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In Situ ^{31}P Nuclear Magnetic Resonance for Observation of Polyphosphate and Catabolite Responses of Chemostat-Cultivated *Saccharomyces cerevisiae* after Alkalinization

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The proposed pH buffering and phosphagenic functions of polyphosphate were investigated by subjecting chemostat-cultivated *Saccharomyces cerevisiae* to alkalinization (NaOH addition) and anaerobiosis. The subsequent changes in intracellular phosphate-containing species were observed in situ by nuclear magnetic resonance (NMR) spectroscopy by using the NMR cultivator we developed. For the alkalinization experiments, changes in catabolite secretion were also measured in parallel experiments. Additionally, a range of potential neutralization capacity was investigated: a dilute culture and concentrated cultures with low or high polyphosphate content. The concentrated cultures displayed increased cytosolic pH and rapid polyphosphate degradation to small chains. The pH changes and extent of polyphosphate degradation depended inversely on initial polyphosphate content. The dilute culture restored extracellular pH rapidly and secreted acetate. The concentrated culture with low polyphosphate reserves also secreted acetate. In contrast to the alkalinization-induced polyphosphate dynamics, anaerobiosis resulted in the complete hydrolysis of polyphosphate to P_i, as opposed to small chains, and reduced cytosolic pH. The results and calculations suggest that the bulk of NMR-observable polyphosphate (vacuolar) degradation to short polymers conceivably contributes to neutralizing added alkalinity. In other circumstances, such as anaerobiosis, degradation serves other functions, such as phosphorylation potential regulation.

Previously, we described the development of a nuclear magnetic resonance (NMR) cultivator for performing in situ NMR experiments on chemostat-cultivated cell suspensions (Fig. 1). The results demonstrated that spectra with high signal-to-noise ratios can be obtained when short repetition times and/or relatively low cell concentrations (5 to 10% volume) are used, because the motion of the fluid reduces the effective spin-lattice relaxation time (13). Other attributes include having the ability to fix or shift cellular growth rates during an NMR experiment by controlling the dilution rate. By the control of medium composition, inclusion product accumulation can also be adjusted prior to observations of the intracellular effects of an environmental perturbation. Lags and other metabolic perturbations associated with cell centrifugation and resuspension, which are required for conventional NMR sample preparation, are also avoided. Finally, convective oxygen transfer ensures that anoxia is avoided.

These attributes enabled us to examine some facets of polyphosphate metabolism in *Saccharomyces cerevisiae* (13). Elevated synthesis in response to the addition of P_i at a fixed growth rate and altered speciation during growth shift-up were observed. The synthesis pattern apparent in the NMR data agreed with the results of prior extraction studies (18). The cultivator thus provides the opportunity to acquire more information that may contribute to ultimately resolving the question of whether and how polyphosphate participates in homeostasis.

Apart from the storage of the micronutrient, phosphate, as an inclusion product, other potential homeostatic functions include detoxification and osmoregulation. This function is believed to be conferred by the polyanionic nature of vacuolar polyphosphate. The ionic association with cations and basic amino acids can be envisioned to provide detoxification and osmoregulation of the cytosol by trapping cations in vacuoles (2, 5, 7, 9, 14). Polyphosphate has also been proposed to participate in ATP homeostasis (9), and evidence for the hydrolysis of cytosolic polyphosphate to maintain membrane energization during impaired oxidative phosphorylation has been presented for strictly aerobic *Acinetobacter johnsonii* 210A (21).

Another proposed function for polyphosphate is buffering of intracellular pH (16). Evidence for buffering is provided by the response of yeast cells to amino acids. Ludwig et al. (10), for example, observed extensive tri- and tetrapolyphosphate accumulation after yeast cells were subjected to either an arginine or a lysine bolus. However, acidic or neutral amino acids did not invoke the same response. Moreover, the extent of polyphosphate degradation corresponded more to the amino acid type than did the growth rate increase induced by amino acid addition. Therefore, Ludwig et al. suggested that low-molecular-weight polyphosphates contribute to buffering against the uptake of basic species as opposed to stimulation of RNA synthesis.

