Interaction of Cations with Phosphate Uptake by *Saccharomyces cerevisiae*

EFFECTS OF SURFACE POTENTIAL

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The effect of bivalent cations on phosphate uptake by *Saccharomyces cerevisiae* was investigated. Phosphate uptake via the Na⁺-dependent transport system at pH 7.2 is stimulated by bivalent cations. The apparent affinity of phosphate for the transport mechanism is increased, but the apparent affinity for Na⁺ is decreased. Uptake of phosphate via the Na⁺-independent transport system is accompanied by a net proton influx of 2H⁺ and an efflux of 1 K⁺ for each phosphate ion taken up. At pH 4.5 phosphate uptake via the Na⁺-independent system is stimulated by bivalent cations, whereas at pH 7.2 uptake is inhibited. The effect of bivalent cations on phosphate uptake can be ascribed to a decrease in the surface potential.

In previous papers (Roomans et al., 1977; Roomans & Borst-Pauwels, 1977) we have demonstrated the existence of two separate transport systems for phosphate in *Saccharomyces cerevisiae*. One of these systems is Na⁺-dependent; it can be kinetically described as a mechanism with one site with affinity of phosphate, and two sites to which Na⁺ or Li⁺ may bind. One of the two cation-binding sites may, however, be apparent, as is discussed in this paper. Other alkali ions have no appreciable affinity for the Na⁺-dependent mechanism. The other phosphate-uptake system occurring in yeast appears to be a system by which phosphate is co-transported with protons or exchanged for cellular hydroxy ions (Cockburn et al., 1975; Borst-Pauwels & Peters, 1977; Roomans & Borst-Pauwels, 1977).

Biological membranes bear a net negative surface charge, which gives rise to an electric potential at the membrane surface (the surface potential) that attracts cations and repels anions. Consequently, the ion concentration in the region adjacent to the membrane will differ from that in the bulk solution, and the difference will depend on the magnitude of the surface potential. Since in kinetical studies of ion transport it is not the ion concentration in the bulk solution but the ion concentration near the membrane that is the relevant parameter, the magnitude of the surface potential and the factors affecting this magnitude are of great importance. Although the absolute value of the surface potential of the yeast cell is not known, it has been shown that the surface potential is affected by pH and polyvalent cations (Theuvenet, 1978). It should be emphasized that the surface potential is not identical with the membrane potential, which is the electrical potential difference across the membrane.

In a theoretical study (Roomans & Borst-Pauwels, 1978) we have shown that the effect of changes in the surface potential on a co-transport mechanism by which anions are co-transported with cations is much more complex than in the case of a transport mechanism by which only cations or anions are transported. Both the magnitude of the effect on the rate of anion uptake, and its direction, may be influenced by the co-substrate (cation) concentration. In addition we have shown that the effects of the surface potential on ion uptake via a co-transport system are markedly affected by the order in which the ions bind to the transport system.

Experimentally it has been shown that the effect of bivalent cations on ion-uptake kinetics could be attributed to the effect of these cations on the surface potential (Theuvenet & Borst-Pauwels, 1976a,b; Roomans et al., 1979). In the present paper we have investigated the effects of the surface potential on phosphate uptake by yeast by determining the effects of bivalent cations on phosphate uptake via the Na⁺-dependent and the Na⁺-independent phosphate-uptake mechanism. The experimental observations are compared with the theory.

