Biochimica et Biophysica Acta 855 (1986) 179–185
Elsevier

BBA 72921

Effects of monovalent cations on derepression of phosphate transport in yeast

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(Received August 22nd, 1985)

Key words: Phosphate transport; Monovalent cation; Derepression; (Yeast)

The effect of monovalent cations on derepression of phosphate transport was studied. It was found that ammonium, K^+ and Rb^+ accelerate the derepression of phosphate transport produced by glucose in yeast *(Saccharomyces cerevisiae)*. Na^+ and Li^+ were ineffective in accelerating derepression; Cs^+ produced only a minor stimulation. The concentration range of both K^+ and NH_4^+ that accelerated derepression was similar to that required for transport to occur. In the case of ammonium, the effects seem to depend exclusively on the so-called low-affinity transport system. The effect was strongly dependent on pH, with an optimum around 6; however, the increase in the pH of the medium did not produce in itself a high increase of the derepression. Derepression was dependent on the presence of glucose, and it was very low with ethanol as substrate. The mechanism seems to depend on the ability that both K^+ and NH_4^+ have to decrease the membrane potential of the cell while transported, and not on the capacity to produce the alkalinization of the cell interior. In addition, the phenomenon depends on the presence of glucose as substrate, which indicates the involvement of some product of glucose metabolism in the mechanism, and possibly some relation to catabolic repression.

Introduction

In early work [1–3], phosphate transport was found to require preincubation with glucose to be observed fully in yeast. Goodman and Rothstein [1] also described the stimulation of phosphate transport by K^+. However, the experimental conditions employed by these authors are difficult to assess from the data reported. Interactions of phosphate transport with divalent cations have also been described [1,2,4–6].

In *Neurospora crassa*, Lowendorf et al. [7], showed that a similar preincubation requirement was due to de novo protein synthesis of the carrier. This phenomenon has been studied also in *Saccharomyces* [8–10], and it seems to be part of a more general increase of many different transport systems when yeast cells are incubated with glucose or other substrates (see Ref. 10). Similar findings were reported for sulfate transport [11]; stimulation of amino acid transport by preincubating yeast cells with K^+ has also been reported [12].

This work was aimed at defining the effects of monovalent cations on derepression of phosphate transport transport in yeast. The data reported here are the rsults of such investigation.

Materials and Methods

30 g of pressed yeast cells, *Saccharomyces cerevisiae* (La Azteca, S.A.) were incubated for 8–10 h

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Abbreviations: TEA, triethanolamine; MES, 4-morpholineethanesulfonic acid; FCCP, carbonyl cyanide trifluoromethoxy phenylhydrazone; $DiSC_3(3)$, 3,3′-dipropylthiacarbocyanine.

with continuous air bubbling in 1 liter of the medium described by De Kloet et al. [13]. The cells were then centrifuged, washed once with deionized water and resuspended in 500 ml of deionized water. After 16–18 h of aeration, the cells were centrifuged again, washed twice and resuspended in deionized water to a ratio of 0.5 g wet weight per ml of the suspension.

Phosphate transport. The cells (50 mg, wet weight) were preincubated for variable times in a medium comprising 20 mM Mes-TEA buffer (pH 6.0)/50 mM glucose, final volume, 1.0 ml. In some experiments, the amount of yeast was adjusted according to the volume used. After preincubation, an adequate volume of NaH_2PO_4 labeled with ^{32}P was added to give a final concentration of 100 μM. After 5 min, an aliquot of the mixture was taken and filtered through a cellulose acetate filter, 0.45 μm mean pore size, and washed several times with unlabeled 10 mM NaH_2PO_4 (pH 6.0). The filters were then dried, placed in a scintillation vial with an adequate scintillator and counted. 5 min incubation time was selected after testing that uptake was linear over very short incubation times. However, for higher precision, the kinetics experiments were performed using 3 min of incubation with phosphate for the measurement of transport.

pH. For the pH curve, the cells were preincubated for 25 min with 20 mM of the buffers succinate-TEA (pH 4.0 or 5.0); Mes-TEA (pH 7.0), and 50 mM glucose, with or without 5 mM KCl or NH_4Cl. Then the cells were centrifuged and resuspended in 20 mM Mes-TEA buffer (pH 6.0)/50 mM glucose; 100 μM [^{32}P]phosphate was added and its transport was measured as described.

