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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, 1976*a*,*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 20 h. 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

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45 mM-Tris/succinate buffer, pH 4.5, in the presence of 3% (w/v) glucose at 25°C. N_2 was bubbled through the suspension continuously. The uptake of phosphate (added to the medium as Tris/phosphate) was studied by using [^{32}P]P_i as a tracer, and the uptake of Na^+ was studied by using ^{22}Na as a tracer, as described earlier (Roomans & Borst-Pauwels, 1977). Nine successive samples of the yeast suspension were taken within 40–60 s, filtered, and washed with ice-cold water (phosphate uptake) or 50 mM-$MgCl_2$ (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 20 s. The P_i concentrations used ranged from carrier-free phosphate (only radioactive P_i added) to 200 μM (pH 4.5) or 1200 μM (pH 7.2). Uptake of ^{45}Ca 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 Ca^{2+} at pH 4.5 was studied with a Ca^{2+}-selective electrode (Philips IS 560); solutions of $CaCl_2$ in Tris/succinate buffer were titrated with NaH_2PO_4 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

P_i 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-Mg^{2+} on uptake of P_i 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 Mg^{2+}, in contrast with uptake via the Na^+-independent mechanism, which appears to be inhibited (except at infinitely high P_i concentrations). We will first discuss the Na^+-dependent mechanism.

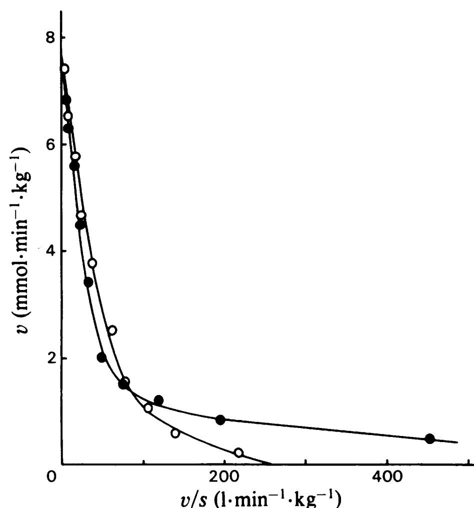

Fig. 1. *Effect of Mg^{2+} on the kinetics of phosphate uptake at pH 7.2 in the presence of 15 mM-NaCl*
○, Control; ●, 4 mM-$MgCl_2$ added. P_i uptake is measured as described in the Experimental section, at a yeast concentration of 1.0% (w/v). P_i concentrations ranged from carrier-free (only radioactive P_i added) to 1.2 mM. Nine successive samples of the suspension were taken within 40–60 s and initial uptake rates determined from the slopes of the tangents to the uptake curves at zero time. The data are plotted as described by Hofstee (1952).

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 P_i, 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 P_i ions bind to the co-transport mechanism in random order (Roomans & Borst-Pauwels, 1978). The kinetic parameters of P_i and Na^+ uptake by the co-transport mechanism are given in the Appendix (eqns. 1, 2 and 4–6).

The effect of Mg^{2+} 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 Mg^{2+} [about 23% of the phosphate is complexed by Mg^{2+} under these conditions (Kobos

& 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 µM. 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+}.

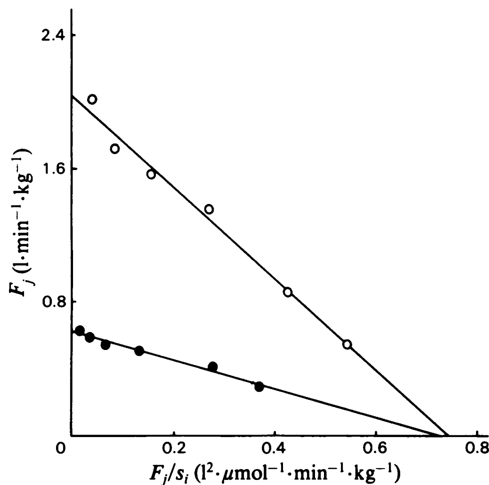

Fig. 2. *Effect of Mg^{2+} on uptake of Na^+ via the Na^+/phosphate co-transport mechanism at pH 7.2*

F_j (v/s at very low Na^+ concentrations) is plotted against the quotient of F_j and the phosphate concentration s_i (in µM). 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 µM, 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 µM.