**Experimental**

Yeast cells, *Saccharomyces cerevisiae* strain Delft II, with a low phosphate content, were suspended in water and starved by aeration for 20h. After starvation, the cells (0.5 or 1.0%, wet wt./volume) were incubated for 60 min in 25 mm-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid]/imidazole buffer, pH 7.4 (final pH 7.2), or for 20 or 60 min in
45 mM-Tris/succinate buffer, pH 4.5, in the presence of 3% (w/v) glucose at 25°C. N₂ was bubbled through the suspension continuously. The uptake of phosphate (added to the medium as Tris/phosphate) was studied by using [32P]P, as a tracer, and the uptake of Na⁺ was studied by using ²²Na as a tracer, as described earlier (Roomans & Borst-Pauwels, 1977). Nine successive samples of the yeast suspension were taken within 40–60s, filtered, and washed with ice-cold water (phosphate uptake) or 50 mM-MgCl₂ (Na⁺ uptake). In most cases uptake kinetics were linear within this period, but at the lowest phosphate concentrations, and if in addition the rate of uptake was high (in the presence of high Na⁺ concentrations), deviations from linearity could occur after about 20s. The Pᵢ concentrations used ranged from carrier-free phosphate (only radioactive Pᵢ added) to 200 μM (pH 4.5) or 1200 μM (pH 7.2). Uptake of ⁴⁰Ca was determined under similar conditions, but the samples were washed with 50 mM-EDTA (adjusted to pH 8.5 with NaOH) and the radioactivity was determined by means of liquid-scintillation analysis. Initial uptake rates were determined from the slopes of the tangents to the uptake curves at zero time.

Complexing of phosphate by Ca²⁺ at pH 4.5 was studied with a Ca²⁺-selective electrode (Philips IS 560); solutions of CaCl₂ in Tris/succinate buffer were titrated with NaH₂PO₄ as described by Kobos & Rechnitz (1976).

Efflux of K⁺ was measured with a K⁺-selective electrode (Philips IS 561) in a buffered (45 mM-Tris/succinate buffer) suspension. Proton fluxes were measured in unbuffered suspension, in the presence of 10 mM-KCl; during preincubation the pH was kept constant at pH 4.5 by means of a pH-stat, with triethanolamine (20 mM) as a titrant. Just before addition of phosphate, the suspension was removed from the pH-stat and the pH changes before and after addition of phosphate were continuously measured. The buffering capacity of the system was determined by titration with ethanolamine. To determine the ratio of phosphate uptake and proton influx, phosphate uptake was measured under similar conditions (Seaston et al., 1973; Cockburn et al., 1975).

Results

P₁ uptake is mediated by two mechanisms, a high-affinity mechanism that is Na⁺-dependent, and a low-affinity mechanism that is Na⁺-independent (Roomans et al., 1977). The effect of addition of 4 mM-Mg²⁺ on uptake of Pᵢ at pH 7.2 in the presence of 15 mM-Na⁺ is shown in Fig. 1. Phosphate uptake via the Na⁺-dependent mechanism appears to be stimulated by Mg²⁺, in contrast with uptake via the Na⁺-independent mechanism, which appears to be inhibited (except at infinitely high Pᵢ concentrations). We will first discuss the Na⁺-dependent mechanism.

![Fig. 1. Effect of Mg²⁺ on the kinetics of phosphate uptake at pH 7.2 in the presence of 15 mM-NaCl](image-url)

We have previously given a kinetic description of the Na⁺-dependent phosphate-uptake mechanism, assuming two sites with affinity for Na⁺ ions (Roomans et al., 1977). We have also shown theoretically that the effect of changes in the surface potential on ion uptake via such a co-transport mechanism is dependent on the order in which the ions bind to the co-transport mechanism (Roomans & Borst-Pauwels, 1978). To determine the order of binding of Na⁺ and Pᵢ, use can be made of the fact that these ions do not influence each other's affinity for the co-transport mechanism (Roomans et al., 1977). From this we may conclude that Na⁺ ions and Pᵢ ions bind to the co-transport mechanism in random order (Roomans & Borst-Pauwels, 1978). The kinetic parameters of Pᵢ and Na⁺ uptake by the co-transport mechanism are given in the Appendix (eqns. 1, 2 and 4–6).