Despite the existence of supporting evidence, there is not a consensus on whether polyphosphate can serve as a phosphagen or buffer or if polyphosphate in different cellular compartments fulfills different functions. For example, a ^{31}P NMR

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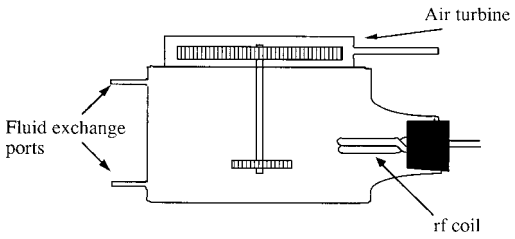

FIG. 1. Cultivator developed for in situ NMR experiments with chemostat-cultivated cells. The top chamber houses an air turbine; an impinging air jet results in turbine rotation and fluid circulation in the lower chamber, which contains suspended cells. The radio frequency (rf) coil is immersed in the culture.

study showed that the stability of mitochondrial polyphosphate increased when oligomycin, an inhibitor of mitochondrial ATP ase, was present. This result suggests that a link which may be provided by polyphosphate kinase activity exists between ATP and polyphosphate (1). On the other hand, Schuddemat et al. (18) found that while the ATP concentration in *S. cerevisiae* dropped more than eightfold after the addition of antimycin (inhibited cytochrome-mediated electron transfer), polyphosphate content remained essentially unchanged.

With regard to buffering, other investigators have found that the addition of basic amino compounds induces polyphosphate degradation (6, 8). However, in some cases, polyphosphate degradation was not firmly concluded to be linked to pH homeostasis. For example, 20 mM NH_4Cl at pH 8 caused extensive polyphosphate degradation in *S. cerevisiae*, whereas the same concentration of methylamine or amantadine did not (6).

To further explore vacuolar polyphosphate's roles in homeostasis, the response of chemostat cultures of *S. cerevisiae* to alkalinization by the addition of NaOH was investigated with the NMR cultivator and by supernatant analyses. The use of NaOH to study alkalinization effects parallels the study of pH and ATP homeostasis in *Platymonas subcordiformis* by Kugel et al. (8). NaOH has the advantage that it is not metabolized, as are amino acids and ammonium salts. Additionally, the effects of amino compound speciation and uptake rates on cytosolic pH changes, which may complicate the comparison of dose-response relationships, are eliminated.

To more fully assess polyphosphate's role in homeostatic mechanisms, three additional step were taken. First, glucose was restricted to diminish carbohydrate reserves. Second, the amount of intracellular polyphosphate accumulated prior to alkalinization was varied by altering the growth medium's P_i content. Our prior work indicates that increased phosphate availability increases the polyphosphate content of chemostat cultures without significantly changing the intracellular and extracellular concentrations of phosphate (13). Third, the polyphosphate dynamics associated with medium alkalinization were contrasted with those observed when *S. cerevisiae* was deprived of oxygen.

The intracellular events that accompany alkalinization when the initial polyphosphate content is varied will be described first. These results are then contrasted with the accompanying shifts in metabolic by-products and the polyphosphate dynamics exhibited after anaerobiosis. We conclude by discussing how the polyphosphate degradation patterns compare with those exhibited after the addition of basic amino acids and what the results suggest for the homeostatic functions of polyphosphate.

MATERIALS AND METHODS

S. cerevisiae BJ2937 was kindly provided by Elizabeth Jones (Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pa.). Details on the design and operation of the cultivator and the preparation of the cell suspension can be found elsewhere (13). Briefly, chemostat cultivation was begun immediately after inoculation with a dense seed culture took place; for all experiments reported, the dilution rate was maintained at 0.05 h^{-1}. The growth medium used for chemostat cultivation contained (in grams per liter) glucose (100), yeast extract (25), $(NH_4)_2SO_4$ (9.48), $MgSO_4 \cdot 7H_2O$ (1.5), KCl (0.30), $FeSO_4 \cdot 7H_2O$ (0.035), and K_2HPO_4 (0.25). The medium with low levels of phosphate referred to in the text contained the same components, except K_2HPO_4 was omitted; hence, the only P_i present was that contained in the yeast extract. The temperature was maintained at 30°C, and aeration was accomplished by bubbling pure oxygen through one of the cultivator's bottom ports. Samples were retrieved periodically to determine the extracellular pH and the cell concentration. Cell concentration measurements were performed by diluting the sample and measuring the optical density at 560 nm. The dilute suspension experiments were performed with the same medium diluted 10-fold. Consequently, yeast cells constituted approximately 1 and 10% by volume, respectively, of the low- and high-concentration cultures referred to in the text.

