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INTERACTION OF PHOSPHATE WITH MONOVALENT CATION UPTAKE IN YEAST

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Summary

The uptake of monovalent cations by yeast via the monovalent cation uptake mechanism is inhibited by phosphate. The inhibition of Rb^+ uptake shows saturation kinetics and the phosphate concentration at which half-maximal inhibition is observed is equal to the K_m of phosphate for the sodium-independent phosphate uptake mechanism. The kinetic coefficients of Rb^+ and Tl^+ uptake are affected by phosphate: the maximal rate of uptake is decreased and the apparent affinity constants for the translocation sites are increased.

In the case of Na^+ uptake, the inhibition by phosphate may be partly or completely compensated by stimulation of Na^+ uptake via a sodium-phosphate cotransport mechanism.

Phosphate effects a transient stimulation of the efflux of the lipophilic cation dibenzyldimethylammonium from preloaded yeast cells and a transient inhibition of dibenzyldimethylammonium uptake. Possibly, the inhibition of monovalent cation uptake in yeast can be explained by a transient depolarization of the cell membrane by phosphate.

Introduction

Recently, we investigated the stimulation of phosphate uptake by Na^+ and of Na^+ uptake by phosphate at pH 7.2 [1]. The presence of a sodium-phosphate contransport mechanism in the yeast cell membrane was proposed. This mechanism has two sites with affinity for Na^+ and Li^+, but not for other alkali cations, and one site with affinity for phosphate. In addition to this mechanism, a sodium-independent phosphate uptake mechanism exists [1,2]. Furthermore, the presence of a transport mechanism for monovalent cations, having two sites to which these ions can bind, has been demonstrated [3]. These sites

Abbreviation: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

are not identical with the two cation sensitive sites on the sodium-phosphate cotransport mechanism.

Borst-Pauwels et al. [4] found that addition of phosphate to phosphate-deficient yeast gives rise to an immediate decrease in the rate of Rb^+ uptake. In this paper, the inhibition of Rb^+ by phosphate is investigated in more detail. It will be shown, that the inhibitory effect is not specific for Rb^+ uptake, but that also Na^+ uptake may be affected. The possible nature of the inhibition will be discussed.

Materials and Methods

Yeast cells, *Saccharomyces cerevisiae*, strain Delft II, with a low phosphate content, were starved under aeration for 20 h. After starvation, the cells (1% w/v) were preincubated anaerobically for 1 h in Tris · succinate buffer, pH 4.5, or in 25 mM HEPES/Imidazole buffer, pH 7.4, in the presence of 3% (w/v) glucose at 25° C. Nitrogen was bubbled through the suspension continuously. During preincubation in Tris buffer the pH remained constant at 4.5, during preincubation in HEPES buffer the pH decreased slowly, reaching a value of 7.2 after 1 h.

The uptake of Rb^+ and Na^+ (added to the medium as chloride salts) was studied using ^{86}Rb or ^{22}Na as a tracer, as described previously [3,5]. 9 successive samples of the yeast suspension were taken within 1 min, washed with ice-cold 50 mM $MgCl_2$, filtered and dried with acetone. The uptake of Tl^+ (added to the medium as thallous sulphate) was studied using ^{204}Tl as a tracer, with a similar method, but in this case the samples were washed with ice-cold 50 mM $MgSO_4$ (instead of $MgCl_2$, to avoid precipitation of TlCl). Studies of phosphate uptake were carried out with ^{32}P as a tracer; phosphate was added as Tris-phosphate. The samples were washed with ice-cold distilled water.

Initial uptake rates were determined from the slopes of the tangents to the uptake curves at zero time. Kinetical constants were determined by fitting the data to a single or double hyperbola with appropriate curve-fitting programmes.

The concentration of Na^+ and K^+ in the supernatant were determined by flame spectrophotometry; typically, values of about 40 μM for Na^+, and 5 μM (at pH 7.2) or 10—15 μM (at pH 4.5) for K^+ were found.

Efflux experiments with dibenzyldimethylammonium were carried out in the following way: the cells were preincubated with 5% glucose at a yeast concentration of 10%, with 1 μM ^{14}C-labelled dibenzyldimethylammonium. At 120 min, 1 volume of yeast was added to 9 volumes of buffer, containing Tris-phosphate or Tris-chloride. 1 ml samples were taken and the radioactivity of the supernatant was determined by means of liquid scintillation analysis. Influx was determined after a similar preincubation with unlabelled dibenzyldimethylammonium, adding the yeast to buffer containing labelled dibenzyldimethylammonium. Samples were taken in a similar way as for Rb^+ uptake, but drying with acetone was omitted [6].