The effect of Mg²⁺ on the kinetic parameters of the Na⁺/phosphate co-transport mechanism is summarized in Table 1. Since phosphate is taken up as the univalent anion only (Goodman & Rothstein, 1957) and at pH 7.2 only 20% of the phosphate is in this form, an appropriate correction was made. The data are also corrected for complex-formation between phosphate and Mg²⁺ [about 23% of the phosphate is complexed by Mg²⁺ under these conditions (Kobos
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& Rechnitz, 1976). The affinity of phosphate for the translocation mechanism, $K_{m,t}$, can be calculated from the experiment shown in Fig. 1. As shown previously (Roomans et al., 1977), $K_{m,t}$ corresponds to the concentration of $P_i$ at which half-maximal stimulation of $Na^+$ uptake is found; this value can be calculated from Fig. 2. The value of $K_{m,t}$ is decreased by addition of $Mg^{2+}$ from about 0.60 to 0.17 $\mu M$. This decrease is in accord with eqn. (2) of the Appendix. Since $K_{m,t}$ is linearly related to $y$, it can be calculated that the value of $y$ at 4 mM-$Mg^{2+}$ is approx. 30% of that of the control. According to eqn. (1) of the Appendix a small decrease of the maximal rate of the co-transport may be expected after a decrease of the surface potential. This decrease may, however, be compensated by a stimulation of the rate of uptake, due to hyperpolarization by $Mg^{2+}$.

The maximal stimulation of $Na^+$ uptake by phosphate, at very low $Na^+$ concentrations, is decreased by $Mg^{2+}$ ions (Fig. 2). Under the experimental conditions applied, about 30 $\mu M$-$Na^+$ is present in the medium, due mainly to leakage of $Na^+$ from the cells, as the radioactive $Na^+$ added was of negligible concentration. According to eqn. (6) of the Appendix the decrease of the maximal stimulation of $Na^+$ uptake (under these conditions approximately equal to $F_{max}$) should be equal to the decrease in $K_{m,t}$ since both parameters are proportional to $y$. It can be seen from Fig. 2 and Table 1 that this is indeed the case.

The effect of $Mg^{2+}$ on the affinity of the co-transport mechanism for $Na^+$ has been determined by studying the stimulation of carrier-free $P_i$ uptake, i.e. under conditions where eqn. (4) of the Appendix applies, as a function of the $Na^+$ concentration, with and without added $Mg^{2+}$ (Fig. 3). The $K_m$ for $Na^+$ of the high-affinity site is increased by the same factor as that by which the $K_m$ for phosphate is decreased (Table 1), as was indeed expected from eqn. (5) of the Appendix. At high concentrations of $Na^+$ the surface potential is already seriously affected by $Na^+$, and the addition of $Mg^{2+}$ ions will have less effect. In fact, it may be expected that at infinitely high concentrations of $Na^+$, where all negative sites are screened, $Mg^{2+}$ will have no effect.

The rate of phosphate uptake at extremely low phosphate concentrations, via the co-transport mechanism with $Na^+$, as a function of the $Mg^{2+}$ and $Ca^{2+}$ concentrations is given in Fig. 4. After correc-

![Graph](image)

Fig. 2. Effect of $Mg^{2+}$ on uptake of $Na^+$ via the $Na^+/phosphate$ co-transport mechanism at pH 7.2

$F_s$ (u/s at very low $Na^+$ concentrations) is plotted against the quotient of $F_i$ and the phosphate concentration $s_i$ (in $\mu M$). The data are corrected for complexing of phosphate by $Mg^{2+}$ ions. ○, Control; ●, 4 mM-$MgCl_2$ added. $Na^+$ uptake was measured as described in the Experimental section, at a yeast concentration of 1.0% (w/v). The concentration of $Na^+$ during the experiment was about 30 $\mu M$, due to $Na^+$ leakage from the cells; the concentration of added $^{22}Na^+$ was negligible. The concentrations of $P_i$ ranged from 1 to 50 $\mu M$.