The NMR experiments were conducted with a 40-cm-bore, 4.7-T Bruker spectrometer. To obtain spectra, 90° pulses at durations of 50 μs each with a 0.1-s repetition time were used. Spectra were accumulated in 8-min blocks which consisted of 4,000 scans; 10-Hz exponential line broadening was applied prior to Fourier transformation, as previously described (13). Resonances were assigned on the basis of prior studies (4, 13). The pH measurements are discussed in more detail in the text.

High-performance liquid chromatography analysis was employed for analysis of the acids present in the medium; filtered 10-μl samples were injected into a Supelco column (SUPELCOGEL C-610H). Acids were eluted isocratically with 0.1% phosphoric acid and detected by UV absorption at 210 nm. Peak assignments in the chromatograms were confirmed by spiking the sample with purified acids.

RESULTS

Response of *S. cerevisiae* to alkaline pH shift for high level of initial polyphosphate content. The cultivator was initially operated to establish steady-state conditions; invariant spectra and constant cell density were the criteria used to conclude when a steady state was established. The spectrum obtained during aerobic growth at a dilution rate of 0.05 h^{-1} is shown in Fig. 2A. The resonances assigned to intracellular species are for phosphomonoesters, cytosolic P_i (P_i^{in}), ATP, NAD(P)H, and polyphosphate (PP1, terminal phosphates; PP4, middle phosphates). The ATP_α resonance overlaps with those of NAD(P)H and other mobile nucleotide diphosphates. Additionally, a resonance originates from heterogeneous phospholipids in the growth medium. This resonance has also been observed by Rayner et al. (17), and its assignment was corroborated by scanning uninoculated medium (data not shown). The vacuolar and extracellular P_i resonances appear on the high shoulder of the phospholipid resonance.

The chemical shifts of the P_i^{in} and PP1 resonances are pH dependent and thus can be used to determine the cytosolic and vacuolar pHs by comparing their chemical shifts with calibration curves (19, 20). The chemical shifts of P_i^{in} and PP1 indicate that the cytosolic and vacuolar pHs were 7.2 and 6.3, respectively. These values are comparable to others reported for growing yeast cells (6). The extracellular pH was measured with an electrode; it was found to be 5.8 prior to medium alkalinization.

At 0.33 h after the spectrum shown in Fig. 2A was obtained, a bolus of sodium hydroxide was added to the cultivator, increasing the extracellular pH to 8.5. Spectra were acquired at intervals to observe the metabolite dynamics. The optical density did not change significantly during the observation period; hence, resonance intensities from different spectra can be compared and used to infer intracellular concentration.

The spectrum acquired immediately after the NaOH bolus is shown in Fig. 2B. The P_i^{in} and PP1 resonances shifted upfield, indicating that medium alkalinization increased cytosolic and vacuolar pH. On the basis of a 0.6-ppm shift for P_i^{in}, the

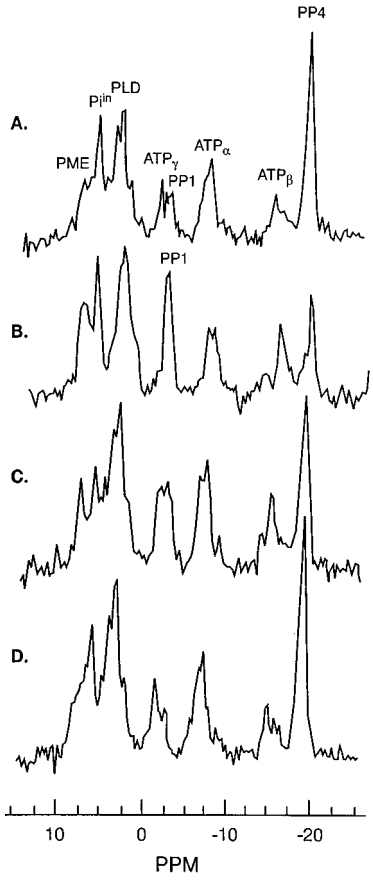

FIG. 2. Response of *S. cerevisiae* cultivated in P_i-supplemented medium following a sudden shift in the pH of the extracellular medium to 8.5. The ^{31}P NMR spectra shown correspond to before the NaOH bolus (A), immediately following the NaOH bolus (B), 30 min after the bolus (C), and 2 h after the bolus (D). PME, phosphomonoesters; P_i^{ex}, extracellular inorganic phosphate; PLD, phospholipid.

