THE ACTIVE TRANSPORT OF PHOSPHATE INTO THE YEAST CELL*

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The exchanges of phosphate between cellular and intracellular compartments have been studied in a number of cell types. In each case the entry of phosphate into the cell seems to be associated with metabolic processes at the cell surface (1). For example, in red blood cells, the uptake of P^{32}-labelled phosphate is to a large measure dependent on glycolysis (2). It has a high temperature coefficient (3) and is blocked by metabolic inhibitors (2). Studies of precursor relationships and also of phosphate turnover in red cell stroma suggest that phosphate enters the cell by incorporation in the membrane into glycolytic intermediates such as 2,3-diphosphoglycerate and ATP (4, 5). In muscle, precursor relationships with P^{32} also suggest that phosphate enters the cell *via* some phosphorylation reaction (6). However, the latter studies involve the technical difficulty of distinguishing extra- and intracellular orthophosphate in the muscle mass. Autoradiographic studies (7) lend support to the phosphorylation concept. In sea urchin eggs formation of ATP at the cell surface seems to be an integral part of the mechanism of phosphate entry (8).

In microorganisms a dependence of phosphate entry on metabolic reactions is well established (9). Yeast has been most intensively studied. In growing yeast, the uptake of P^{32}-labelled phosphate requires the presence of sugar and is blocked at low temperature (10), and by metabolic inhibitors (9). The phosphate which is absorbed is largely converted to polymerized forms of phosphate (11, 12). The total amount of uptake is dependent on the presence of cations such as potassium and magnesium which are also absorbed (13). It is not clear whether the effect of potassium is due to a stimulation of the entry process for phosphate, or to a maintenance of cation-anion balance within the cell, or to stimulation of metaphosphate formation within the cell.

In the present study some of the properties of the process of phosphate entry into yeast cells have been investigated, including the kinetics of the reaction, the nature of the substrate, the nature of the entering ion, the effect of potassium, and certain other related phenomena.

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Methods

Non-growing populations of *Saccharomyces cerevisiae* were used. Bakers' yeast (Standard Brands, Inc.) was washed three times with distilled water and then aerated and starved for 18 to 24 hours. Cells were centrifuged again, resuspended in distilled water, and their concentration measured turbidometrically. In order to avoid known effects of inorganic cations, trishydroxyaminomethane, (THAM) or triethylamine (TEA) were used in a buffer mixture with succinic acid (14). In the pH range of 6.5–7.5 in which the buffer is ineffective, the suspensions were continuously titrated with TEA or with HCl.

The suspensions were bubbled with either nitrogen or air for 30 minutes with substrate in order to establish a uniform rate of metabolism. Orthophosphoric acid was then added, and the time of this addition was considered zero for all subsequent measurement. At specified times aliquots were withdrawn from the suspensions, centrifuged quickly, and the decanted supernatant was analyzed for phosphate. In cases in which cells were to be analyzed, they were washed at least twice before analyses were begun. Extraction of soluble cellular phosphate was carried out with cold 10 per cent trichloracetic acid. Specific procedures used in analysis were as follows:

Phosphate—method of Fiske and Subbarow (15) modified by the addition of 15 per cent ethyl alcohol before color development.

P^{32} activity—Geiger counter.

O_2 consumption and CO_2 production—standard Warburg procedure.

Glucose—method of Nelson (16).

RESULTS

In the absence of exogenous substrate, there is only a very slow exchange of cellular and extracellular phosphate (10), an observation confirmed in the present studies. Nevertheless, the extracellular phosphate was found to distribute into a cellular space representing about 10 per cent of the total cell volume, presumably the "cell wall space" demonstrated by Conway (17). This space is permeable to sugars, sodium chloride, and amino acids, but not to peptone or insulin.

Little binding or adsorption of phosphate by the cell surface was observed, even at very low concentrations of phosphate (1×10^{-6} M).

Beyond the initial distribution phenomena, no redistributions of phosphate take place between the resting cell and the medium. However, when glucose is added, there is a rapid uptake of orthophosphate by the cells as measured by its disappearance from the medium (Fig. 1). Furthermore, the rate of disappearance of phosphate is the same when it is measured chemically or by P^{32} activity. Thus the specific activity of the phosphate remaining in the medium does not change even after a large proportion of the phosphate has been absorbed, indicating the absence of exchange of cellular and extracellular phosphate. It must be concluded that the flow of the phosphate is essentially unidirectional, from the medium to the cell.