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 µM-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 $F_{max.,j}$) 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-

Table 1. *Effect of 4 mM-Mg^{2+} on the kinetical 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.

	Control	4 mM-$MgCl_2$ added
K_m for phosphate, $K_{m,t}$ (µM)		
From Fig. 1	0.65 ± 0.20	0.17 ± 0.04
From Fig. 2	0.55 ± 0.04	0.17 ± 0.02
Maximal rate of phosphate uptake, V_t (mmol/min per kg), from Fig. 1	0.70 ± 0.20	0.85 ± 0.10
K_m for Na^+ (high-affinity site), $K_{m,j1}$ (mM), from Fig. 3, see eqn. (3) of the Appendix	0.07 ± 0.01	0.24 ± 0.01
$F_{max.,j}$ (l·min^{-1}·kg^{-1}), from Fig. 2, see eqn. (6) of the Appendix	2.05 ± 0.06	0.61 ± 0.02

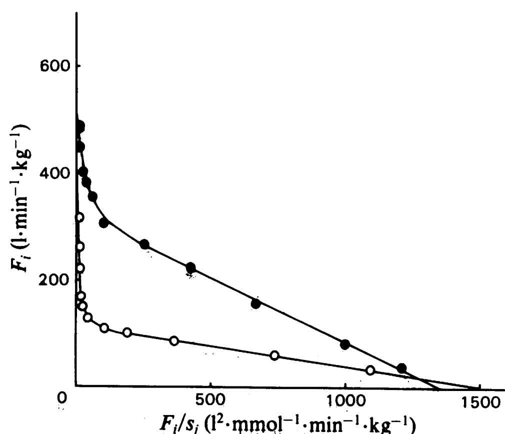

Fig. 3. *Effect of Mg^{2+} on the apparent affinity constants of the co-transport mechanism for Na^+*

F_i (v/s at very low phosphate concentrations) 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; ●, 4mM-$MgCl_2$ 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.

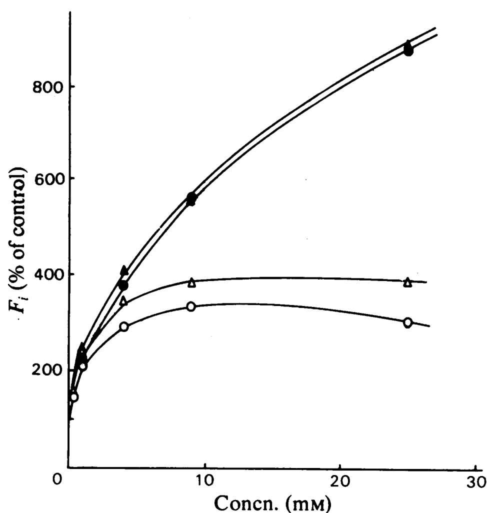

Fig. 4. *Effect of Ca^{2+} and Mg^{2+} on phosphate uptake by the Na^+/phosphate co-transport mechanism at pH7.2*

○, 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 15mM. The other experimental details are the same as for the experiment shown in Fig. 1.

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:

$$\frac{1}{y} = c_1 + c_2 s_b^{\frac{1}{2}} \quad (1)$$

where c_1 and c_2 are constants, depending on the bivalent cation species and the pH; this relation is based on experimental findings (Theuvenet & Borst-Pauwels, 1976*a*). If the maximal rate of phosphate uptake in the presence of 15mM-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_i) can be approximated by:

$$F_i = \frac{V_i}{K_i y} \quad (2)$$

and, combining eqns. (1) and (2), by approximation:

$$F_i = \frac{V_i}{K_i}(c_1 + c_2 s_b^{\frac{1}{2}}) \quad (3)$$

According to eqn. (3) we may, by approximation, expect a linear relationship between F_i 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

