THE PARTICIPATION OF PHOSPHATE IN THE FORMATION OF A "CARRIER" FOR THE TRANSPORT OF MG^{++} AND MN^{++} IONS INTO YEAST CELLS*

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ABSTRACT

During the absorption of phosphate by yeast, the cells acquire the capacity to absorb Mn^{++} and Mg^{++}, a capacity which is retained even after phosphate is no longer present in the medium. Cells pretreated with phosphate and then washed, slowly lose their ability to absorb Mn^{++}, the rate of loss depending on the temperature and on the metabolic state. The fermentation of sugars induces a very rapid loss of absorptive capacity, whereas the respiration of ethyl alcohol, lactate, or pyruvate has little effect. Inhibitor studies with sodium acetate, redox dyes, and arsenate, reveal parallel effects on Mn^{++} absorption, and on phosphate absorption. It is concluded that the synthesis of a carrier for the transport of Mg^{++} and Mn^{++} involves a phosphorylation step closely coupled with reactions involved in the absorption of phosphate.

Yeast cells in the absence of glucose bind bivalent cations to the cell surface (1). Cations so bound are completely exchangeable with the cations of the medium. Certain of these cations, particularly Mg^{++} and Mn^{++}, can be specifically absorbed by yeast, in a non-exchangeable manner, provided that glucose is available and that phosphate is also absorbed (2). This cation absorption is stimulated by low concentrations of K^+, but inhibited at higher concentrations. The inhibitory effect of K^+ is due to the accumulation of a large surplus of K^+ within the cell, whereas the stimulation of uptake at the lower concentrations is the result of the enhancement of phosphate accumulation (3). The rate of Mn^{++} uptake is also conditioned by the concentration of phosphate; and, although the rate of uptake is never greater than the rate of

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phosphate absorption when these occur simultaneously, Mn^{++} uptake may continue for a time after all of the phosphate has disappeared from the medium. It is apparent, therefore, that the *absorption*, rather than the presence of phosphate, is the important factor in Mn^{++} accumulation. It is suggested from the time course studies that a phosphorylation product responsible for the accumulation of Mn^{++} may be formed during the course of phosphate uptake (2). In the present paper, information is presented concerning the synthesis and decay of a phosphorylated "carrier" for bivalent cations. These data, together with those from inhibitor studies, suggest that the synthesis of carrier is closely related to the process of phosphate absorption.

Methods

Fresh bakers' yeast was thoroughly washed and suspended in a metabolically inert buffer containing triethylamine, tartaric, and succinic acids in 22.5 mM concentration and with a pH of 5.0. The temperature was 25°C., and aeration and stirring of the suspensions were by a stream of air. Nitrogen replaced air in anaerobic experiments.

The general procedure for the experiments was to pretreat the cells with 0.1 M glucose and 0.5 mM potassium chloride for half an hour (to stimulate maximum phosphate absorption (4) and then to add 1.0 mM phosphate to the suspension). After an hour, by which time all the phosphate had been absorbed, the yeast cells were washed twice and resuspended in inert buffer. Manganous chloride and those substances which might affect the Mn^{++} uptake were then added at the appropriate concentrations.

Disappearance of Mn^{++} from the medium was followed using Mn^{54} isotope. Samples of the suspension were taken at the times indicated, centrifuged, and the supernatant decanted for analysis with a scintillation counter. Surface binding was determined using control experiments with no glucose. It has been convenient not to present the data from such controls, as the initial (3 minutes) reading during absorption studies gives an almost exact value of the amount of binding. It should be emphasized at this point that the transport system under study shows the strongest preference for Mg^{++} (2). However, Mn^{++} was chosen for the experimental work, because of the suitability of its radioactive isotope. P^{32} was used as an isotope for those experiments in which phosphate was determined. It was counted with a Geiger counter.

