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COTRANSPORT OF PHOSPHATE AND SODIUM BY YEAST

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Summary

Phosphate uptake by yeast at pH 7.2 is mediated by two mechanisms, one of which has a K_m of 30 μM and is independent of sodium, and a sodium-dependent mechanism with a K_m of 0.6 μM, both K_m values with respect to monovalent phosphate. The sodium-dependent mechanism has two sites with affinity for Na^+, with affinity constants of 0.04 and 29 mM. Also lithium enhances phosphate uptake; the affinity constants for lithium are 0.3 and 36 mM. Other alkali ions do not stimulate phosphate uptake at pH 7.2. Rubidium has no effect on the stimulation of phosphate uptake by sodium.

Phosphate and arsenate enhance sodium uptake at pH 7.2. The K_m of this stimulation with regard to monovalent orthophosphate is about equal to that of the sodium-dependent phosphate uptake.

The properties of the cation binding sites of the phosphate uptake mechanism and those of the phosphate-dependent cation transport mechanism have been compared. The existence of a separate sodium-phosphate cotransport system is proposed.

Introduction

Borst-Pauwels et al. [1] showed, that at pH 7.2, phosphate stimulated sodium uptake by yeast cells, but not rubidium uptake. Phosphate uptake was enhanced by sodium. It was suggested that this was due to the presence of a phosphate-sodium ion symport system. In this paper, the properties of the phosphate-sodium ion cotransport have been investigated. A model is proposed, in which, in addition to a sodium-independent phosphate uptake mechanism and a cation transport mechanism with affinity for all alkali cations, an additional phosphate-sodium ion cotransport system exists.

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

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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 25 mM HEPES/Imidazol buffer, pH 7.4, in the presence of 3% (w/v) glucose at 25°C. Nitrogen was bubbled through the suspension continuously. At the end of the preincubation period, the pH of the suspension was 7.2. The uptake of Na^+ (added to the medium as NaCl), using ^{22}Na as a tracer, was studied according to the method of Borst-Pauwels et al. [2] as modified by Theuvenet and Borst-Pauwels [3]. The uptake of phosphate (added to the medium as Tris/phosphate), using ^{32}P as a tracer, was studied by a similar method, but in this case, the yeast was washed with ice-cold distilled water instead of with a 50 mM $MgCl_2$ solution.

Initial uptake rates were determined from the slopes of the tangents to the uptake curves at zero time. The concentrations of sodium and potassium in the supernatant were determined by flame spectrophotometry; typically, concentrations of about 5 μM for K^+ and 30—40 μM for Na^+ were found. The pH of the cells was determined after freezing and boiling the cells, as described by Borst-Pauwels and Dobbelmann [4]. Kinetical constants were determined by fitting the data to a single or double hyperbola with appropriate curve fitting programs.

Results

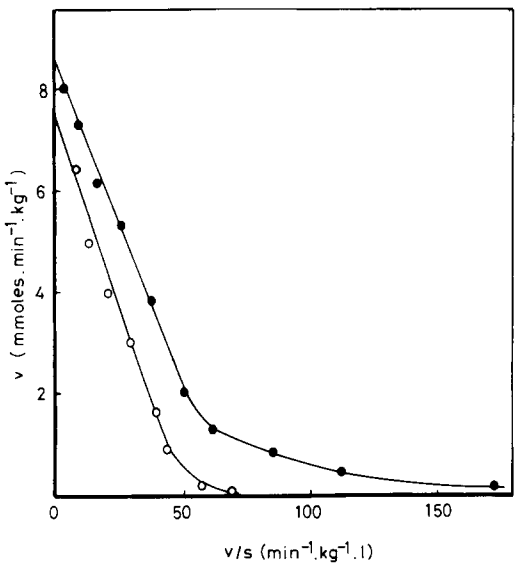
Phosphate uptake by yeast at pH 7.2 is enhanced by Na^+, especially at low concentrations of phosphate. The effect of Na^+ on the kinetics of phosphate uptake is shown in Fig. 1. The data are represented in a Hofstee or Eadie plot. When only a single Michaelis-Menten equation applies, a straight line is expected according to Eqn. 1:

$$v = \frac{Vs}{K_m + s} = V - K_m(v/s) \qquad (1)$$

In the absence of added Na^+ (and neglecting the small deviation from a straight line found at low phosphate concentrations) the uptake of phosphate can be described by single Michaelis-Menten kinetics. The K_m of this process with respect to monovalent orthophosphate is 30 μM (as phosphate is taken up in the form of the monovalent anion only [5], and at pH 7.2 only 20% of the phosphate is in this form, the apparent K_m as found in Fig. 1 has to be multiplied by 0.2).

In the presence of 15 mM Na^+, a strong deviation from linearity is found, which points to the operation of a second uptake mechanism; the K_m of this process is 0.6 μM. The K_m of the low affinity phosphate uptake mechanism is not affected by Na^+. The slight deviation from linearity shown by the points at low phosphate concentration in the control plot may be attributed to the small amount of extracellular Na^+ present.

Also other cations were tested for their ability to stimulate phosphate uptake at pH 7.2. These experiments were carried out at an external phosphate concentration of 1 μM. Only lithium was found to enhance phosphate uptake.



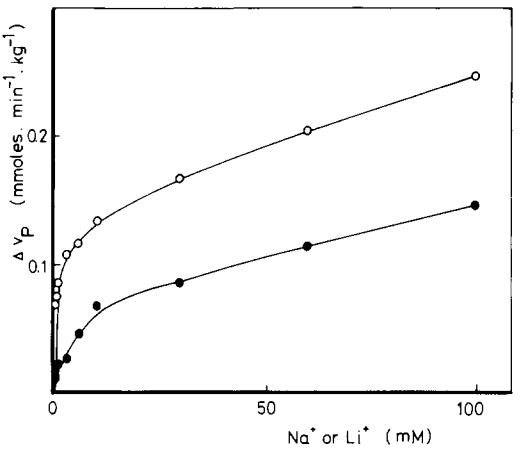


Fig. 1. Effect of sodium on the kinetics of phosphate uptake at pH 7.2, ○, control; ●, 15 mM Na^+ added.

Fig. 2. Effect of sodium and lithium on the rate of phosphate uptake. Δv_P is the rate of phospate uptake at a particular sodium or lithium concentration minus the rate of phosphate uptake at zero concentration of sodium (extrapolated) or lithium. The phosphate concentration is 1 μM. ○, sodium; ●, lithium.

At a concentration of 10 mM, potassium, rubidium and caesium, added as chloride salts, did not stimulate phosphate uptake.

At a concentration of 100 mM Na^+, about a 5-fold stimulation of phosphate uptake was measured. The dependence of the rate of phosphate uptake upon Na^+ and Li^+ is shown in Fig. 2, where the extra phosphate uptake (Δv_P) is plotted as a function of the concentration of Na^+ (a correction was applied for the residual Na^+ in the control) or Li^+.

A plot may be made of Δv_P against $\Delta v_P/s_{Na}$, analogous to the conventional Hofstee plot. In such a plot, a linear relationship between Δv_P and $\Delta v_P/s_{Na}$ would indicate, that only one Na^+-sensitive site is involved in the stimulation of phosphate uptake by Na^+. A deviation from linearity would point to the presence of more than one Na^+-sensitive site. In Fig. 3 it is shown, that such a deviation indeed occurs. A good fit to the experimental data was obtained by the assumption that two Na^+ binding sites are present. By computer analysis, values of 0.04 and 29 mM for the affinity constants were found. A similar curve was obtained for lithium; affinity constants of 0.3 and 36 mM were found (these values may, in fact, be somewhat to high, due to competition of the small amount of sodium still present).