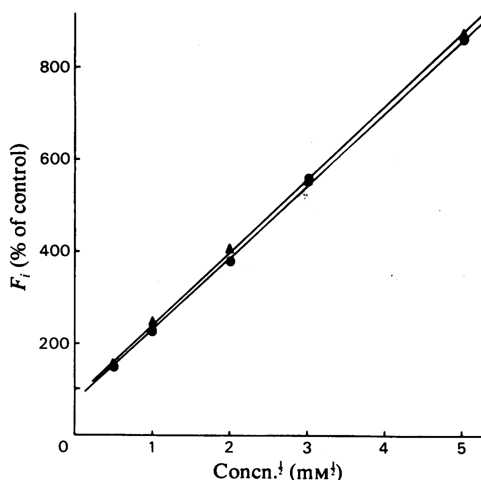

Fig. 5. *Effect of bivalent cations on phosphate uptake by the Na^+/phosphate co-transport mechanism at pH7.2*

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+}.

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

Phosphate uptake via the Na^+-independent transport system is accompanied by net proton influx and extrusion of K^+ ions (Cockburn *et al.*, 1975). We have determined the ratios of the proton, phosphate and K^+ 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^+ ions for each phosphate ion taken up (mean ± S.D. of five experiments), whereas a net proton influx of 2.01 ± 0.30 H^+ 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^- ions); electroneutrality is maintained by efflux of one K^+ 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_t 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_t is independent of the cation concentration and of the surface potential.

Theuvenet & Borst-Pauwels (1976*b*) showed that at pH 4.5 Mg^{2+} did not significantly affect the maximal rate of phosphate uptake via the Na^+-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^{2+} on phosphate uptake at extremely low P_i concentrations via the Na^+-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^{2+}. At pH 4.5 phosphate uptake is enhanced by Ca^{2+} until maximal stimulation is reached at about 9 mM-Ca^{2+}. The decrease of the stimulation at higher Ca^{2+} concentrations can be explained by complexing of phosphate. At pH 7.2 phosphate uptake is slightly inhibited by Ca^{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^+ 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

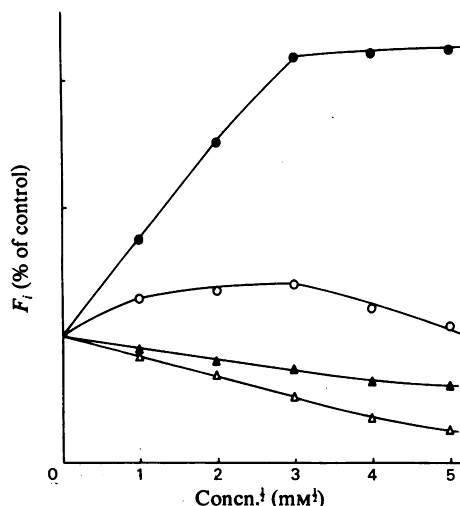

Fig. 6. *Effect of Ca^{2+} on phosphate uptake via the Na^+-independent transport mechanism*

F_i is plotted against the square root of the Ca^{2+} concentration. ○, pH 4.5; ●, same data corrected for complex-formation of phosphate with Ca^{2+}. At pH 4.5 the experiment was carried out at a yeast concentration of 0.5% (w/v). △, pH 7.2; ▲, corrected for complex-formation. At pH 7.2 a 1.0% yeast suspension was used. Carrier-free phosphate was used in all experiments. Sampling time was 40–60 s, as described in the Experimental section.

uptake. At relatively high concentrations (>10 mM) of Ca^{2+} other effects of Ca^{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 (Theuvenet, 1978). In that case the decrease in the surface potential by Ca^{2+} may become less at higher Ca^{2+} concentrations.