Results

The effect of phosphate on the rate of Na^+ and Rb^+ uptake is shown in Figs. 1 and 2. At pH 7.2 Na^+ uptake is stimulated at low phosphate concentra-

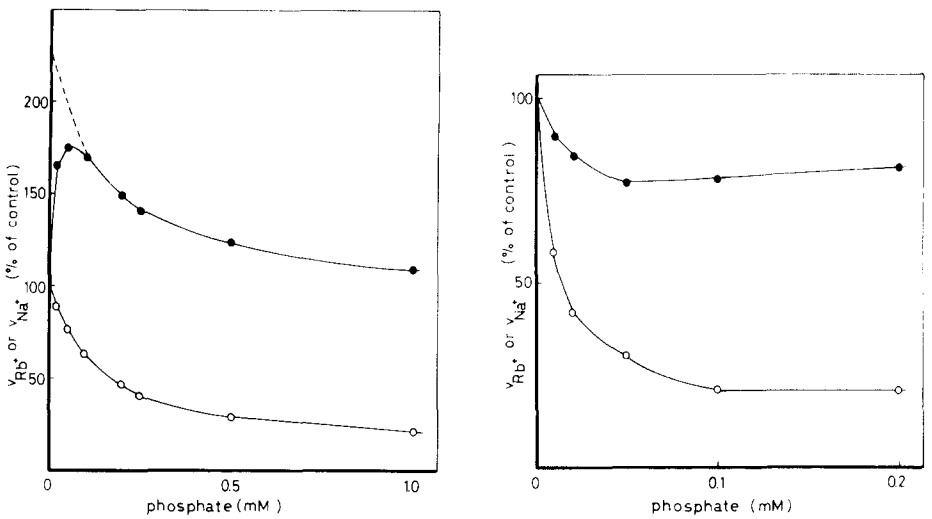


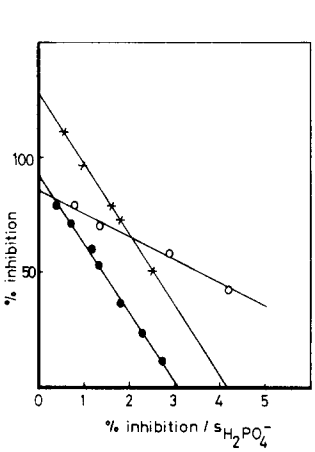
Fig. 1. Effect of phosphate on Rb^+ and Na^+ uptake at pH 7.2. The relative rates of Rb^+ and Na^+ uptake are plotted against the total phosphate concentration. Broken line; extrapolation of the right-hand part of the curve for Na^+ (for explanation see text). The Rb^+ concentration is 50 μM, the Na^+ concentration is 40 μM. (○), Rubidium; (●), sodium.

Fig. 2. Effect of phosphate on Rb^+ and Na^+ uptake at pH 4.5. The relative rates of Rb^+ and Na^+ uptake are plotted against the total phosphate concentration. The Rb^+ concentration is 0.2 mM, the Na^+ concentration is 40 μM. (○), Rubidium; (●), sodium.

tions. This stimulation has been the subject of a previous paper [1] where it was shown that phosphate and Na^+ may be cotransported by a transport mechanism having two sites with affinity for Na^+ and one site with affinity for phosphate. At concentrations of phosphate higher than 50 μM, stimulation decreases. Rb^+ uptake is inhibited by phosphate. No signs of stimulation are apparent, which is in accordance with the finding that Rb^+ has no affinity for the sodium-phosphate cotransport mechanism. Also, at pH 4.5 Rb^+ uptake is inhibited by phosphate; Na^+ uptake is inhibited to a lesser extent (Fig. 2).

The inhibition of Rb^+ uptake by phosphate shows a saturation curve. We have investigated whether the inhibition of cation uptake by phosphate was correlated with phosphate uptake via the Na^+-independent transport process by comparing the K_m of phosphate for this process with the phosphate concentration at which half-maximal inhibition of cation uptake is observed. Since phosphate is taken up as the monovalent cation only [7], and at pH 7.2 only 20% of the phosphate is in this form, an appropriate correction was made. Fig. 3 shows a plot of the relative inhibition of Rb^+ uptake versus the quotient of this inhibition and the $H_2PO_4^-$-concentration. From the slope of this plot, the inhibition constant can be determined; at pH 7.2 this is 30 ± 1 μM, as compared to a K_m of 31 ± 2 μM for phosphate uptake via the Na^+-independent phosphate transport mechanism. The K_m of phosphate for the Na^+-phosphate cotransport mechanism is much lower, about 0.6 μM [1]. At pH 4.5, a value of 10 ± 1 μM is found for the inhibition constant, which compares well with a K_m of 11 ± 1 μM for phosphate uptake via the Na^+-independent mechanism at this pH.