RESULTS

In a previous study (2), it was found that if Mn^{++} was added simultaneously with K^+, phosphate, and glucose to a suspension of yeast cells, there was no Mn^{++} uptake by the cells until some phosphate had been absorbed (2). In contrast, cells pretreated with glucose, K^+, and phosphate, and then washed thoroughly and resuspended in a K^+- and phosphate-free buffer, were capable of absorbing Mn^{++} without delay, provided that glucose was supplied. In fact, such treatment did not result in any reduction of the amount of Mn^{++} absorbed, when compared with the quantity absorbed during a similar period of time with phosphate present in the medium. The pretreatment procedure

provides a method for studying the properties of the Mn^{++} transport system in isolation from the process of phosphate uptake, which is necessary for its initiation.

Although a large quantity of Mn^{++} was absorbed immediately after pretreatment, the cells gradually lost absorptive power on standing (Fig. 1), with the rate of loss conditioned by metabolic factors, such as temperature and substrates. For example, after 36 hours at 2°C., the pretreated cells lost

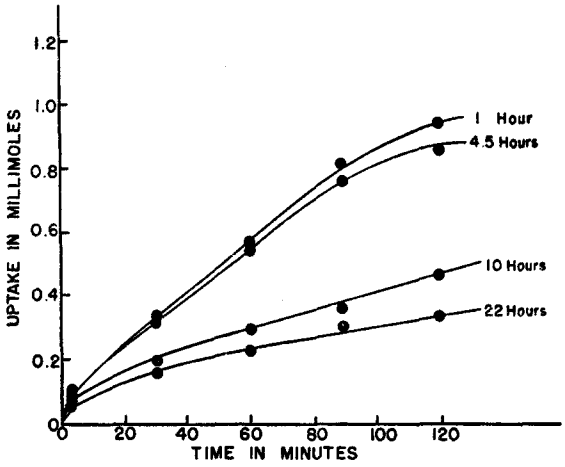

FIG. 1. The loss of ability of pretreated yeast cells to absorb Mn^{++}. The yeast concentration was 50 mg./ml.; pH, 5.0; temperature, 25°C. Yeast cells were pretreated with 1.0 mM phosphate and 0.1 M glucose, for 75 minutes. Mn^{++} uptake from 1.0 mM Mn^{++} was measured at the times indicated in the presence of 0.1 M glucose and 0.5 mM potassium chloride.

only 33 per cent of their absorptive capacity, but after only 8½ hours at 25°C., they lost 60 per cent. In the presence of glucose, at 25°C., the loss was especially rapid, over 50 per cent in 2 hours (up to 80 per cent in some experiments). The substrate effect was specific for sugars (Table I). Glucose and fructose were equally effective, whereas the respiration of alcohol, pyruvate, and lactate had little or no effect. The loss of absorptive capacity is apparently related to activity of the glycolytic rather than the respiratory pathways of metabolism, since the glucose effect was as great under anaerobic as under aerobic conditions. None of the treatments had any effect on the ability of the cells to acquire the capacity to absorb Mn^{++}, if given phosphate plus glucose.

The intimate relation between phosphate and Mn^{++} absorption suggested a study of the effect of certain inhibitors on each of the two processes.