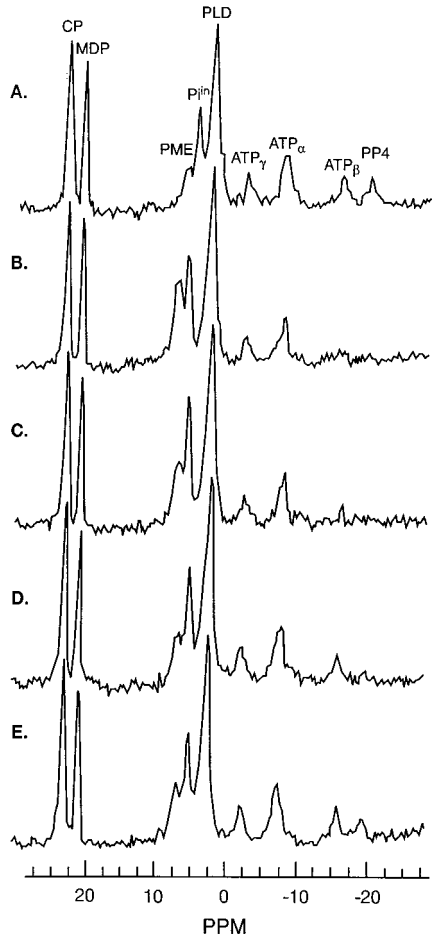

FIG. 3. Response of *S. cerevisiae* following a sudden increase in the extracellular pH. The conditions were similar to those of the previous experiment, except the P_i content of the growth medium was limited to that provided by the yeast extract. The ^{31}P NMR spectra shown correspond to before the NaOH bolus (A), immediately following the NaOH bolus (B), and 10 (C), 25 (D), and 50 min (E) after the bolus. CP, cyclic nucleosides; MDP, reference; PME, phosphomonoesters; PLD, phospholipids.

cytosolic pH increased to 7.8. In addition to chemical shifts, the PP4 resonance decreased significantly. This decrease was accompanied by increased PP1 as opposed to increased P_i^{in}, indicating that degradation predominantly yields short chains. The phosphomonoester resonance also increased. In contrast to those of other species, the peaks where the α- and β-phosphates of ATP resonate did not appear to change appreciably. However, the apparent lack of change may not reflect ATP homeostasis. The ATP_β resonance is bracketed by the penultimate phosphate resonances from polyphosphate. Additionally, resonances from NAD(P)H and nucleotide diphosphates overlap with the ATP_α resonance. Thus, for the high level of initial polyphosphate content, the extent to which the intracellular pH (pH^{in}) changes and the time to restore pH^{in} and PP4 indicate the impact of alkaline stress and dissipation; only tentative assessments of ATP dynamics can be made.

The spectrum acquired at 30 min (Fig. 2C) indicates that pH^{in} decreased to nearly its initial value. Moreover, the PP4 and PP1 resonances were increasing and decreasing, respectively, to their initial intensities. Ultimately, the culture recovered from the pH perturbation, which is reflected by the similarity of the initial spectrum (Fig. 2A) and the one acquired 2 h after the NaOH bolus (Fig. 2D).

Response of *S. cerevisiae* to alkaline pH shift for low level of initial polyphosphate content. The medium alkalinization experiment was repeated with a growth medium containing less excess phosphate in order to lower the cellular polyphosphate content. The aim was to determine if a decreased level of polyphosphate would alter the dynamics of polyphosphate, pH^{in}, and metabolites after alkalinization. The lowering of cellular polyphosphate content was also anticipated to improve the resolution of the ATP_β resonance, since the contributions from penultimate phosphates would be considerably less. Improved resolution is helpful because the ATP_β resonance is highly indicative of the ATP level, while the ATP_α and ATP_γ resonances, which, as noted earlier, overlap with the NAD(P)H and nucleotide diphosphate resonances, are not. Thus, any interrelationship between polyphosphate degradation and ATP dynamics would be more clearly observable.