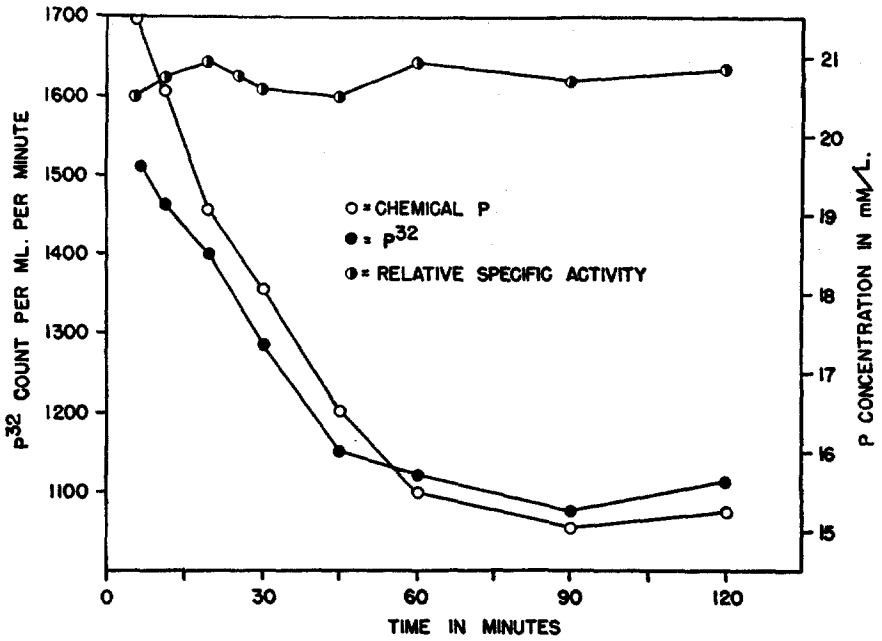

FIG. 1. The disappearance of P^{32} and of chemical phosphate from the medium during the fermentation of glucose by yeast. The yeast concentration was 50 mg./ml. of suspension, pH, 7.0; glucose, 0.2 M; and initial phosphate, 0.022 M.

TABLE I

The Dependence of Phosphate Uptake on Specific Substrate

Experiments were carried out aerobically, and pH was always in the range of 4.5–5.0. Initial concentration: yeast, 100 mg./ml.; substrate, 0.3 M; KCl, 0.02 M; H_3PO_4, 0.01 to 0.02 M.

Substrate	No. of experiments	Rate of phosphate uptake in mM of P/kg. yeast
Glucose	5	254.8
Fructose	4	201.6
Mannose	3	161.4
Lactate	2	10.3
Pyruvate	5	9.1
Acetate	4	0
Ethyl alcohol	4	49.5

Not every substrate will support a rapid uptake of phosphate. In fact, the reaction was relatively specific for the fermentable sugar, glucose, fructose, and mannose (Table I). The respiration of alcohol supported a rate of phosphate uptake only 20 per cent of that found with glucose and the respiration of lactate, pyruvate, and acetate could support only a very low rate of uptake.

Furthermore, the finding that the phosphate uptake induced by glucose is just as rapid under anaerobic as under aerobic conditions (18) was readily confirmed. Thus glycolysis reactions seem to provide the major source of energy for the reaction.[1]

Other evidence that glycolysis rather than respiration supplies the energy for phosphate uptake is found in the time relationship of the processes. If glucose is added 30 minutes prior to phosphate, a steady rate of phosphate uptake is immediately established. If, however, the glucose and phosphate