Membrane potential. This was estimated as described before [14], by following the fluorescence changes of $DiSC_3(3)$ at 540–590 nm. The cyanine was added after incubating the cells in the buffers indicated in the last paragraph, depending on the pH used, and 50 mM glucose. By recording the fluorescence values, the changes produced by the cations could be observed.

Results

As found by others [8–10], when the starved cells prepared by our procedure were incubated with glucose, and ^{32}P uptake was measured also in the presence of glucose after different times of preincubation, practically no phosphate was taken up during the first minutes. In 15–20 min the rate of uptake started to increase, and after approximately 50–60 min it reached a constant value (Fig. 1). If 5 mM of either KCl or NH_4Cl were added to the medium, the rate of increase of the uptake was accelerated, and the maximum valuel of uptake reached was higher. Fig. 2 shows that the phenomenon was dependent on the concentration of the cations, and that rather low concentrations of either K^+ or NH_4^+ were required to produce the effect. Higher concentrations than those shown in Fig. 2 did not produce a further acceleration of derepression; in addition, it was found that Na^+ and Li^+, added under the same conditions failed to produce the increase of derepression observed. Cs^+ produced only a minor increase. Rb^+ produced a smaller effect than potassium, and ammonium was more effective than K^+, but the difference shown in Fig. 1 was not consistent (see Fig. 3). It should be mentioned also that the incubation of the cells in the absence of a substrate with or without cations added did not result in any transport of phoshate (only the results in

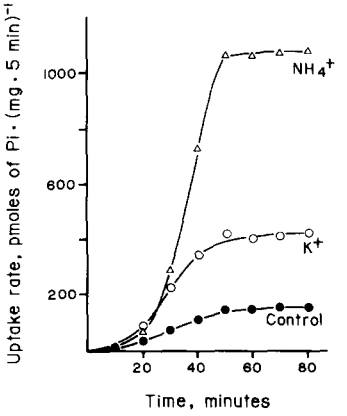

Fig. 1. Effects of K^+ and NH_4^+ on derepression of phopshate transport by yeast. The cells (50 mg, wet weight) were preincubated with 20 mM Mes-TEA (ph 6.0) and 50 mM glucose. Where indicated, 5 mM KCl or NH_4Cl was included. At the indicated times, 100 μM $NaH_2{}^{32}PO_4$ was added. Incubation was allowed to proceed for 5 min. Then an aliquot was taken, filtered, washed and counted as described under Materials and Methods. Final volume was 1.0 ml; incubation was performed at room temperature.

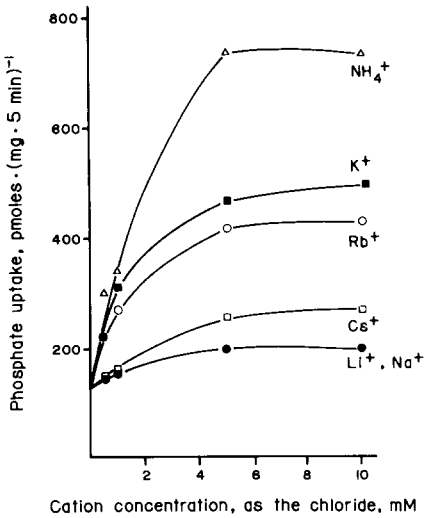

Fig. 2. Effects of variable concentrations of monovalent cations on derepression of phosphate transport in yeast. The experiment was carried out as described for Fig. 1, but variable concentrations of the cations were used and the preincubation time was always 25 min.

TABLE I

EFFECTS OF AMMONIUM AND K^+ ON PHOSPHATE TRANSPORT AND ON THE DEREPRESSION OF PHOSPHATE TRANSPORT IN YEAST

The cells, 50 mg wet weight, were preincubated for 25 min in 20 mM Mes-TEA buffer (pH 6.0)/50 mM glucose, with the additions indicated: 5 mM KCl, NH_4Cl, or 200 μM cycloheximide (CH) were used. After preincubation, the additions of the second column were made, 100 μM [^{32}P]phosphate, either alone, or with 5 mM KCl or NH_4Cl or cycloheximide. Results of two different experiments are presented.