A comparison was made using the following procedure: during the phos-

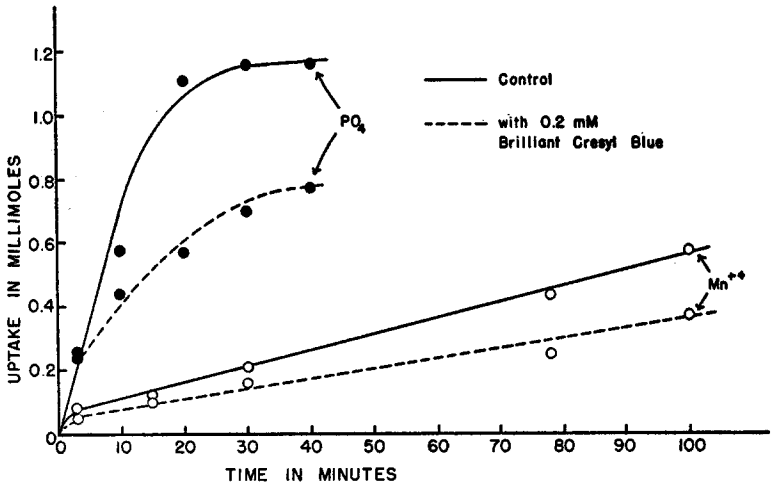

FIG. 2. The influence of 0.2 mM brilliant cresyl blue upon phosphate and Mn^{++} uptake. The yeast concentration was 50 mg./ml.; pH, 5.0; temperature, 25°C.; phosphate, 1.25 mM; and Mn^{++}, 1.0 mM. The yeast cells were pretreated with 1.25 mM phosphate for 40 minutes. Both phosphate and Mn^{++} uptake were measured in the presence of 0.1 M glucose and 0.5 mM potassium chloride.

TABLE I

The Influence of Specific Substrates on the Loss of Ability of Pretreated Yeast Cells to Absorb Mn^{++}

The yeast concentration was 50 mg./ml.; pH, 5.0; temperature 25°. The yeast cells were pretreated with 1.0 mM phosphate and 0.1 M glucose for 1 hour, followed, after washing, by 2 hours of treatment with the respective substrates (at 0.1 M) and 0.5 mM potassium chloride. After a further washing, manganese uptake from 1.0 mM Mn^{++} was studied in the presence of 0.1 M glucose and 0.5 mM potassium chloride.

Substrate	Mn^{++} uptake after 120 min.
	*m*M
Control	0.61
Glucose (aerobic)	0.31
Glucose (anaerobic)	0.31
Fructose	0.24
Pyruvate	0.53
Lactate	0.70
Alcohol	0.61
No substrate	0.59

phate pretreatment period, inhibitor and phosphate were added to samples which paralleled those to which Mn^{++} was to be added later. A control was used to measure the phosphate uptake without inhibitor. Similarly, inhibitor and Mn^{++} were added to samples which had been pretreated with phosphate

but without inhibitor. In the latter case, the capacity to absorb Mn^{++} was already present; consequently, the effect of the inhibitor on the process of absorption could be directly observed.

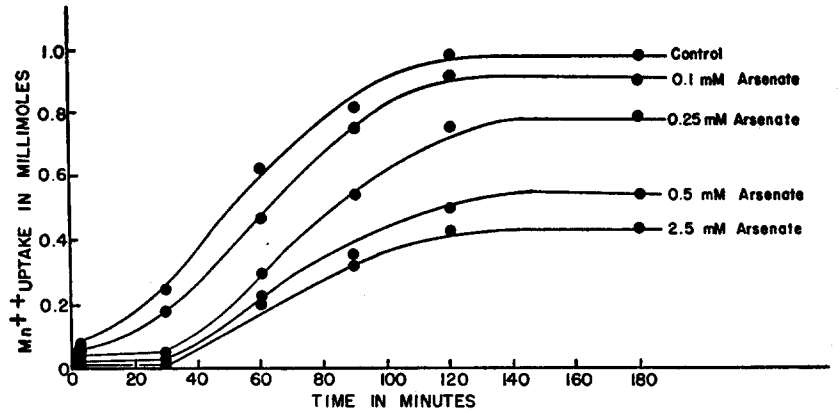

FIG. 3. The influence of sodium arsenate upon Mn^{++} uptake. The yeast concentration was 50 mg./ml.; pH, 5.0; temperature, 25°C.; Mn^{++}, 1.0 mM; glucose 0.1 M; and potassium chloride, 0.5 mM. The yeast cells were pretreated with 1.0 mM phosphate and 0.1 M glucose for 60 minutes.