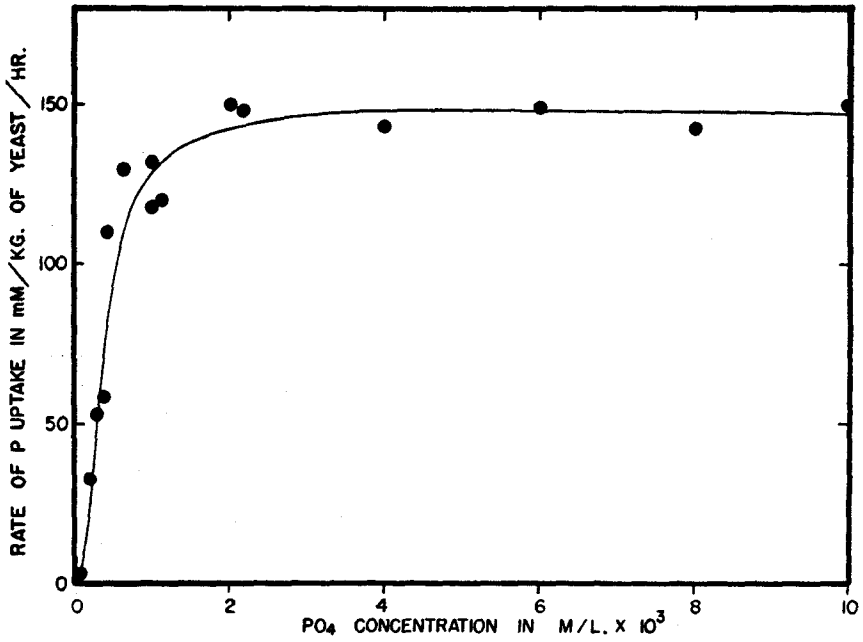

FIG. 2. The relationship between the initial rate of phosphate uptake and the initial phosphate concentration. The yeast concentrations varied from 10 to 50 mg./ml., the pH was 4.0; glucose, 0.2 M; and K^+, 0.02 M.

are added simultaneously, there is a 10 to 15 minute lag period before a steady rate of phosphate uptake is achieved. A similar lag period in the attainment of the maximal rate of fermentation can be demonstrated, whereas no such lag period occurs in the case of respiration.

Although phosphate uptake is dependent on the fermentation of glucose, no apparent stoichiometric relationship exists between the two processes. The maximal rate of phosphate uptake observed was 260 mM per kg. of cells

[1] In this strain of yeast there is a high rate of aerobic glycolysis. Thus even in the aerobic experiment, about 40 per cent of the glucose was fermented.

per hour. The sugar uptake under the same conditions was 1500 mM per kg. of cells per hour. In the absence of phosphate, the rate of sugar uptake was slightly reduced.

The rate of phosphate uptake as a function of the phosphate concentration follows an asymptotic relationship (Fig. 2). The maximal rate for this experiment of 160 mM per kg. of cells per hour, was attained at phosphate concentration greater than 2×10^{-3} M. The data can be fitted by the Michaelis-Menten equation with a Km of 4×10^{-4} M.

An appreciable phosphate uptake was observed at a concentration as low as 2×10^{-4} M. In view of the fact that the orthophosphate concentration within the cells (determined by cold trichloracetic acid extraction) was 2×10^{-2} M or 100 times as high, it is apparent that phosphate can be taken up against a

TABLE II

Changes in Cellular Phosphate Fractions during Phosphate Uptake

The experiment was carried out under anaerobic conditions at pH 4.5. The yeast concentration was 250 mg./ml. of suspension, glucose 0.3 M, phosphate 0.05 M, and potassium when present 0.02 M.

Time	TCA-extractable						Non-extractable		Total cellular	
	Inorganic		Labile		Stable					
	No K	K	No K	K	No K	K	No K	K	No K	K
min.										
0	19.7	26.7	3.8	3.4	17.5	12.1	61.1	71.5	115.1	128.8
10	25.0	36.9	5.8	9.2	22.2	13.8	64.9	86.4	131.7	164.6
20	25.0	25.0	8.0	6.9	25.9	23.5	72.3	119.4	146.6	200.3
40	25.8	26.9	12.2	10.3	21.8	15.9	86.4	125.5	164.6	205.2
60	25.8	25.3	12.9	11.2	24.4	14.9	78.3	108.7	158.1	183.3

Values are in mM of P/kg. of yeast.

large concentration gradient. Furthermore, the gradient was not altered by the influx of phosphate, for the cellular orthophosphate concentration remained constant during the course of the experiment (Table II), the phosphate taken up being entirely channelled into phosphate compounds.