Addition during preincubation (25 min)	Additions at 25 min	Phosphate transport (nmol/g per 5 min)	
		Expt. I	Expt. II
Substrate omitted	P_i	7	14
None	P_i	269	235
None	P_i + CH	215	185
CH	P_i	10	20
K^+	P_i	1014	864
None	$P_i + K^+$	309	284
K^+	P_i + CH	921	849
K^+ + CH	P_i	26	32
NH_4^+	P_i	1166	1023
None	$P_i + NH_4^+$	377	302
NH_4^+	P_i + CH	1140	957
NH_4^+ + CH	P_i	29	38
NH_4^+ + K	P_i	1209	975
None	$P_i + K + NH_4^+$	326	290

the absence of a substrate and cations are shown in Table I).

Fig. 3 shows that, in agreement with other authors [8–10], cycloheximide could block derepression observed both in the absence or in the presence of the cations. This experiment was carried out by preincubating the cells with glucose either in the absence or in the presence of K^+ or NH_4^+ for 25 min, but cycloheximide was added at different times during preincubation. After this, phosphate was added to measure its transport as described under Materials and Methods. The inhibition of derepression, depended on the time of addition of cycloheximide; if added before the first 15 min during preincubation, it produced a large inhibition of derepression. If cycloheximide was added later than 15 min during preincubation, the inhibition was small. Cycloheximide was added later than 15 min during preincubation, the inhibition was small. Cycloheximide, at the concentration used (200 μM), did not produce any significant inhibition of transport itself (Table I).

It was important to find out whether the effects of ammonium and K^+ could be additive, because this might give an indication about the possibility

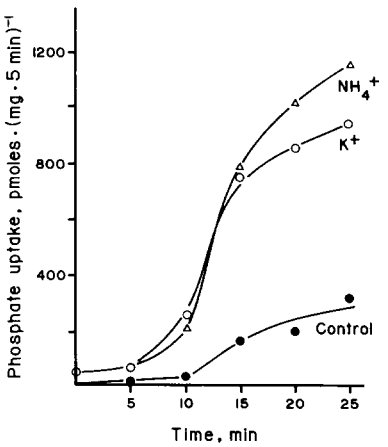

Fig. 3. Effect of time of addition of cycloheximide on derepression of phosphate transport with or without the addition of K^+ or NH_4^+ in yeast. Preincubation was carried out for 25 min for all experimental points as follows: 20 mM Mes-TEA (pH 6.0); 50 mM glucose; 50 mg wet weight of yeast; final volume, 1.0 ml; room temperature. 200 μM cycloheximide was added at the indicated times and preincubation was allowed to proceed for 25 min. The 100 μM [^{32}]phosphate was added and its transport was measured as described under Materials and Methods. K^+ and NH_4^+ were used at 5 mM concentration.

of both cations acting by different mechanisms. The results of Table I show that this was not the case; the simultaneous addition of K^+ and NH_4^+ hardly increased derepression as compared to that produced by NH_4^+ separately. This same table shows that the addition of either NH^+ or K^+ after the preincubation with glucose alone produced only a small stimulation of phosphate transport by the cells.