Table 1. Effect of 4 mM-$MgCl_2$ on the kinetic parameters of $Na^+$ and phosphate uptake via the co-transport mechanism

Kinetic constants were calculated by the method of Cleland (1967); the calculated constant and the standard error are given. The data are corrected for complexing of phosphate by $Mg^{2+}$ ions. If applicable, the data refer to univalent phosphate.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>4 mM-$MgCl_2$ added</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_m$ for phosphate, $K_{m,t}$ ($\mu M$)</td>
<td>0.65 ± 0.20</td>
<td>0.17 ± 0.04</td>
</tr>
<tr>
<td>From Fig. 1</td>
<td>0.55 ± 0.04</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>From Fig. 2</td>
<td>0.70 ± 0.20</td>
<td>0.85 ± 0.10</td>
</tr>
<tr>
<td>Maximal rate of phosphate uptake, $V_i$ (mmol/min per kg), from Fig. 1</td>
<td>0.07 ± 0.01</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>$K_m$ for $Na^+$ (high-affinity site), $K_{m,t}$ (mm), from Fig. 3, see eqn. (3) of the Appendix</td>
<td>2.05 ± 0.06</td>
<td>0.61 ± 0.02</td>
</tr>
<tr>
<td>$F_{max}$ (1 = min$^{-1}$ kg$^{-1}$), from Fig. 2, see eqn. (6) of the Appendix</td>
<td>2.05 ± 0.06</td>
<td>0.61 ± 0.02</td>
</tr>
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tion for complex-formation of these ions with phosphate (Kobos & Rechnitz, 1976), it can be seen that 
Mg$^{2+}$ and Ca$^{2+}$ have about the same effect. Theuvenet (1978) found the following relation between 
the concentration of the bivalent cation ($s_b$) and $y$:
\[
\frac{1}{y} = c_1 + c_2 s_b^+ \tag{1}
\]
where $c_1$ and $c_2$ are constants, depending on the bi-
valent cation species and the pH; this relation is based 
on experimental findings (Theuvenet & Borst-
Pauwels, 1976a). If the maximal rate of phosphate 
uptake in the presence of 15 mM-Na$^+$ is not 
significantly affected by changes in the surface potential, 
as indeed suggested by Fig. 1, the rate of uptake at 
very low phosphate concentrations ($F_1$) can be 
approximated by:
\[
F_1 = \frac{V_i}{K_i y} \tag{2}
\]
and, combining eqns. (1) and (2), by approximation:
\[
F_1 = \frac{V_i}{K_i} (c_1 + c_2 s_b^+) \tag{3}
\]
According to eqn. (3) we may, by approximation, 
expect a linear relationship between $F_1$ and the square 
root of the bivalent cation concentration. Fig. 5 
shows that this is indeed the case.

As an alternative hypothesis, the possibility was 
considered that bivalent cations might substitute for 
Na$^+$ and be co-transported with phosphate, and by 
that mechanism stimulate phosphate uptake. Uptake

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**Fig. 3. Effect of Mg$^{2+}$ on the apparent affinity constants of the co-transport mechanism for Na$^+$**

$F_i (\mu l:min^{-1}.kg^{-1})$ is plotted against the quotient of $F_i$ and the Na$^+$ concentration $s_j$ (in mM). The data are corrected for complexing of phosphate by Mg$^{2+}$ ions. ○, Control; ●, 4 mM-MgCl$_2$ added. Carrier-free phosphate was used. The pH of the suspension during the experiment was 7.2. The other experimental details are the same as for the experiment in Fig. 1.

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**Fig. 4. Effect of Ca$^{2+}$ and Mg$^{2+}$ on phosphate uptake by the Na$^+$/phosphate co-transport mechanism at pH7.2**

○, Mg$^{2+}$; ●, Mg$^{2+}$, data corrected for complex-
formation with phosphate; △, Ca$^{2+}$; ▲, Ca$^{2+}$, data corrected for complex-formation with phosphate. Carrier-free radioactive phosphate was used; the concentration of Na$^+$ was 15 mM. The other experimental details are the same as for the experiment shown in Fig. 1.

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**Fig. 5. Effect of bivalent cations on phosphate uptake by the Na$^+$/phosphate co-transport mechanism at pH7.2**

$F_i$ is plotted against the square root of the Ca$^{2+}$ or 
Mg$^{2+}$ concentration. The data are from Fig. 4, 
corrected for complex-formation. ●, Mg$^{2+}$; ▲, Ca$^{2+}$. 