The spectra acquired are shown in Fig. 3. In these spectra, a reference and an additional medium constituent appears (2′,3′-cyclic nucleosides) CP (17). As anticipated, the polyphosphate content was lower when phosphate was reduced (compare Fig. 2A and 3A).

After the NaOH bolus, the PP4 resonance vanished (Fig. 3B). The P_i^{in} resonance did not increase significantly, but it shifted by 1.1 ppm, indicating increased pH^{in}. The ATP_β res-

onance became undetectable (compare Fig. 2B and 3B), whereas the phosphomonoester peak increased. Overall, the initial discernible events displayed by yeast cells with low levels of polyphosphate largely resemble those exhibited by the counterparts with high levels of polyphosphate. However, a decrease in the initial polyphosphate content results in a greater loss of PP4 and a greater rise in pH^{in}. Additionally, ATP homeostasis is not maintained.

After 25 min (Fig. 3D), polyphosphate synthesis resumed, as was indicated by the increasing PP4 resonance. At 50 min, pH^{in}, ATP_β, and PP4 nearly recovered to levels comparable to their initial values (Fig. 3E). Interestingly, ATP_β appeared to recover before PP4, suggesting that ATP is either a polyphosphate precursor or a regulator of polyphosphate synthesis. Alternately, extension of short-chain polyphosphates leading to an increased number of penultimate phosphates preceded the restoration of PP4, which is consistent with observed synthesis patterns (13, 18).

Comparison of levels of acid production. To assess the role of acid production, supernatants from a series of chemostat experiments were analyzed. The series comprised cultures corresponding to those examined by NMR and a dilute yeast culture. Thus, a range of potential neutralization capacity was examined. At one end was high metabolic capacity and high or low levels of polyphosphate content. At the other end was a combination of low metabolic capacity and polyphosphate content.

After the extracellular pH was raised to 8.5, all three cultures rapidly dropped the pH to 6.5 (typically, within 30 min). However, the relative changes in acetate concentration differed, as is shown in Fig. 4A and B. After alkalinization, the dilute culture increased its acetate excretion (Fig. 4A), as did the high-concentration culture with an initial low level of polyphosphate (Fig. 4B). The concentrated culture with high levels of polyphosphate, in turn, imported acetate from the extracellular medium (Fig. 4B).

Response of *S. cerevisiae* to anaerobiosis. To contrast the polyphosphate dynamics following anaerobiosis to those observed after alkalinization, the oxygenation of the culture was terminated after a steady growth rate ($0.05\ h^{-1}$) was established. The NMR spectra acquired before and after the termination of oxygenation are shown in Fig. 5. Polyphosphate was extensively degraded to P_i within 20 min, as was indicated by the decreased PP4 resonance and increased P_i^{in} resonance. Moreover, cytoplasmic pH decreased from 7.2 to 6.7. The intensity of the resonances from ATP also appeared to decrease. The cytoplasmic pH, ATP, and P_i changes we observed for chemostat-cultivated yeast cells parallel those reported by den Hollander et al. (3), who contrasted aerobic and anaerobic yeast suspensions. The finding of a difference and replications of other results indicate that the cultivator indeed provides for controlled aerobic or anaerobic chemostat cultivation.