The transport of K^+ into yeast cells is indirectly responsible for an increased alkalinization of the cell interior, depending on the external value of pH [15]. Unpublished results from our laboratory have shown that the presence of NH_4^+ in the medium also produces increased alkalinization of the cell interior. It has been found also that the increase in the pH of the medium in the presence of a substrate increases the proton pumping activity of the cells, and this produces also their internal alkalinization [14,16]. If the internal alkalinization of the cells were the factor responsible for the accelerated derepression of the phosphate transport system, this should be produced also by increasing the pH of the medium during preincubation. It has been demonstrated also that phosphate transport itself depends to a great extent on both the internal and the external values of pH [17]. An experiment (Fig. 4) was performed by preincubating the cells with glucose at different pH values with or without the addition of K^+ or NH_4^+. After 25 min the cells were centrifuged and all of them were resuspended and incubated in 20 mM Mes-TEA buffer (pH 6.0)/50 mM glucose. 100 μM [^{32}P]phosphate was added, and transport was allowed to continue during 5 min; an aliquot was taken, filtered and treated as usually. It was found that transport increased when changing from pH 6.0 to pH 7.0; however, K^+ and NH_4^+ produced a much larger increase than the pH increase alone. Also, this increase was much higher at pH 6.0 and 7.0, which were the highest pH values studied (Fig. 4).

It has been shown that the addition of K^+ decreases the membrane potential of yeast [14,16]. It might be also possible that the addition of NH_4^+ produces the same effect if this cation is transported into the cells. The decrease in the membrane potential might be the triggering mechanism for derepression of phosphate transport; if so, this

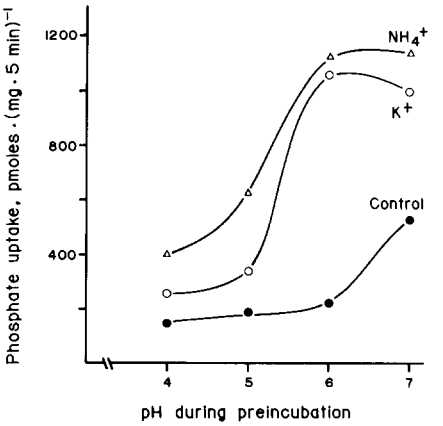

Fig. 4. Effect of preincubation pH and K^+ or NH_4^+ on derepression of phosphate transport in yeast. The cells (50 mg, wet weight), were preincubated as usual, but using the buffers described under Materials and Methods for the different pH values in the absence or in the presence of 5 mM KCl or NH_4Cl. After preincubation, they were centrifuged and resuspended in 20 mm MEs-TEA buffer (pH 6.0)/50 mm glucose; final volume, 1 ml. 100 μM [^{32}P]phosphate was then added and the transport of phosphate was allowed to proceed for 5 min before following the general procedure already described under Materials and Methods.

decrease not only should be produced both by K^+ and NH_4^+, but it also should be observed at all pH values tested so far, i.e., from 4.0 to 7.0. The experiment of Fig. 5 shows that this was in fact the case. At all pH values tested, the addition of the cations could produce a clear decrease in the fluorescence of $DiSC_3(3)$ which can be interpreted as a decrease in the membrane potential of the cell [14].

It was also necessary to determine whether the cation-stimulated derepression of phosphate transport involved the same or other system as compared with that derepressed only in the presence of glucose. To this purpose, experiments were carried out to determine the kinetic properties of the carrier system under different conditions. Fig. 6 shows that there was only a small change in the K_m value from 96 μM to 54 and 48 μM with K^+ or NH^+, respectively. The V_{max} values, however, increased more than 3-fold with K^+, and more than 6-fold with ammonium. Experiments are in progress to define the effects of monovalent cations on transport at lower phosphate concentrations.

