Vacuolar Function in the Phosphate Homeostasis of the Yeast Saccharomyces cerevisiae

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We studied physiological roles of the yeast vacuole in the phosphate metabolism using 31P-in vivo nuclear magnetic resonance (NMR) spectroscopy. Under phosphate starvation wild-type yeast cells continued to grow for two to three generations, implying that wild-type cells contain large phosphate pool to sustain the growth. During the first four hours under the phosphate starved condition, the cytosolic phosphate level was maintained almost constant, while the vacuolar pool of phosphate decreased significantly. 31P-NMR spectroscopy on the intact cells and perchloric acid (PCA) extracts showed that drastic decrease of polyphosphate took place during this phase. In contrast, Δslp1 cells, which were defective in the vacuolar compartment, thus lacked polyphosphate, ceased their growth immediately when they faced to phosphate starvation. Taken together, we conclude that vacuolar polyphosphate provides an active pool for phosphate and is mobilized to cytosol during phosphate starvation and sustained cell growth for a couple rounds of cell cycle.

Key words: NMR — Phosphate metabolism — Polyphosphate — Vacuole — Yeast.

Phosphate is one of the most important nutrients for living organisms. It is a component of nucleotide, phospholipid, and nucleic acid. In addition, chemical energy of phosphate bonds functions as the major source of free energy required for many cellular activities. Therefore, depletion of phosphate is a serious problem to living organisms. For understanding the phosphate metabolism, it is important to consider intracellular compartmentation of phosphorous compounds. Yeast cell accumulates considerable amounts of phosphate as polyphosphate which is a linear polymer of orthophosphates with acid anhydride linkage (Wood and Clark 1988). It accounts for 40 % of the total phosphate content in a certain condition (Kulaev 1979). The length of polyphosphate varies from three residues to greater than 1000 residues in yeast cells, the bulk of the polyphosphate localizes in the vacuole (Indge 1968, Urech et al. 1978, Dürr et al. 1979, Kornberg 1995). However, the metabolism and function of polyphosphate have not been established in yeast.

In this study, we showed a dynamic role of the vacuole on maintaining the phosphate level in the cytosol. Using 31P-NMR, we showed that the levels of cytosolic phosphorous compounds are maintained constant for about four hours after shift to phosphate starvation medium, whereas the amount of polyphosphate decreased. This suggests that the vacuolar polyphosphate is mobilized to the cytosol to maintain the cytosolic phosphate level. These results showed that the vacuole plays critical roles in the phosphate metabolism.

Materials and Methods

Strains, media, and culture conditions—Yeast strains used were X2180-1A (MATa SUC2 mal mel gal2 CUP1), YWS-1B (MATa leu2 trp1 ura3 SUC2 mal mel gal2 CUP1), and YW10-2B (MATa adel slp1::LEU2 SUC2 mal mel gal2 CUP1). YEPD medium contained 1% yeast extract (Difco Laboratories, Inc., Detroit, MI), 2% polypepton (Nippon Seiyaku, Tokyo, Japan), and 2% glucose. The composition of synthetic medium (SD) was 0.17% yeast nitrogen base (Difco Laboratories, Inc., 0.5% ammonium sulfate, 2% glucose and, if needed, appropriate amino acids. For synthetic phosphate free medium [SD(−P)]2, potassium phosphate was replaced by potassium chloride. Liquid cultures were grown in flasks or tubes at 30°C with shaking. For standard starvation-experiments, exponentially growing cells at a density of 2~3×107 cells ml−1 were collected by centrifugation, washed twice with the starvation-medium, and resuspended in the starvation-medium.

31P-NMR spectroscopy—31P-NMR spectra were obtained at 202.3 MHz using a Varian VXR-500S spectrometer operating in the Fourier-transform mode. Each spectrum was acquired with 45° pulses at a repetition rate of 0.35 s and 1024 scans were accumulated. Methylene diphosphonate (MDP) in Tris buffer (pH 8.9) in a capillary tube was used as an external standard reference. Temperature was kept at 20°C throughout the measurements. Cells were transferred to 10-mm NMR tube and suspended at a density of 2×107 cells ml−1 in SD(−P) medium with final concentrations of 10% (v/v) D2O and 6 mM EDTA. To maintain aerobic conditions in the NMR tube, pure oxygen was bubbled at a rate of 4.8 ml min−1.

