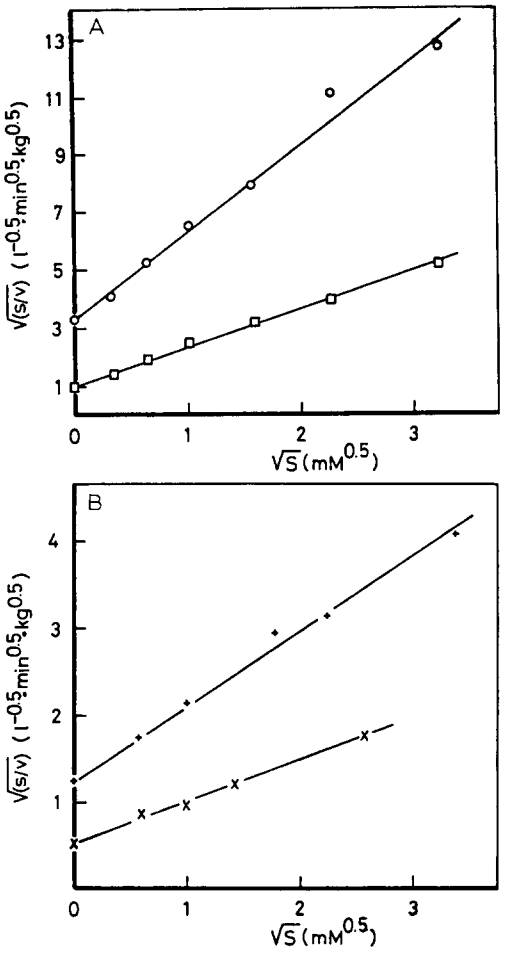

Fig. 5. Linearisation of the concentration dependence of the uptake rate according to Eqn. 9. (A) Ca^{2+} uptake; (B) Sr^{2+} uptake. See further legend to Fig. 2.

that the values of a do not much depend upon the way they are calculated, see Table I.

Discussion

Ca^{2+} and Sr^{2+} uptake in yeast can be described formally by a dual mechanism of two simultaneously operating single-site transport processes or alternatively by a single two-site transport mechanism [12]. This is in accordance with the finding of other authors [1–5]. According to the dual mechanism model one of the two carriers has a high affinity for the divalent cations with a K_m of approx. 0.1 mM and the other one has a low affinity for the ions with a K_m varying from 2 to 9 mM. Our study, however, shows that it is more likely that divalent cations are absorbed in yeast by way of a simple diffusion process or by means of a single-site translocation mechanism of which the affinity to the cations is extremely low. This conclusion is reached from the striking similarity between the dependence of the rate of radioactive isotope uptake upon the divalent cation concentratoin (Fig. 2) and the dependence of ζ upon the cation concentration (Fig. 3) resulting in a linear single relation between $\ln(v/s)$ and ζ (Fig. 4). The apparent saturation kinetics found can be accounted for quantitatively by the reduction in ψ on increasing the divalent cation concentration in the medium. This causes a decrease in the quotient of the interfacial divalent cation concentration near the membrane and the bulk aqueous phase concentration. Not only the apparent saturation kinetics but also the effect of pH_o upon divalent cation uptake can be almost quantitatively accounted for by changes in ψ. It should be stressed that this is only found after correcting the uptake rates for the effect of pH_i [5].

Fig. 2 shows that the reduction in the rate of radioactive Ca^{2+} uptake caused by increasing the non-radioactive Ca^{2+} concentration is much larger than the reduction in the rate of radioactive Sr^{2+} uptake in the presence of non-radioactive Sr^{2+}. Similarly the reduction in ζ is much greater with Ca^{2+} than with Sr^{2+}. This shows that differences in the concentration dependence of these two divalent cations should be ascribed to differences in effectivity by which ψ is reduced instead of to differences in the affinity of a hypothetical carrier for the two ions. The effectivity by which ψ is reduced is expressed by the magnitude of the coefficient a, see Eqns. 8 and 9. This value appears to depend upon pH_o and also upon the loading of the cells with phosphate, see Table I. In addition a depends upon the divalent cation examined. At pH_o 6.5 a smaller value is found for Sr^{2+} uptake than for Ca^{2+} uptake. This indicates that Ca^{2+} is more strongly bound to the negative groups on the cell membrane than Sr^{2+}. This is in accordance with earlier findings. Rb^+ uptake in yeast is inhibited by divalent cations by way of an apparent competitive inhibition [11]. This inhibition is in fact due to an increase in K_m caused by the reduction in the negative surface potential. The

effect of Ca^{2+} is larger than the effect of Sr^{2+} [13].

The enhancement of divalent cation uptake by preloading the cells with phosphate was initially attributed to an increase in the divalent carrier concentration [14–16]. An other possible cause for the increase in the rate of divalent cation uptake considered is that this increase is due to the increase in cell ATP [6,17]. The results obtained in this study provide an alternative explanation, namely that enhancement of divalent cation uptake caused by loading the cells with phosphate is due to the concomitant increase in the negative surface potential.

In an earlier paper [7] we have shown that a surface potential regulated cation transport process that is mediated by a saturable system with a single binding site may show similar saturation kinetics as is found for a dual mechanism or for a multi-site system. The present paper shows that even a simple diffusion process may show this complicated saturation kinetics if the process is influenced by the surface potential.

The zeta potential of the yeast cells appears to be much lower than the electrostatic potential experienced by the divalent cations in their translocation across the membrane. This is possible if the negative charges are not uniformly distributed over the surface of the membrane and the transport of the cations occurs in areas of the membrane that are in close proximity of these negatively charged groups. In such a case a relatively high discrete charge potential is experienced in the transport process, while the zeta potential, which depends upon the average charge density, may be appreciably lower. In a recent study [9] we have calculated that the electrostatic potential experienced by the monovalent cation carrier of yeast cells is also an order of magnitude larger than the zeta potential. It might be hypothesized therefore that both monovalent and divalent cation transport occurs in regions of the yeast plasmamembrane with a high density of negative charges. The observation of Schmidt et al. [18] that cationic silica microbeads are clustered in spots and are not evenly distributed over the plasmamembrane surface of the yeast cell is indicative for the existence of localized charged areas on the membrane surface.

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