Another way to detect differences between the

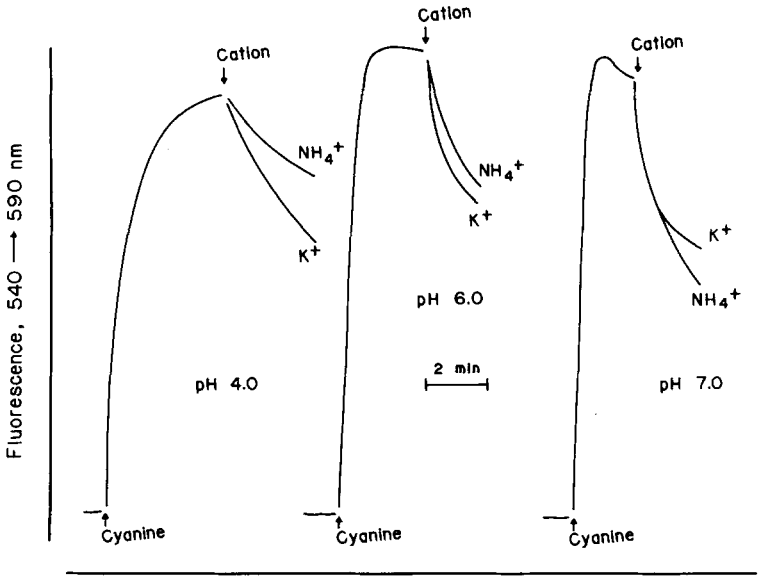

Fig. 5. Effects of K^+ or NH_4^+ on the membrane potential in yeast at different pH values. 50 mg of cells were incubated for 1 min in 20 mM buffer of the indicated pH values, as described under materials and Methods, with 50 mM glucose and 10 μM FCCP. Then, 0.5 μM $DiSC_3(3)$ was added and fluorescence was followed at 540–590 nm. Where indicated, 5 mM KCl or NH_4Cl was added.

derepressed transport systems consisted in defining the rate of disappearance of the transport system after stopping protein synthesis with cycloheximide. It might be also that the apparent increased derepression was due to a decreased degradation of the carrier, and not necessarily to an increased rate of synthesis. Fig. 7 shows that, after a preincubation of 1 h, upon the addition of cycloheximide, no important differences were observed in the rate of decay. Also, in all three cases, the phenomenon was found to be a first-order process.

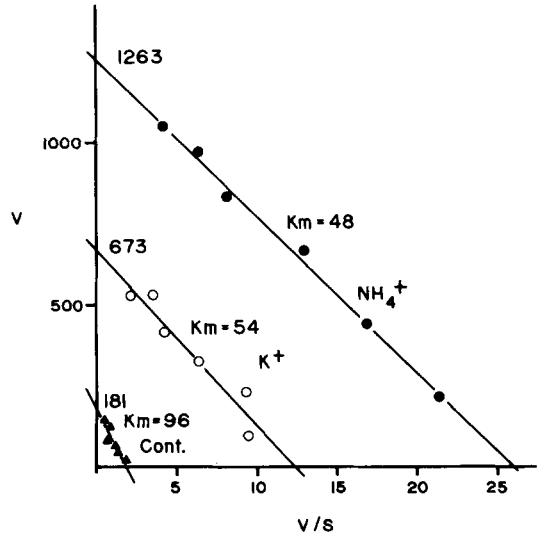

Fig. 6. Kinetics of phosphate transport derepressed in the absence or in the presence of 5 mM K^+ or NH_4^+. The cells (50 mg wet weight) were preincubated for 25 min in 20 mM Mes-TEA (pH 6.0)/50 mM glucose. After preincubation, variable concentrations of [^{32}P]phosphate were added, to measure its transport with an incubation time of 5 min, as described under Materials and Methods. Final volume, 1.0 ml; room temperature. The most probable straight lines and V_{max} and K_m values were obtained by the least-squares method.

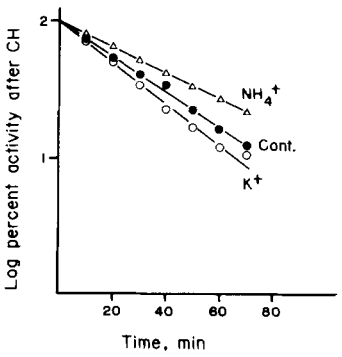

Fig. 7. Degradation of the transport system after derepression. The cells (50 mg, wet weight), were preincubated for 1 h in 20 mM Mes-TEA (pH 6.0)/50 mM glucose, with or without 5 mM K^+ or NH_4^+. At this point, 200 μM cycloheximide, as well as [^{32}P]phosphate, was added at the indicated times to measure its transport as described under Materials and Methods, using incubation times of 5 min. Each tracing represents the percentage of its own initial value after the time in which cycloheximide was added (after 1 h of preincubation).