Fig. 1 shows that at pH 7.2, phosphate has a dual effect on Na^+ uptake: a



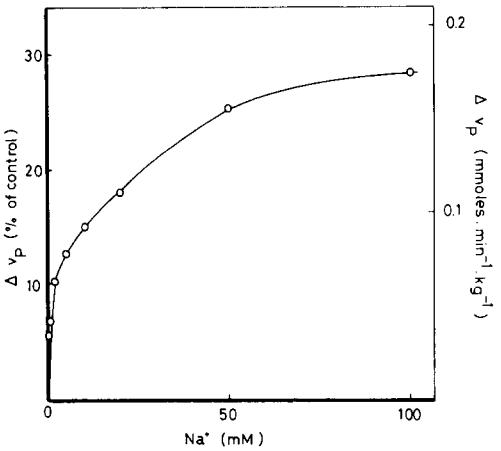


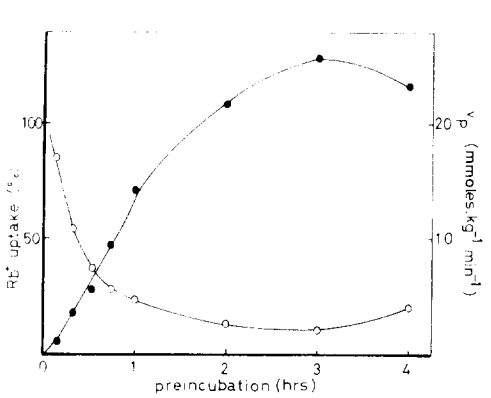
Fig. 3. Plot of the relative inhibition of Rb^+ or Na^+ uptake versus the quotient of this inhibition and the concentration of $H_2PO_4^-$. The relative inhibition is defined as the difference between the relative rate of uptake at a particular phosphate concentration and the relative rate of uptake when no phosphate has been added (in the case of Na^+ uptake, the extrapolated value, Fig. 1). (○), Rb^+, pH 4.5; (●), Rb^+, pH 7.2; (X), Na^+, pH 7.2. Data from Figs. 1 and 2.

Fig. 4. Effect of Na^+ on phosphate uptake at pH 4.5. The concentration of phosphate was 1 μM; Na^+ was added as NaCl.

stimulatory effect via the sodium-phosphate cotransport mechanism, and an inhibitory effect. By extrapolating the right-hand part of the curve towards 0 mM phosphate (broken line in Fig. 1), an estimation can be made of the relative inhibition of Na^+ uptake by phosphate and the concentration dependence of this inhibition. The data are given in Fig. 3, and the inhibition constant can be estimated at about 30 μM with respect to $H_2PO_4^-$ at pH 7.2. Inhibition of cation uptake by phosphate seems thus indeed to be correlated with the Na^+-independent phosphate uptake mechanism.

At pH 4.5 no stimulation of Na^+ uptake by phosphate is observed (Fig. 2); this is in accordance with earlier findings [4]. Bearing in mind however, that at pH 7.2 phosphate has a dual effect on Na^+ uptake, the plot of the relative rate of Na^+ uptake at pH 4.5 versus the phosphate concentration may equally well reveal two effects: an inhibition of Na^+ uptake by the Na^+-independent phosphate uptake mechanism and a stimulation via the sodium phosphate cotransport mechanism. This notion is supported by the finding that phosphate uptake at pH 4.5 is stimulated by Na^+ (Fig. 4). The relative stimulation by 100 mM Na^+ is much less than at pH 7.2 (30% as compared to about 500% at pH 7.2) but the absolute stimulation compares reasonably well with the values found at pH 7.2 (0.18 mmol · kg^{-1} · min^{-1} at pH 4.5 compared to 0.24 mmol · kg^{-1} · min^{-1} at pH 7.2). This suggests strongly, that the sodium phosphate cotransport is also functioning at pH 4.5.