It seems thus, that two sites with affinity for Na^+ and Li^+ are involved in the stimulation of phosphate uptake by these ions. As also the uptake of Na^+ by yeast occurs via a transport mechanism with two sites [2], it was investigated whether these sites are identical. The results of this comparison between the two transport systems is summarized in Table I. The affinity of Na^+ for the cation transport system at pH 7.2 was determined by measuring the uptake of $^{22}Na^+$ at various external Na^+ concentrations (see also ref. 6). The affinity of Li^+

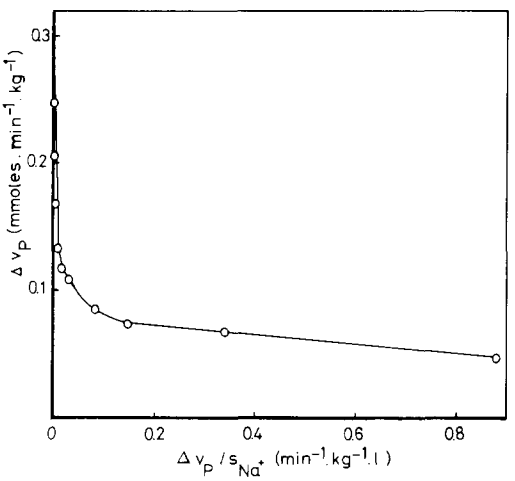


Fig. 3. Plot of Δv_P versus $\Delta v_P/s_{Na}$; data of Fig. 2.

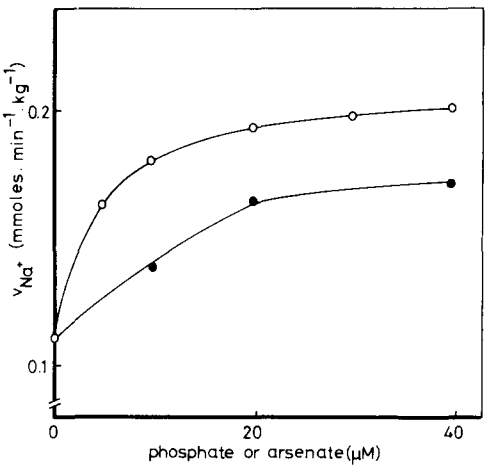


Fig. 4. Effect of phosphate and arsenate on the rate of sodium uptake at pH 7.2. The Na^+ concentration is 40 μM. ○, phosphate; ●, arsenate.

for the cation uptake system was determined by measuring the inhibition constants of Li^+ for the uptake of sodium at pH 7.2. The affinity constants of Rb^+ for this system were determined earlier [6]. Addition of 0.1 mM RbCl has no effect on the affinity constants of sodium for the activation sites for the phosphate transport. At this concentration of Rb^+, uptake of sodium via the cation uptake system is markedly affected. It may hence be concluded that Rb^+ has no appreciable affinity to the sodium-sensitive sites on the sodium ion phosphate cotransport mechanism.

Uptake of Na^+ is enhanced by phosphate, already at very low phosphate concentrations (Fig. 4). The K_m of this process with respect to monovalent orthophosphate is about 0.7 μM, which is in good agreement with the K_m found for the sodium-dependent phosphate uptake mechanism. Maximal stimulation is reached at a (total) phosphate concentration of 40—50 μM. If the concentration of phosphate is raised further, the stimulation decreases.

Several indirect effects by which phosphate might stimulate sodium uptake were investigated.

TABLE I

COMPARISON OF THE AFFINITIES OF ALKALI IONS FOR THE CATION TRANSPORT SYSTEM AND THE CATION-SENSITIVE SITES ON THE PHOSPHATE TRANSPORT MECHANISM

Values of K_m in mM.