At pH 4.5 also univalent cations (K^+, Rb^+, Cs^+) stimulate phosphate uptake via the Na^+-independent uptake mechanism. Univalent cations are less effective than bivalent cations; 30 mM-K^+ 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^+ 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^+, one phosphate ion may be co-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, 1976*c*) 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^+-dependent 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 (1976*a*,*b*) concerning effects of bivalent cations on Rb^+ uptake by yeast, we found little difference between the effect of Ca^{2+} and Mg^{2+} 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

Borst-Pauwels, G. W. F. H. & Peters, P. H. J. (1977) *Biochim. Biophys. Acta* **466**, 488–495

Cleland, W. W. (1967) *Adv. Enzymol. Relat. Subj. Biochem.* **29**, 1–32

Cockburn, M., Earnshaw, P. & Eddy, A. A. (1975) *Biochem. J.* **146**, 705–712

Goodman, J. & Rothstein, A. (1957) *J. Gen. Physiol.* **40**, 915–923
Hofstee, B. H. J. (1952) *Science* **116**, 329–331
Kobos, R. K. & Rechnitz, G. A. (1976) *Arch. Biochem. Biophys.* **175**, 11–20
McLaughlin, S. (1977) *Curr. Top. Membr. Transp.* **9**, 71–144
Roomans, G. M. & Borst-Pauwels, G. W. F. H. (1977) *Biochim. Biophys. Acta* **470**, 84–91
Roomans, G. M. & Borst-Pauwels, G. W. F. H. (1978) *J. Theor. Biol.* **73**, 453–468
Roomans, G. M., Blasco, F. & Borst-Pauwels, G. W. F. H. (1977) *Biochim. Biophys. Acta* **467**, 65–71
Roomans, G. M., Kuypers, G. A. J., Theuvenet, A. P. R. & Borst-Pauwels, G. W. F. H. (1979) *Biochem. Biophys. Acta.* in the press
Seaston, A., Inkson, C. & Eddy, A. A. (1973) *Biochem. J.* **134**, 1031–1043
Theuvenet, A. P. R. (1978) Ph.D. Thesis, University of Nijmegen
Theuvenet, A. P. R. & Borst-Pauwels, G. W. F. H. (1976*a*) *Biochim. Biophys. Acta* **426**, 745–756
Theuvenet, A. P. R. & Borst-Pauwels, G. W. F. H. (1976*b*) *Bioelectrochem. Bioenerg.* **3**, 230–240
Theuvenet, A. P. R. & Borst-Pauwels, G. W. F. H. (1976*c*) *J. Theor. Biol.* **57**, 313–329

APPENDIX

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:

$$V_i = \left(\frac{(K_{j2}a_1 + K_{j1}a_2)s_j y + bs_j^2 y^2}{K_{j1}K_{j2} + (K_{j1} + K_{j2})s_j y + s_j^2 y^2}\right) V \quad (1)$$

$$K_{m,i} = K_i y \quad (2)$$

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 ψ_0 by:

$$y = \exp(-q\psi_0/kT) \quad (3)$$

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 v_i/s_i at infinitely low values of s_i is given by:

$$F_i = \frac{V}{K_i}\left(\frac{(K_{j2}a_1 + K_{j1}a_2)s_j + bs_j^2 y}{K_{j1}K_{j2} + (K_{j1} + K_{j2})s_j y + s_j^2 y^2}\right) \quad (4)$$

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:

$$K_{m,j1} = K_{j1}/y \quad \text{and} \quad K_{m,j2} = K_{j2}/y \quad (5)$$

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

$$F_j = \frac{\left(\frac{(K_{j2}a_1 + K_{j1}a_2)}{K_{j1}K_{j2}} V s_i y\right)}{K_i y + s_i} = \frac{F_{max.,j} s_i}{K_{m,i} + s_i} \quad (6)$$

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

$$V_i = bv \quad (7)$$

$$K_{m,i} = K_i\left(\frac{K_{j1}K_{j2} + (K_{j1} + K_{j2})s_j y + s_j^2 y^2}{s_j^2 y}\right) \quad (8)$$

Reference

Roomans, G. M. & Borst-Pauwels, G. W. F. H. (1978) *J. Theor. Biol.* **73**, 453–468