Phosphate uptake increases with the period of anaerobic preincubation [8]. It was investigated, whether this also applied to the inhibition of Rb^+ uptake by phosphate. Fig. 5 shows an increase in inhibition of Rb^+ uptake by 200 μM phosphate with increased length of the preincubation period reaching a maximum about 3 h. In a parallel experiment, the rate of phosphate uptake at the



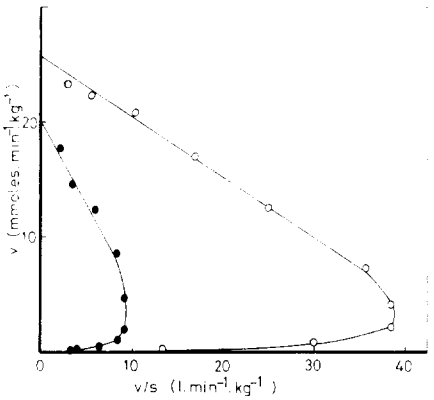


Fig. 5. Effect of the anaerobic preincubation period with glucose on the inhibition of Rb^+ uptake by phosphate at pH 4.5. The Rb^+ concentration was 1 μM. (○), Rb^+ uptake, in the presence of 200 μM phosphate in % of the control (without added phosphate); (●), rate of phosphate uptake at an external phosphate concentration of 200 μM.

Fig. 6. Effect of phosphate on the kinetics of Tl^+ uptake at pH 4.5 (Hofstee-plot). (○), Control; (●), 200 μM phosphate added.

same external phosphate concentration was measured, It can be concluded that a correlation exists between the rate of phosphate uptake and the inhibition of Rb^+ uptake by phosphate.

The effect of phosphate on the kinetics of monovalent cation uptake was studied for Rb^+ and Tl^+ uptake. Tl^+ is accumulated more rapidly than Rb^+ (this can be seen from Table I), and the uptake kinetics can be determined with a greater accuracy. Tl^+ has affinity for the same two-site transport mechanism as Rb^+; the affinity constants of Rb^+ for the Tl^+ transport sites are equal to the affinity constants of Rb^+ for the Rb^+ uptake sites. Fig. 6 shows the effect of phosphate on the kinetics of Tl^+ uptake in yeast. The data are represented according to Hofstee [9]. By computer analysis, values for V and the affinity constants for the two transport sites were obtained. The effect of phosphate is a significant decrease in V and an increase in the apparent affinity constants K_1 and K_2 (Table I).

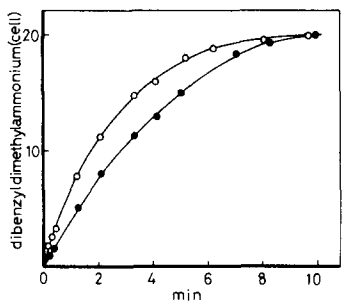
The increase in K_1 is only to a small extent due to an indirect effect of phosphate, namely the enhancement of K^+ efflux [10]. The rise in extracellular K^+ concentration causes about a 20% increase in K_1 whereas the observed increase

TABLE I

EFFECT OF 200 μM PHOSPHATE ON THE KINETIC COEFFICIENTS OF Rb^+ AND Tl^+ UPTAKE

	pH	V		K_1		K_2	
			+ Phosphate		+ Phosphate		+ Phosphate
Rb^+	4.5	17.0 ± 1.1	14.2 ± 1.2	0.13	1.3	1.5	2.6
	7.2	13.4 ± 0.3	12.0 ± 0.3	0.019	0.04	0.32	0.56
Tl^+	4.5	24.8 ± 0.3	19.9 ± 2.0	0.023	0.20	0.43	1.1

Dimensions: V, mmol · kg^{-1} · min^{-1} (±standard deviation); K, mM.



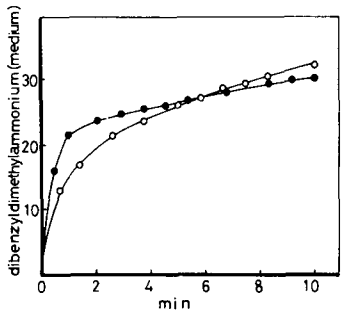


Fig. 7. Effect of phosphate on the uptake of radioactive dibenzyldimethylammonium at pH 4.5. On the vertical axis, radioactivity in the cells in arbitrary units. (○), Control (200 μM Tris-chloride added); (●), (200 μM Tris-chloride added); (●), 200 μM Tris-phosphate added.