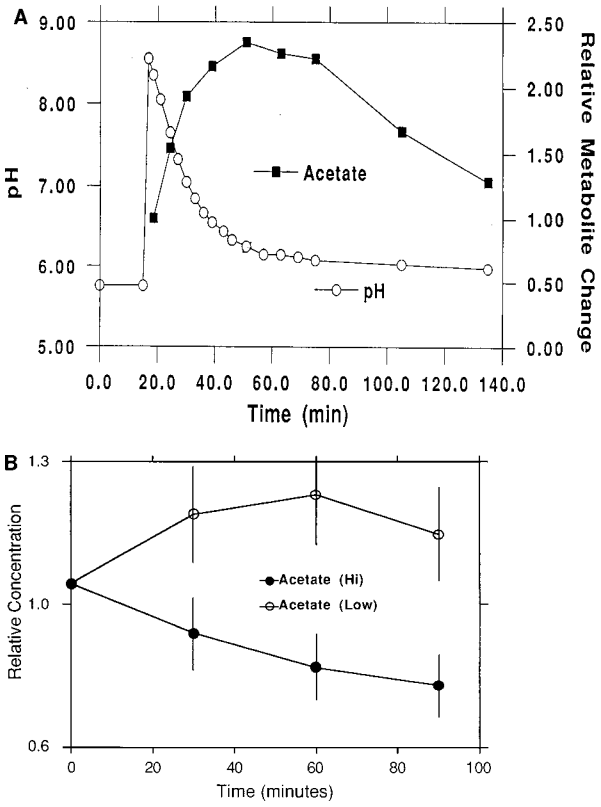

FIG. 4. Dynamics for different cell concentrations and polyphosphate contents of acetate secreted by *S. cerevisiae* after alkalinization. Shown are the responses of the dilute (ca. 1% volume) culture (A) and more concentrated cultures (B) with high or low levels of polyphosphate content. Estimated errors correspond to the ability to replicate standards and samples and typically are based on three to four trials.

DISCUSSION

For aerobic yeast cultures, cytosolic pH increases and polyphosphate degrades to short chains immediately after the addition of NaOH. However, the extent of polyphosphate degradation and the amount pH^{in} increases both depend inversely on the initial polyphosphate content. Additionally, acetate production depends on the combined metabolic capacity (cell concentration) and initial polyphosphate content. The time for pH restoration, however, is rapid, despite variations in metabolic capacity and polyphosphate content.

The polyphosphate degradation evident in Fig. 2 indicates that PP1 and PP4 increase and decrease, respectively, by approximately twofold. Comparing the NaOH-induced changes in PP1 and PP4 to changes in polyphosphate speciation after the addition of amino compounds will indicate the degree to which a correspondence exists. For the acid-soluble vacuolar pool, representative values of chain lengths are 10 (11), 12 (15), and 7 to 15 (6). Tripolyphosphate is the predominant degradation product formed after yeast cells are subjected to arginine (10) or other basic amino compounds (11). With regard to the PP1 resonance, one excision doubles PP1, another triples PP1, etc. For PP4, excising one tripolyphosphate from a chain of length 10 changes PP4 fourfold. One tripolyphosphate excision from a chain containing 12 to 15 residues, in turn, changes PP4 by circa twofold. Therefore, a linear combination of tripolyphosphate excisions from chains containing 10 to 15 residues can account for the NMR data. Thus, in yeast cells, the NaOH-induced polyphosphate degradation pattern resembles that exhibited after the additions of basic amino acids (10) and ammonia (6). The halotolerant green alga, *Dunaliella salina* (16), also displays the pattern.

The concentrated culture with a high level of polyphosphate content exhibits acetate consumption during the recovery from alkalinization. While the reimport of catabolites is not unusual, the utility of importing acetate remains to be fully explained. The importation of acetate suggests that the intracellular concentrations of acetate precursors decrease, thereby establishing a concentration gradient that is favorable for acetate influx. One possible explanation for the importation is to replenish the large polyphosphate pool; the acetate precursor, 1,3-

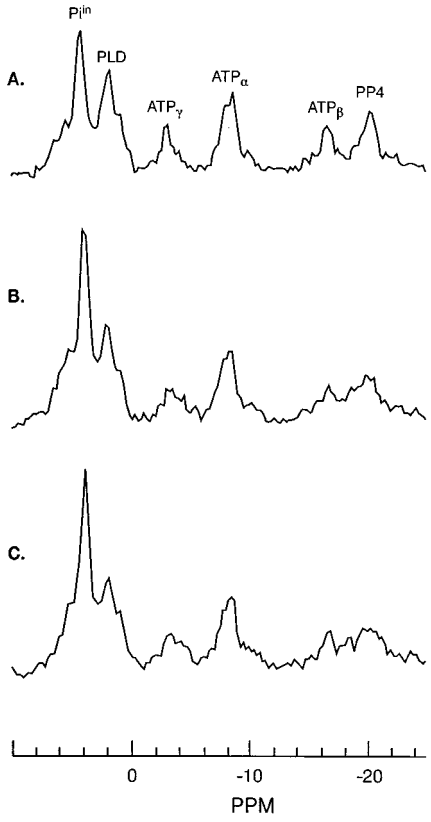

FIG. 5. Response of *S. cerevisiae* to anaerobiosis after a steady growth rate of 0.05 h^{-1} has been established. The ^{31}P NMR spectra correspond to oxygenated culture (A), culture 5 min after the termination of oxygenation (B), and culture 20 min after the termination of oxygenation (C). PLD, phospholipids.