The effects of potassium and of extracellular pH on phosphate uptake are of some interest. Previous studies (15) have demonstrated a marked stimulation of phosphate uptake by potassium. The extent of the potassium effect was found to be dependent on the pH of the medium (Fig. 3). For example, in the range of pH 7–9, there was little K-effect, whereas in the range of pH 3–6, stimulation was observed with a maximum of 700 per cent at pH 4.0. In consequence, the pH optimum for phosphate uptake was shifted in the presence of potassium from pH 6.5 to pH 4.8.

The effect of potassium is a specific one, for sodium was unable to stimulate the phosphate uptake.

The right hand legs of the pH curves of Fig. 3, lie in a position similar to that for the second dissociation of phosphoric acid, suggesting that the monovalent anion, $H_2PO_4^-$ is selectively absorbed in preference to the bivalent anion, HPO_4^{--}.

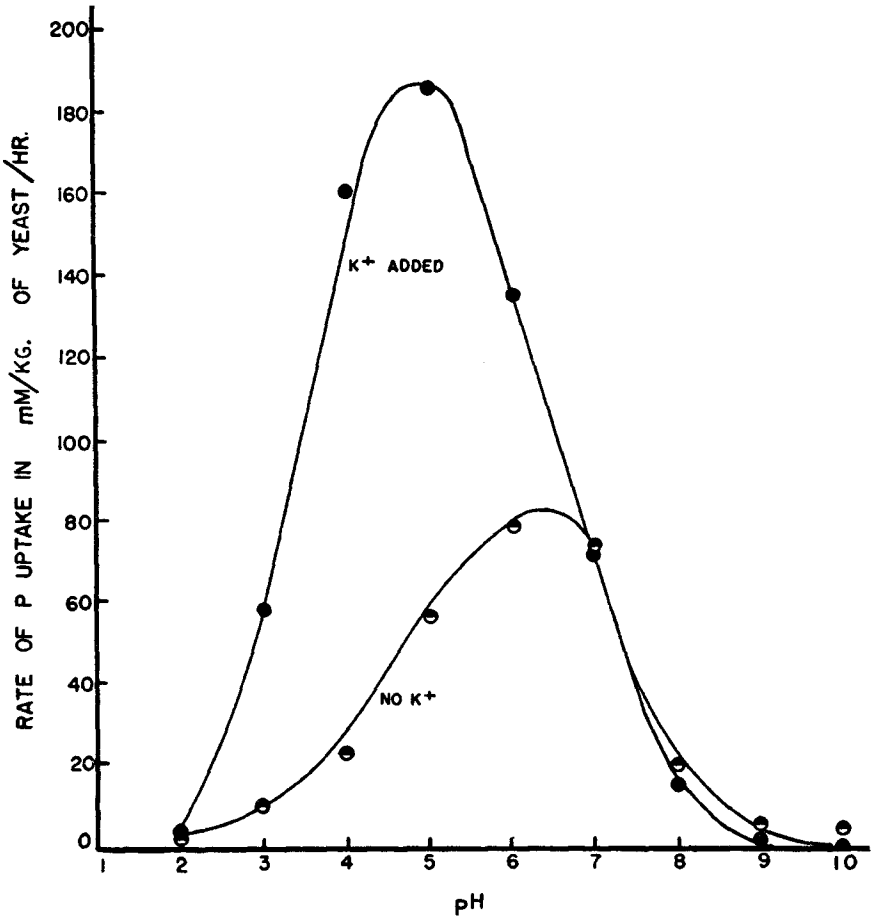

FIG. 3. The effect of pH on the uptake of phosphate in the presence and absence of K^+. The yeast concentration was 50 mg./ml.; the glucose, 0.2 M; and the K^+, 0.02 M.

DISCUSSION

Data have been presented indicating that the entry of phosphate into the yeast cell involves an active transport system:

(*a*) Orthophosphate is taken up against a concentration gradient as large as 100 to 1.

(*b*) The uptake is dependent on specific substrates.

(*c*) The relationship between phosphate concentration and rate of phosphate uptake is asymptotic, suggesting that entry of phosphate involves its combination with a limited quantity of a cellular constituent.

(*d*) During the uptake of phosphate, there is almost no efflux of the same ion, indicating that transport take place across a permeability barrier with a high resistance to phosphate diffusion.