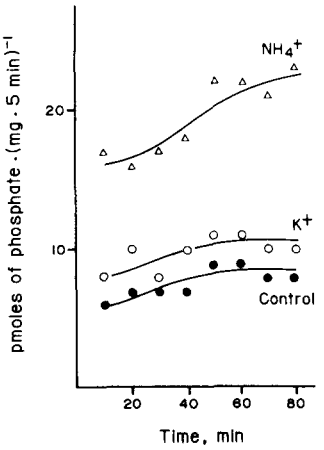

Fig. 8. Derepression of phosphate transport in yeast, preincubating with ethanol. The experiment was performed as described for Fig. 1, but 100 mM ethanol supplemented with 0.015% H_2O_2 was used as the substrate.

Due to the high rate of metabolism of glucose, and the possibility that some of its metabolites might be involved in the derepression phenomenon, another substrate, ethanol, accompanied by H_2O_2, was used as substrate during the preincubation of the cells and transport. Ethanol, either alone or in the presence of K^+ or NH_4^+, did not produce any significant derepression of phosphate transport (Fig. 8). Even though NH_4^+ seemed to increase transport, the maximal rate attained was negligible compared to that reached with glucose (see Fig. 6, for instance).

Discussion

The data obtained from this work show that it is the derepression [8–10] that is mostly stimulated by the presence of both K^+ and NH_4^+ in the medium. The derepression was blocked by cycloheximide, it was not due to differences in the rate of degradation of the carrier, and it seems that the same carrier was synthesized in the presence or in the absence of the cations. Additionally, neither K^+ nor NH_4^+ produced any significant stimulation of the transport system for phosphate once it was synthesized (Table I). It should be noted that Knotková and Kotyk [8] and Kotyk et al. [10] did not find this stimulation of derepression when adding 40 mM NH_4^+ to the incubation medium in the presence of glucose. However, the preparation of their cells, their experimental conditions and the strains used were different to ours.

The observed stimulation seems to be related to the transport of the cations; in the case of K^+, the concentrations at which derepression was observed were similar to those at which transport occurs [18]. In the case of NH_4^+, the transport system involved would be that of low affinity [18]. At least in the case of the monovalent cations, the selectivity of the system studied was similar to that of the transport system for monovalent cations in yeast [18]. Also, the fact that the effects of ammonium and K^+ were not additive may indicate a comon mechanism for both, that may be secondary to their transport.

It seemed possible that the internal pH, modified by the transport of the cations, triggered derepression of phosphate transport. However, the effects observed in the pH curve indicated that at least the internal pH change was not the only factor involved; even though a stimulation of derepression was observed at pH 7.0, it was not large, and still under these conditions, both K^+ and NH_4^+ produced an important effect additional to that of pH. This is not the case for the changes of the internal pH produced by K^+ at a high medium pH; under these conditions, K^+ has only a small effect on internal pH or metabolism, and vice versa, in the presence of K^+, the increased pH of the medium does not have any effect on metabolism or internal pH [15]. In any case, however, these results seem different to the effects that both the internal and external variationso f pH have on the uptake of phosphate [17].

Another factor that might be involved in the mechanism of this derepression is the decrease of the membrane potential that both NH_4^+ and K^+ produce upon their addition to the medium [14,20]. This effect was produced at low and high medium pH. However, it has been described that low pH of the medium produces a rather marked decrease of the electric potential of the yeast membrane [16], and no correlation was found between membrane potential and derepression of phosphate and other transport systems [10].

Finally, the glucose requirement for derepression to be produced, and the failure of ethanol to produce it, might indicate that, whatever the trig-

gering mechanism, the phenomenon depends on some metabolite of glucose. However, our results showing almost no derepression with ethanol as substrate are in some disagreement with those reported by other authors [8–10]; for the moment, it is difficult to know which of the metabolites, primary or secondary, could be involved, the more so in view of the analysis of other metabolites such as inorganic phosphate and the adenine nucleotides that has been performed and which showed no clear correlation with the phenomenon [11]. The glucose requirement reported here, might indicate some relationship of this phenomenon to that of catabolic repression.

Acknowledgements

This work was partially supported by grant No. ICCBBXNA-01877, from the Consejo Nacional de Ciencia y Tecnologia, de México.

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