	Cation transport mechanism		Cation binding sites of the phosphate transport system	
	$K_{m,1}$	$K_{m,2}$	$K_{m,1}$	$K_{m,2}$
Na^+	0.09	20	0.04	29
Li^+	17	17	0.3	36
Rb^+	0.03	0.2	no measurable affinity	

(1) Cell pH. As phosphate is exchanged for hydroxyl ions or cotransported with protons [7,8], the pH of the cell is lowered as a consequence of phosphate absorption. A decrease in cell pH may increase the rate of uptake of cations by yeast (ref. 9 and Theuvenet, A.P.R., Roomans, G.M. and Borst-Pauwels, G.W.F.H., unpublished). It could thus be imagined, that the stimulating effect of phosphate on Na^+ uptake is an indirect effect via the cell pH. Phosphate stimulates Na^+ uptake already at low concentrations where the effect on the cell pH is very small. Addition of 50 μM phosphate decreased the cell pH only about 0.03 within 1 min. This decrease is not sufficient to affect the rate of Na^+ uptake significantly.

(2) Extracellular potassium. Absorption of phosphate by yeast cells is associated with efflux of K^+ from the cells. K^+ stimulates the uptake of Na^+ over a limited range of Na^+ concentrations [2]. At the very low concentrations of phosphate, where stimulation of sodium uptake occurs, the raise in extracellular K^+ concentration was about 5 μM within the first minute after addition of phosphate. This change in extracellular K^+ concentration is not sufficient to account quantitatively for the stimulation of sodium uptake by phosphate. Furthermore, phosphate was found to enhance Na^+ uptake at all sodium concentrations. This cannot be explained by an indirect effect of phosphate via K^+ efflux.

(3) Intracellular ATP level. It might be conceived, that phosphate stimulates Na^+ uptake by increasing the intracellular ATP level. The uptake of Na^+ is however, also enhanced by low concentrations of arsenate, which competes for the same transport sites as phosphate [10], but does not rise the intracellular ATP concentration. The stimulation of Na^+ uptake by arsenate was studied under the same experimental conditions as the stimulation by phosphate (Fig. 4). The stimulating effect of arsenate is somewhat lower than that of phosphate, which

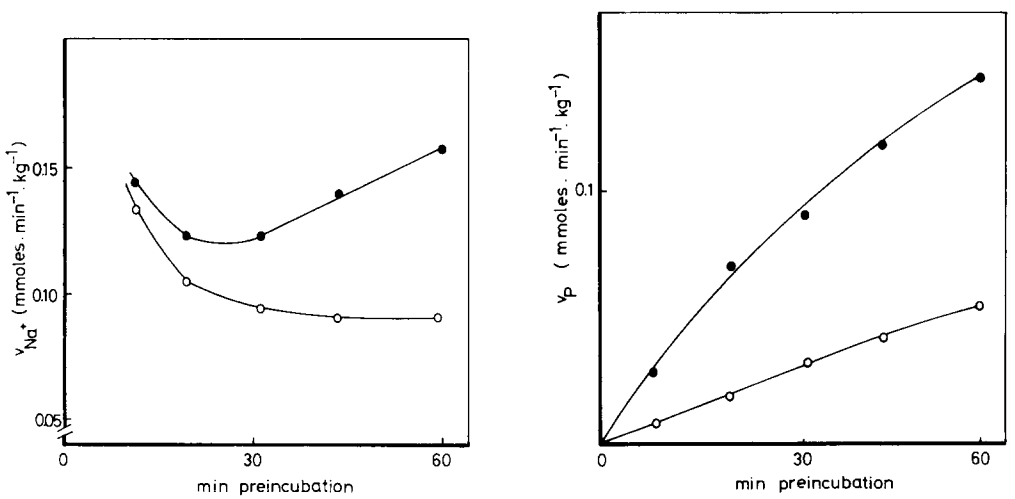


Fig. 5. Effect of the anaerobic preincubation on sodium uptake and phosphate-stimulated sodium uptake (40 μM sodium). ○, control; ●, 50 μM phosphate.