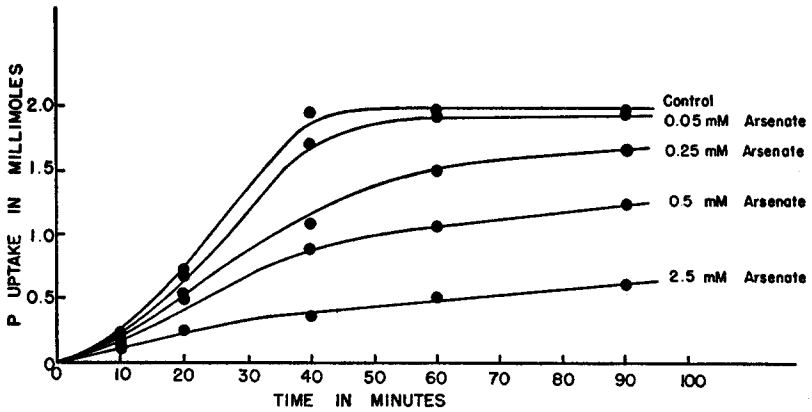

FIG. 4. The influence of sodium arsenate upon phosphate uptake. The yeast concentration was 50 mg./ml.; pH, 5.0; temperature 25°C.; glucose, 0.1 M; potassium chloride, 0.5 mM; and phosphate, 2.0 mM.

Three inhibitors were used, sodium acetate, redox dyes, and sodium arsenate. Sodium acetate blocks phosphate uptake by yeast but has no effect upon fermentation (5). The two redox dyes used, methylene blue and brilliant cresyl blue, both markedly increase the permeability of yeast cells to K^+, but, at the concentrations used, do not reduce the rate of fermentation (6, 7).

Sodium arsenate, while interfering with the phosphorylation mechanisms at the triose phosphate dehydrogenase reaction (8, 9), does not inhibit the fermentation even with concentrations as high as 0.01 M (10).[1]

With each inhibitor, parallel inhibitions of phosphate absorption and of Mn^{++} absorption were obtained. Those shown in Fig. 2 for 0.2 mm. cresyl blue are also typical of the data obtained with the same concentration of methylene blue, or with 0.2 M acetate. A more detailed study was carried out with arsenate (Figs. 3 and 4). The closely parallel effects of different concentrations of aresnate on Mn^{++} and on phosphate absorption are readily apparent.

DISCUSSION

During the course of phosphate absorption, the yeast cell becomes physiologically altered, gaining the capacity to absorb Mn^{++} or Mg^{++}. The nature of the alteration is the primary concern of the present discussion. The simplest possibility consists of an alteration in the permeability of the cell membrane to the bivalent cations. However, the absence of exchanges of cations across the cell membrane even during the course of their absorption, excludes such a hypothesis. Furthermore the absorption process is specifically dependent on the fermentation of sugar (2), and it is highly selective for Mg^{++} and for Mn^{++}. On the basis of such observations, the uptake process can best be classified as an active transport system, involving energy expenditure by the cell. The role of phosphate must thereby involve the activation or the synthesis of an essential part of the transport mechanism.

It has long been postulated that active transport of specific ions is accomplished by "carrier" molecules, but few substances with the necessary chemical characteristics to fill the role have been found in biological systems. Pyridoxial (11), polyphosphates (12), and phosphoryl choline (13) have been suggested as possible compounds responsible for active transport of cations, while a heterogeneous material has been extracted from blood that has the necessary properties that would be required for a carrrier of univalent cations (14).