PCA extract for 31P-NMR—Cell extracts were prepared by PCA extraction as described by Navon et al. (1979) with modifications. Cells were collected by centrifugation at 4°C, and washed once with cold distilled water. Cells were then suspended in cold distilled water and 60% PCA (prechilled at −20°C) was added at
a final concentration of 10%. Cell extracts were prepared by shaking the cell suspension vigorously, freezing and thawing three times, and pelleting the cell debris by centrifugation. The supernatant was neutralized with 5 M KOH and centrifuged to remove the deposits. Finally, D$_2$O and EDTA were added at final concentrations of 10% (v/v) and 10 mM, respectively.

**Results**

Deprivation of phosphate is a critical situation to living organisms. We examined growth response of yeast to phosphate starvation. Wild-type cells (YW5-1B) grown to exponential phase in YEPD were transferred to synthetic medium depleted for phosphate [SD(—P)]. The growth rate during the first four hours in SD(—P) medium was similar to that in synthetic medium (SD) (Fig. 1A). It gradually decreased in prolonged incubation in SD(—P). The population of budded cells decreased during phosphate starvation (Fig. 1B). After incubation for 10 hours in SD(—P) medium, bud index decreased to below 5%, indicating that cells were arrested in the G1 phase of the cell cycle. The response to phosphate starvation was unique, since yeast cells can not enter the new cell-cycle under the nitrogen or carbon starvation (Pringle and Hartwell 1982). This obvious contrast in the growth response to the nutrient starvation conditions may reflect the difference in the amount of intracellular pool for each nutrient. Yeast cells contain a considerable amount of phosphate in various forms (Kulaev 1979). This large pool may allow the cells to undergo two to three cell cycles.

To understand physiological responses to phosphate starvation, it is necessary to consider the intracellular compartmentation of phosphorous metabolites because yeast cells have a large pool for phosphate as polyphosphate (Indge 1968, Dürr et al. 1979, Kornberg 1995). By using $^3$P-NMR, we examined the changes of intracellular phosphorous metabolites both in the cytosol and the vacuole under the phosphate starvation. Wild-type (YW5-1B) cells were grown to exponential phase in SD medium, and transferred to SD(—P) medium, then the amounts of intracellular phosphorous compounds were measured by $^3$P-NMR (Fig. 2A). The amounts of cytosolic phosphorous compounds did not change during phosphate starvation, indicating that the amount of inorganic phosphate was kept constant (Fig. 2A). The intensity of signal assigned to inorganic phosphate in the cytosol did not change during the first four hours. For additional two hours, the intensity of signal of cytoplasmic inorganic phosphate decreased to about 20% of the initial level. The intensities of signals assigned to sugar phosphates showed similar decrease to that of cytosolic inorganic phosphate.

The $^3$P-NMR spectrum of wild-type (YW5-1B) cells grown in SD medium showed a large signal of core phosphate of polyphosphate (Fig. 2A, peak 1). During phosphate starvation, intensity of signal assigned to core phosphate group of polyphosphate decreased (compare a-f), and disappeared after four hours. Examination of PCA extracts by $^3$P-NMR also confirmed these observations (Fig. 2B). While the terminal phosphate signal (peak 6) showed transient increase at one hour and maintained long period, then disappeared. During incubation of two hours in SD(—P) medium, the intensity of signal assigned to core phosphate group of polyphosphate decreased to approximately 25%, while the intensity of the terminal phosphate signal increased four-fold. This indicates that polyphosphate is digested with endopolyphosphatase and cleaved to shorter chains. We could not observe any change in the chemical shift of polyphosphate signal so that the cleavage of polyphosphate should occur within the vacu-
Vacuolar phosphate in yeast

The levels of cytoplasmic phosphorus compounds are maintained constant during phosphate starvation. (A) $^{31}$P-NMR spectra of wild-type cells. Exponentially growing cells of wild-type (YW5-1B) were transferred to SD(−P). At the time indicated, cells were harvested and measured with $^{31}$P-NMR. The chemical shift values are expressed in ppm relative to an external standard of MDP. (a) 0 h, (b) 1 h, (c) 2 h, (d) 3 h, (e) 4 h, (f) 5 h. Signals were assigned as follows: 1, MDP; 2, sugar phosphates; 3, cytoplasmic inorganic phosphate; 4, vacuolar inorganic phosphate; 5, NTP-$\beta$, 6, terminal phosphate group of polyphosphate; 7, NTP-$\alpha$; 8, NADP; 9, NTP-$\beta$; 10, the third phosphate group of polyphosphate; 11, core phosphate group of polyphosphate. (B) Changes in the intensities of phosphorous compounds measured with $^{31}$P-NMR. Wild-type (YW5-1B) cells were grown to the exponential phase in SD, then transferred to SD(−Pi). At the time indicated, cells were harvested, and 10% PCA extracts were measured by $^{31}$P-NMR as described in Materials and Methods. ◊, sugar phosphates; □, core phosphate group of polyphosphate; △, inorganic phosphate.