Fig. 6. Effect of the anaerobic preincubation on phosphate uptake and sodium-stimulated phosphate uptake (1 μM phosphate). ○, control; ●, 15 mM Na^+ added.

may be attributed to inhibition of cell metabolism.

Both cation uptake and phosphate uptake in yeast depend on the period of anaerobic preincubation with glucose, although in different ways: phosphate uptake increases with the period of preincubation [11], cation uptake decreases (Theuvenet, A.P.R., Roomans, G.M. and Borst-Pauwels, G.W.F.H., unpublished). It was therefore investigated how the sodium ion-phosphate cotransport depended on the anaerobic preincubation. The stimulation of Na^+ uptake by phosphate increases with the period of preincubation (Fig. 5). The same applies to the stimulation of phosphate uptake by sodium (Fig. 6).

Discussion

The mutual stimulation of phosphate uptake by sodium and of sodium uptake by phosphate has been tentatively explained by assuming the existence of a sodium ion-phosphate cotransport mechanism [1]. Several possible indirect effects have been considered in this study, but these fail to give a sufficient explanation for the observed phenomena. It has already been demonstrated earlier [1], that phosphate uptake in the presence of Na^+ at pH 7.2 can be described by a dual mechanism of transport. The low affinity mechanism, with a K_m of 30 μM, is sodium independent. According to Cockburn et al. [7] phosphate is taken up together with protons via this mechanism, which may mean that a potassium ion-phosphate cotransport is involved. This has also been suggested to be the case for phosphate uptake in *Neurospora crassa* [12] and *Candida tropicalis* [13].

Two sites with affinity for Na^+ and Li^+ are involved in the uptake of phosphate via the sodium-dependent high affinity mechanism. A comparison between these two sites, and the two sites of the cation uptake mechanism showed, that the affinity constants for Na^+ in both systems are in the same order of magnitude. Rb^+ has no appreciable affinity to the sodium-sensitive sites of the sodium ion-phosphate cotransport system. The same applies probably to potassium and caesium. Lithium, on the other hand, has a relatively high affinity to one of the sites of the cotransport system. The cation transport system has no site with such a high affinity to lithium. In view of these marked differences it can be concluded that the sodium-sensitive sites of the cotransport system are probably not identical with the cation transport system. This indicates the existence of a separate sodium ion (lithium ion)-phosphate cotransport system.

The rate of uptake of phosphate via the sodium-independent process increases with the length of the preincubation time with glucose. This applies also to the sodium ion-phosphate cotransport, in contrast to the uptake of Na^+ via the cation transport system. Also this does not suggest, that the extra phosphate uptake site is located on the cation transport system.

Based on the experimental results in this study, a model can be proposed for the mechanism of sodium and phosphate uptake at high pH. This model assumes the existence of three ion transport systems: (1) a cation transport system, having two sites to which cations can bind, and having affinity towards all alkali cations; (2) a phosphate transport system, having one site, with a K_m of 30 μM at pH 7.2 to which phosphate can bind; (3) a sodium-phosphate cotrans-

port system, having one site with affinity for phosphate (with a K_m of approx. 0.6 μM at pH 7.2) and arsenate, and two sites with affinity for sodium and lithium, but not for other alkali ions.

The fact that phosphate uptake is enhanced already by low sodium concentrations, whereas the intracellular sodium concentration as determined by flame spectrophotometry of ashed cells is about 10 mM, does not suggest that a sodium gradient would be the driving force for the sodium-phosphate cotransport, as seems to be the case in animal cells [14,15]. On the other hand, combination of one monovalent negative phosphate ion with two Na^+ may give rise to a positively charged complex, which could be transported along the electrical gradient.

In a number of plant cells, specific stimulation of phosphate uptake by Na^+ has been found [16—18]. This points to the possibility, that the occurrence of a sodium ion-phosphate cotransport system as described in this study for yeast may be a more wide-spread property of single plant cells.

Acknowledgements

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