The process of phosphate absorption has been investigated in some detail in yeast, but it is incompletely understood (3). Its specific dependence on glycolysis suggests a central role for the glyceraldehyde-3-phosphate reaction, the only step in fermentation in which the esterification of inorganic phosphate occurs. However, the esterification reaction is reversible, in contrast to the lack of isotopic exchange during the absorption process. It is suggested that an essentially irreversible transport step across a membrane is interposed

[1] The fermentation of the yeast used in the present experiment is partially sensitive to arsenate. However, the effects are too small to account directly for the effects of the inhibitor on the absorption of Mn^{++} or of phosphate.

between the extracellular phosphate and its participation in the metabolism of the cell. It is more likely that the close connection between phosphate absorption and glycolysis lies in the provision of energy by the latter for the transport process, rather than a means for trapping phosphate within the cell by chemical conversion into phosphate compounds. Such a connection may account for the fact that although the glycolytic reactions are essential for the transfer of phosphate across the membrane, the coupling is neither obligatory nor stoichiometric. Thus the glycolytic reactions proceed at a normal rate even in the absence of extracellular phosphate, by the cycling of intracellular phosphate, while in the presence of extracellular phosphate, the rate of absorption is only a variable fraction of the rate of glycolysis. Furthermore, a variety of chemical agents can uncouple the glycolysis from the phosphate absorption.

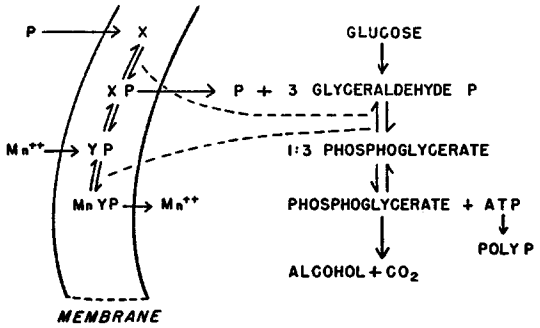

FIG. 5. A scheme for the transport of Mn^{+} and of phosphate into the yeast cell.

In Fig. 5, a simplified scheme is presented to take into account the available information concerning phosphate and Mn^{++} transport. It is assumed that both phosphate and the Mn^{++} are transferred across a membrane in an essentially irreversible step, by systems represented as *X* and *Y*, which are coupled in an unknown manner to the glycolysis system at the glyceraldehyde-3-phosphate dehydrogenase step. Phosphate once transferred, mixes with the intracellular phosphate and may be incorporated into ATP and eventually into the storage form, polyphosphate, and into other phosphorylated products (15, 16). Yeast cells ordinarily absorb phosphate only after a lag period of about one-half hour, but if pretreated with glucose and K^{+}, no delay occurs. Therefore, it is suggested that the phosphate carrier, *X*, is synthesized during active glycolysis. In contrast, the Mn^{++} carrier cannot be synthesized by glycolytic reactions alone. A transfer of extracellular phosphate into the cell is an essential prerequisite. It is suggested, therefore, that the Mn^{++} carrier, *YP*, is a phosphorylated compound formed in the cell membrane as a direct product of the phosphate-transferring systems, chemically related to the phos-

phate carrier. For this reason both the phosphate and the Mn^{++} absorption are similarly inhibited by a variety of substances. The rapid decay in the activity of the Mn^{++} carrier during glycolysis in the absence of extracellular phosphate is readily understandable, if the synthesis of the Mn^{++} carrier is a reversible reaction. The exact chemical nature of the Mn^{++} carrier is not known. Its absence in cells undergoing glycolysis eliminates as candidates any known intermediates of metabolism. The specificity pattern, however, is similar to that of many enzymatic phosphorylation reactions, so that possibly a protein component is involved. Whatever the exact structure, the actual amount of the carrier substance must be small. Likewise, only a small fraction of the phosphate which enters the cell must be involved in its formation, for during the synthesis of the carrier, the major part of the absorbed phosphate can be accounted for as polyphosphate, ATP, and glycolytic intermediates. In spite of this, the amount of Mg^{++} and Mn^{++} that can be absorbed may be very much more than the total amount of absorbed phosphate. One must conclude that there is a rapid cycling of YP and $Mn^{++}YP$, the rate of which might be determined by glycolysis, which is essential for Mn^{++} absorption even in cells in which the carrier substance has already been formed.

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