The specific dependence of phosphate uptake on the fermentation of sugars, particularly glucose, is of interest. Of the fermentation reactions in the Emden-Meyerhof scheme, the only one that might be implicated in the esterification of orthophosphate is the glyceraldehyde-dehydrogenase step.

$$\text{Glyceraldehyde-3-PO}_4 + \text{PO}_4 \sim \text{1,3-diphosphoglycerate}$$

The same reaction is strongly implicated in phosphate uptake by red blood cells, on the basis of precursor relationship (5). The phosphate compound which shows the highest P^{32} specific activity early in the course of phosphate uptake is 2,3-diphospholgycerate, a compound in enzymic equilibrium with the 1,3-diphosphoglycerate in the red cell.

Another possibility is phosphorylation by the reverse reaction of phosphatases (20). However, in yeast, the phosphatases of the cell surface can be completely blocked by molybdate, with no effect on phosphate uptake (21).

If a phosphorylation reaction is indeed involved in phosphate transport, this reaction must be located at or near the surface of the cell where contact with extracellular phosphate is possible. Furthermore the reaction must be isolated from the interior of the cell by a permeability barrier to phosphate which prevents exchanges of intra- and extracellular phosphate. Evidence has been presented elsewhere (22) that such is the case, that the glycolytic reactions in the yeast cell are located in a compartment in the periphery of the cell. Phosphorylation at the cell surface *per se* does not explain the absence of phosphate exchanges, for the reactions are themselves reversible and a phosphorylation reaction in equilibrium with both extra- and intracellular phosphate would allow exchanges to occur. It may be presumed, therefore, that an essentially irreversible phosphate transport step across a membrane is involved, in association with the phosphorylation reaction.

The effects of potassium and pH on phosphate uptake are complex. The pH is due in part to the selective uptake of $H_2PO_4^-$ in preference to HPO_4^{--}. In addition the rate of fermentation is influenced by the extracellular pH. For example, in the absence of potassium there are two pH optima, at 4.5 and 8.2 (23). However, neither of these corresponds to the optimum for phosphate uptake at pH 6.5. In the presence of potassium, the fermentation is independent of pH over a broad range (pH 2.5–8.5 for the potassium concentration in the present experiment). Again there is no apparent relation-

ship to phosphate uptake. Thus, the pH effect on phosphate uptake seems to reflect in large a direct action of H^+ on the phosphate transport mechanism.

The stimulation of phosphate uptake by potassium may involve three phenomena:

(*a*) A direct stimulating effect of potassium on the transport mechanism.

(*b*) A shift of the pH in the cell surface region by a membrane-hydrolysis phenomenon. The outer membrane of the cell is impermeable to anions, but permeable to K^+ and H^+. Therefore an increase in the extracellular concentration of K^+ results in alkalinization of the cell surface compartment by an exchange reaction (22). This mechanism accounts for the reversal of the inhibitory effects of H^+ on fermentation by K^+ (14), and perhaps also for the shift in the optima of the pH curves for phosphate uptake (Fig. 3).

(*c*) A balancing of cellular cations and anions. Small quantities of $H_2PO_4^-$ can be taken up without cation absorption at the expense of cellular buffer capacity (cell more acid, medium more alkaline). However, the uptake of phosphate in large quantities for an extended period can continue only if a cation such as K^+ is also absorbed. The uptake of K^+ and $H_2PO_4^-$ are not directly linked. If both ions are presented to the cell at the same time the K^+ is taken up more rapidly, largely in exchange for H^+ (19), but after 10 to 15 minutes the potassium uptake slows down and the uptakes of the two ions become roughly equal.

SUMMARY

Phosphate can distribute in the cell wall space, but is not bound to an appreciable extent at the cell surface in non-metabolizing yeast. During metabolism of sugars, phosphate is actively transported into the yeast cell by a mechanism specifically involving glycolysis reactions. The movement of phosphate is in the inward direction only (no appreciable efflux), and it can proceed against a concentration gradient of 100 to 1. It is dependent on external phosphate concentrations in an asymptotic relationship, but is independent of the cellular orthophosphate concentration. The pH optimum for the phosphate uptake of 6.5 is shifted to the acid side by potassium. At certain values of pH a stimulation of 700 per cent by potassium can be observed. The nature of the effects of K^+ and H^+ are discussed.

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