Fig. 8. Effect of phosphate on the efflux of radioactive dibenzyldimethylammonium from preloaded yeast cells (pH 4.5). On the vertical axis, radioactivity in the medium in arbitrary units. (○), Control (200 μM Tris-phosphate added); (●), 200 μM Tris-phosphate added.

is much larger. At low concentrations of Rb^+ and Na^+, the uptake of these ions may be slightly stimulated by K^+ [3]; this can also be seen in Fig. 2 at the highest phosphate concentration. The concentrations of Rb^+ employed in the experiments of Figs. 1 and 2 were chosen to minimize the effect of K^+ efflux. Use of lower concentrations does not have a significant effect on the quantitative results if suitable corrections for the increase in K^+ concentration in the medium are applied.

According to Cockburn et al. [10] each phosphate ion is cotransported with 2 or 3 protons. Combination of a monovalent negative phosphate ion with more than one proton may give rise to a positively charged complex; transport of such a complex may cause depolarization. To investigate whether phosphate affected the membrane potential of yeast we used the lipophylic cation dibenzyldimethylammonium. This ion has been used as a probe for the determination of the membrane potential in small cells [6,11,12]. We found that phosphate caused a transient increase in dibenzyldimethylammonium efflux from preloaded cells and a transient decrease of the influx of dibenzyldimethylammonium (Figs. 7 and 8). These findings point to a possible transient depolarization of the yeast cell membrane by phosphate.

Discussion

Addition of phosphate to the external medium causes an immediate decrease of the rate of Rb^+ uptaken in phosphate deficient yeast. The extent of inhibition is correlated with the amount of phosphate added, but this is not the only factor on which the inhibition depends. As, at a constant external phosphate concentration, the inhibition is seen to depend on the period of anaerobic preincubation with glucose, a second factor must be involved. The external phosphate concentration at which half-maximal inhibition of Rb^+ uptake is observed is, both at pH 4.5 and 7.2, equal to the K_m of the Na^+-independent phosphate transport mechanism. This points strongly to the possibility that this transport mechanism is involved in the inhibition of Rb^+ uptake. The increased

inhibition of Rb^+ uptake with prolonged anaerobic preincubation would reflect increased binding of phosphate to this mechanism or increased rate of uptake via this transport mechanism. The inhibitory effect of phosphate is not specific for Rb^+ but affects all cations taken up via the two-site monovalent cation transport mechanism, including Na^+. The inhibitory effect of Na^+ uptake is, however, at pH 7.2 more than completely, and at pH 4.5 to a great extent, compensated by extra Na^+ uptake via the sodium phosphate cotransport system.

The effect of phosphate on the kinetics of Rb^+ and Tl^+ uptake resembles the effect of 2,4-dinitrophenol on the kinetics of Rb^+ uptake. Also in that case, a decrease in V and an increase in affinity constants were observed [13]. This suggested that phosphate might inhibit cation uptake via depolarization of the cell membrane. The results of the experiments with dibenzyldimethylammonium are in accordance with this notion.

Although it has not yet been fully proved that the dibenzyldimethylammonium ion is distributed quantitatively in accordance with the membrane potential there is evidence to indicate that compounds that depolarize the membrane affect the partition coefficient of the dibenzyldimethylammonium ion [6,11,14].

The Na^+-independent phosphate uptake mechanism in fungal cells has been assumed to be a H^+-phosphate cotransport [10,15,16] enabling phosphate to be transported into the cell along the electrochemical gradient. Cotransport with protons has been found in several cases of uptake of negatively charged or uncharged substrates and in some cases depolarization of the membrane by the cotransport has been demonstrated [17,18].

Since phosphate is cotransported with protons (or exchanged for cellular hydroxyl ions) [2,10] the pH of the cell will decrease as a result of phosphate uptake. This may explain the transient character of the depolarization. An increase in intracellular proton concentration may cause an increased activity of the proton pump, and consequently hyperpolarization. We have in fact, found a stimulation of Rb^+ uptake as a consequence of decrease in intracellular pH [19]. The transient character of the depolarization is also in accordance with earlier findings, that inhibition of Rb^+ uptake is less if Rb^+ is added some time after addition of phosphate, under conditions where the relative decrease of the extracellular phosphate concentration is negligible [4].

Whereas maximal inhibition of Rb^+ uptake by phosphate, at the Rb^+ concentrations applied, is about 90% the uptake of Na^+ can be inhibited to more than 100% of the control (Fig. 3). This can be explained by assuming, that not only Na^+ uptake via the two-site cation uptake mechanism is inhibited, but also Na^+ uptake via the sodium-phosphate cotransport mechanism. In an earlier paper [1], we suggested that coupling of phosphate uptake to Na^+ uptake would permit phosphate uptake to take place along the electrical gradient. If this is the case transport would occur by a positively charged complex and one might indeed expect inhibition of this transport by depolarization.

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