Aslpl (YW10-2B) cells have no detectable vacuolar compartment and they also lack vacuolar polyphosphate (Fig. 3A). We compared the growth response of wild-type and Aslpl cells to phosphate starvation. Cells of wild-type (X2180-1A) and Aslpl (YW10-2B) were grown to exponential phase in YEPD medium, and then transferred to SD (−Pi) medium. Wild-type cells continued to grow for more than six hours, while Aslpl cells immediately stopped their growth under phosphate-starvation (Fig. 3B). This result suggests that polyphosphate in the vacuole has certain role as phosphate reserve and supports cell to proliferate under phosphate starvation.

Discussion

In this study, we investigated the response of intracellular phosphate levels in yeast under phosphate starvation. The vacuole of yeast is known to be a storage compartment for various primary and secondary metabolites. Phosphate is also stored in the vacuole as a polymeric form, polyphosphate. We used the $^{31}$P-NMR spectroscopy to distinguish the phosphate in the cytosol and the vacuole as well as their chemical forms. We found that yeast cells continued to grow for two to three rounds of the cell-cycles during phosphate-starvation. During this phase, the levels of cytoplasmic phosphorous compounds remained constant despite of the phosphate deprivation, whereas the vacuolar phosphate compounds decreased significantly. Aslpl mutant cell which lacks the functional vacuolar compartment (Kitamoto et al. 1988, Wada et al. 1990), thus lacks vacuolar polyphosphate, ceased to grow immediately when faced to phosphate depletion. The changes in the cytosolic and vacuolar phosphate levels and the response of the mutant cells indicate a dynamic flow of phosphorous compounds during the phosphate starvation.

The exponentially growing cells contain a large amount of phosphate as polyphosphate in the vacuoles. Storage of phosphate as polymers may provide an apparent advantage for reducing osmolarity. It is also likely that the polyphosphate may participate in the storage of divalent cations and basic amino acids in the vacuole (Dürr et al. 1979). The vacuole is also known to be a storage compartment of calcium (Dunn et al. 1994): it contains several millimolar order of calcium under certain condition, where the cytoplasmic calcium level should be regulated within the submicro molar levels. This large difference in calcium concentration between the two compartments will generate a large electrochemical potential difference across the vacuolar membrane (Ohsumi and Anraku 1983). Polyphosphate sequesters calcium very effectively, thus the vacuolar polyphosphate can contribute to reduce the concentration of free calcium ion in the vacuole. Despite of its physiological importance, the biogenesis of polyphosphate is not well...
examined. Accumulation of polyphosphate requires the active vacuolar H\textsuperscript{+}-ATPase (Beauvoit et al. 1991), thus energization of the vacuolar membrane may play an important role.

We showed here that the vacuolar polyphosphate provides a physiologically active pool for phosphate. The vacuolar polyphosphate dynamically mobilized from the vacuole to the cytosol during the phosphate starvation and supported cell growth for a couple rounds of the cell cycle. This cellular response to phosphate starvation is unique: the vacuolar phosphate and induce several responses before significant decrease of phosphate in the cytosol. 

It is important to elucidate the mechanisms of how the cells sense the extracellular phosphate-level, transduce the signals and mobilize the vacuolar polyphosphate. In this paper, we showed polyphosphate was first digested with endopolyphosphatase in the vacuole. Recently, Wurst et al. (1995) reported the isolation and the characterization of cytosolic exopolyphosphatase of yeast. To understand the polyphosphate metabolism, it is essential to elucidate the mechanism of phosphate transport across the vacuolar membrane. Genetic and molecular biological approaches on enzymes involved in the polyphosphate metabolism, especially import and export system, will provide clues for further understanding on the dynamic mechanism.

References


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