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of Ca\textsuperscript{2+} was, however, not stimulated by phosphate, neither in the presence nor in the absence of Na\textsuperscript{+} ions.

Phosphate uptake via the Na\textsuperscript{+}-independent transport system is accompanied by net proton influx and extrusion of K\textsuperscript{+} ions (Cockburn et al., 1975). We have determined the ratios of the proton, phosphate and K\textsuperscript{+} fluxes at pH 4.5 at a phosphate concentration of 0.5 mM. It was found that addition of phosphate to the yeast suspension caused an efflux of 0.95 ± 0.09 K\textsuperscript{+} ions for each phosphate ion taken up (mean ± S.D. of five experiments), whereas a net proton influx of 2.01 ± 0.30 H\textsuperscript{+} for each phosphate ion taken up could be measured (means ± S.D. of ten experiments). Probably phosphate is co-transported with 2 protons (or exchanged for cellular OH\textsuperscript{-} ions); electroneutrality is maintained by efflux of one K\textsuperscript{+} ion.

It has been shown by Borst-Pauwels & Peters (1977) that the dependence of the maximal rate of phosphate uptake on the pH of the suspending medium is, in fact, only apparent; \( V_I \) depends only on the cell pH. The independency of the maximal rate of phosphate uptake on the extracellular proton concentration may be explained by assuming that protons bind to the carrier before phosphate (Roomans & Borst-Pauwels, 1978) (see eqn. 7 of the Appendix). In that case, \( V_I \) is independent of the cation concentration and of the surface potential.

Theeuvenet & Borst-Pauwels (1976b) showed that at pH 4.5 Mg\textsuperscript{2+} did not significantly affect the maximal rate of phosphate uptake via the Na\textsuperscript{+}-independent system. This appears to be also the case at pH 7.2 (Fig. 1). The effects of bivalent cations on phosphate uptake may then be ascribed to effects on the apparent \( K_m \) of the carrier for phosphate.

The effect of Ca\textsuperscript{2+} on phosphate uptake at extremely low P\textsubscript{i} concentrations via the Na\textsuperscript{+}-independent system at pH 4.5 and 7.2 is shown in Fig. 6. The data are given with and without correction for complexing of phosphate ions by Ca\textsuperscript{2+}. At pH 4.5 phosphate uptake is enhanced by Ca\textsuperscript{2+} until maximal stimulation is reached at about 9 mM-Ca\textsuperscript{2+}. The decrease of the stimulation at higher Ca\textsuperscript{2+} concentrations can be explained by complexing of phosphate. At pH 7.2 phosphate uptake is slightly inhibited by Ca\textsuperscript{2+}.

We have shown theoretically (Roomans & Borst-Pauwels, 1978) that, if an anion is co-transported with cations, not only the magnitude of the effect of a decrease of the surface potential, but also its direction (stimulation, inhibition), depends on the cation concentration. The decrease of the proton concentration near the membrane due to the decrease of the surface potential may cause inhibition of the co-transport at high pH, where the H\textsuperscript{+} concentration is the limiting factor, whereas at low pH the occupation of proton-binding sites may not be significantly affected and the increase of the concentration of phosphate near the membrane will result in stimulation of phosphate uptake. At relatively high concentrations (>10 mM) of Ca\textsuperscript{2+} other effects of Ca\textsuperscript{2+} may come to the fore, such as depolarization of the membrane (J. A. Hoeberichts, A. Klaassen, P. Barts & G. W. F. H. Borst-Pauwels, unpublished work). It should also be realized that at pH 4.5 the surface potential may be rather low owing to protonation of negative sites on the membrane (Theeuvenet, 1978). In that case the decrease in the surface potential by Ca\textsuperscript{2+} may become less at higher Ca\textsuperscript{2+} concentrations.

At pH 4.5 also univalent cations (K\textsuperscript{+}, Rb\textsuperscript{+}, Cs\textsuperscript{+}) stimulate phosphate uptake via the Na\textsuperscript{+}-independent uptake mechanism. Univalent cations are less effective than bivalent cations; 30 mM-K\textsuperscript{+} is needed to obtain a stimulation of about 40%. This is indeed expected if the effect is due to a decrease of the surface potential, and has also been found with sulphate uptake (Roomans et al., 1979).

**Discussion**

The observed effects of bivalent cations on phosphate and Na\textsuperscript{+} uptake via the co-transport mechanism compare very well with the theoretically predicted effects of a decrease of the surface potential. Although also other effects of bivalent cations, such as hyper-
polarization, may play a role in the observed stimulation, it appears that the effect of bivalent cations via a decrease of the surface potential is quantitatively the most important. The finding that univalent cations, which depolarize the membrane, stimulate the Na⁺-independent phosphate uptake at pH 4.5 also points to an effect of the surface potential rather than of the membrane potential. Since univalent cations and bivalent cations in concentrations where they affect the membrane potential in an opposed way (J. A. Hoeberichts, A. Klaassen, P. Barts & G. W. F. H. Borst-Pauwels, unpublished work) affect P_i uptake qualitatively in the same way, it appears that changes in the membrane potential cannot provide a satisfactory explanation of the results. The surface potential is decreased both by bivalent and by univalent cations, though less effectively by univalent cations (McLaughlin, 1977).

The results confirm the notion that Na⁺ and phosphate ions bind to the co-transport mechanism in random order. From the experiment in Fig. 2 it may also be concluded that the rate constants for the incompletely loaded carrier \( a_1 \) and \( a_2 \) are non-zero (eqn. 6 of the Appendix). Hence, at low concentrations of \( Na_0^+ \), one phosphate ion may be transported with only one Na⁺ ion, and the ratio between Na⁺ uptake and \( P_i \) uptake via the co-transport mechanism may be lower than 2.

It has been shown theoretically (Theuvenet & Borst-Pauwels, 1976c) that uptake of a univalent cation across a negatively charged membrane may show apparent two-site kinetics even though the translocation mechanism has only one site; this is due to an increase in apparent \( K_m \) at high cation concentrations, which cause a decrease in the surface potential. In a similar way it is possible that the deviation from single-site kinetics shown in Fig. 3 should not be attributed to a specific Na⁺-binding site on the Na⁺-independent transport mechanism, but to charged groups on the membrane surface, which are screened by Na⁺ ions. If this is the case, the Na⁺-dependent phosphate-transport mechanism would have only one real binding site with affinity for Na⁺. The effects of bivalent cations on such a mechanism would, however, be similar to the effects on a co-transport mechanism to which two Na⁺ ions can bind in a range of Na⁺ concentrations that do not appreciably affect the surface potential (Roomans & Borst-Pauwels, 1978).

We have as yet not been able to determine the ratio between Na⁺ uptake and phosphate uptake via the Na⁺-dependent phosphate-uptake mechanism with sufficient accuracy to allow us to distinguish between a mechanism with one or two sites with affinity for Na⁺. It should be realized that, in addition to the co-transport mechanism, there is an Na⁺-independent phosphate-uptake mechanism, which may be, however, affected by a high Na⁺ concentration via a decrease in the surface potential, and a univalent-cation-transport mechanism, by which Na⁺ can be taken up, that is inhibited by phosphate via depolarization of the membrane (Roomans & Borst-Pauwels, 1977), so that the interactions between Na⁺ and phosphate uptake are rather complex.

To some extent, the effect of cations on phosphate uptake via the Na⁺-independent mechanism resembles the effect of cations on sulphate uptake by yeast (Roomans et al., 1979). Sulphate uptake can be described as a co-transport of one sulphate ion with three protons. In this case, however, a maximum was found if the rate of sulphate uptake was plotted against the cation concentration; the decrease of stimulation at high cation concentration could not be explained by complex-formation with sulphate. Also, the stimulation of sulphate uptake by the same concentration of bivalent cations was less than the stimulation of phosphate uptake. Both differences may have a common reason: it was hypothesized that one or two of the proton-binding sites of the sulphate-uptake mechanism would have a relatively low affinity for protons. Under these conditions a maximum may be found if the rate of uptake is plotted as a function of the surface potential (Roomans & Borst-Pauwels, 1978; Roomans et al., 1979), and, since even at low pH the effect of a decrease of the surface potential on the occupation of the proton-binding sites is not negligible, stimulation of anion uptake will be less.

Our results show some differences from those of Cockburn et al. (1975), who found that at high phosphate concentrations the ratio of K⁺ efflux to phosphate uptake was about 2; the ratio of net H⁺ uptake to phosphate uptake was also 2, similar to our findings. It may be considered that the increased K⁺ efflux may be found in cells in which metabolism is impaired, whereas we used metabolizing cells.

In contrast with findings by Theuvenet & Borst-Pauwels (1976a,b) concerning effects of bivalent cations on Rb⁺ uptake by yeast, we found little difference between the effect of Ca²⁺ and Mg²⁺ on phosphate uptake. This may point to the involvement of different negative groups determining the surface potential around the phosphate-binding sites and the univalent-cation-binding sites.

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References

The general rate equation describing ion uptake via a co-transport mechanism by which one anion is co-transported with two cations has been given previously (eqn. A3 in Roomans & Borst-Pauwels, 1978). In the case of co-transport of a univalent anion \( s_i \) with two univalent cations \( s_j \), where the ions bind to the translocation mechanism in random order, the rate of anion uptake is given by the Michaelis-Menten equation:

\[
v = \frac{V_i s_i}{K_{m,i} + s_i}
\]

where the kinetic constants of anion uptake are given by:

\[
V_i = \left( \frac{(K_{j2}a_1 + K_{j1}a_2)s_j y + b s_j^2 y^2}{K_{j1}K_{j2} + (K_{j1} + K_{j2})s_j y + s_j^2 y^2} \right) V
\]

\[
K_{m,i} = K_i y
\]

where \( K_{j1} \) and \( K_{j2} \) are the dissociation constants of the cation with the two cation-binding sites on the translocation mechanism, \( K_i \) is the dissociation constant of the anion, \( a_1 \) and \( a_2 \) are rate constants for ion uptake if the translocation mechanism is loaded with one anion and one cation only, \( b \) is the rate constant for uptake via the fully loaded translocation mechanism (it is assumed that \( a_1 \) and \( a_2 < b \)); \( y \) is related to the surface potential \( \psi_0 \) by:

\[
y = \exp(-q \psi_0/kT)
\]

where \( q \) is the absolute value of the charge of the electron, \( k \) the Boltzmann constant and \( T \) the absolute temperature; for negatively charged membranes \( y > 1 \).

The kinetic parameter \( F_i = V_i/K_{m,i} \), which equals the value of \( s_i/s_i \) at infinitely low values of \( s_i \) is given by:

\[
F_i = \frac{V}{K_i} \left( \frac{(K_{j2}a_1 + K_{j1}a_2)s_j y + b s_j^2 y^2}{K_{j1}K_{j2} + (K_{j1} + K_{j2})s_j y + s_j^2 y^2} \right)
\]

The rate of cation uptake via such a mechanism is described by a more-complex quadratic relation; the affinity constants of the cation for the translocation mechanism depend in the following way on the surface potential:

\[
K_{m,j1} = K_{j1}/y \quad \text{and} \quad K_{m,j2} = K_{j2}/y
\]

\( F_j \) (the value of \( v_j/s_j \) at infinitely low values of \( s_j \)) is given by:

\[
F_j = \frac{(K_{j2}a_1 + K_{j1}a_2)V_{51} y}{K_{j1}K_{j2} + (K_{j1} + K_{j2})s_j y + s_j^2 y^2}
\]

\[
F_j = \frac{V_{51} y}{K_{m,j} + s_j}
\]

If the cations bind to the translocation mechanism before the anion, the kinetic parameters of anion uptake are:

\[
v_i = bv
\]

\[
K_{m,i} = K_i \left( \frac{K_{j1}K_{j2} + (K_{j1} + K_{j2})s_j y + s_j^2 y^2}{s_j^2 y} \right)
\]