diphosphoglycerate, is diverted to the vacuolar compartment for polyphosphate synthesis via 1,3-diphosphoglycerate–polyphosphate phosphotransferase. Schuddemat et al. (18) suggest that the transferase as opposed to polyphosphate kinase may be responsible for polyphosphate synthesis. Alternately, the mitochondrial oxidation of the imported metabolites may help to replace the loss of ATP when 1,3-diphosphoglycerate is not used for substrate-level ATP formation.

The inverse relationship between the pH^{in} rise and polyphosphate content suggests that polyphosphate degradation contributes to pH homeostasis, as has been suggested for an alga (16). The tendency to excrete acids when the level of polyphosphate in the total culture is low is also consistent with the buffering function. However, if polyphosphate degradation indeed contributes to buffering, then other aspects require further consideration. For example, how and why does degradation to tripolyphosphate produce acidity, and how much acidity is produced?

The degradation to triphosphoric acid rather than P_i may occur because polyphosphoric acids are stronger than phosphoric acid. The pK_a values for pyrophosphoric acid are 1.52, 2.36, 6.60, and 9.25, and those for P_i are 2.23, 7.21, and 12.32. Higher polyphosphates have even lower pK_a values; mainly, the terminal phosphates of pentapolyphosphate tend to be protonated, with pK_a values of 5.94 and 6.76 (12). When polyphosphates are complexed with Mg^{2+}, which is typical for vacuolar polyphosphates (14), the pK_a values decrease further to 3.77 and 4.42 (12).

The different pK_as of the degradation products may lead to different H^+ yields from hydrolysis. Assuming that P_i has two basic sites and the predominant chain length of vacuolar polyphosphate is 12, hydrolyzing one chain to three P_is will yield six H^+ ions and the total number of basic sites will be seven. If one site is initially occupied, then no net H^+ will be yielded. Assuming that tripolyphosphates and higher have one basic site, then one net H^+ is yielded for degradation to tripolyphosphate. Thus, when quality and quantity are simultaneously considered, degradation to tripolyphosphate instead of P_i may be a means to implement the higher net H^+-yielding strategy.

On the basis of the estimated net H^+ yield from hydrolysis, the impact of polyphosphate hydrolysis on neutralization can be approximated. Polyphosphate can constitute 0 to 20% of cellular dry weight. Assuming that 10% of cellular dry weight is polyphosphate and the representative chain length is 12, then 3×10^{-5} mol of H^+ per cm^3 of cells is generated when each chain undergoes one tripolyphosphate excision. If the cells constitute 10% (by volume) of the cultivator volume, then 3×10^{-3} mol of H^+ per liter of cultivator volume is generated. Such H^+ generation can neutralize up to a 2.5 pH unit change. Not all the H^+ ions will be available for NaOH neutralization because of the titration of other bases (e.g., proteins), but the estimate suggests that the potential contribution of polyphosphate hydrolysis to H^+ generation is not insignificant.

With regard to whether polyphosphate degradation performed other functions in the aerobic yeast cells, ATP homeostasis was not observed to be maintained at the expense of polyphosphate degradation when the polyphosphate content was restricted. The ATP decrease may arise from decreased acid oxidation and/or a temporary reduction in the transmitochondrial pH gradient available for ADP phosphorylation. For the high-level polyphosphate content, the results in Fig. 3 were noted earlier to require cautious interpretation because of the overlap of ATP resonances with other species. Therefore, it is difficult to discern whether polyphosphate contributed to ATP homeostasis. Subsequent work with inhibitors may prove helpful.

The polyphosphate dynamics displayed after anaerobiosis differed in some respects from those exhibited after medium alkalinization. Although degradation occurred, the extent of degradation to P_i was greater for anaerobiosis. Moreover, cytosolic pH decreased and the phosphorylation potential ($[ATP]/[ADP][P_i]$) decreased. The changes in phosphorylation potential and pH^{in} due to anaerobiosis are consistent with findings from other studies (4). In this case, polyphosphate degradation can be envisioned as establishing a new phosphorylation potential or manifesting another regulatory function.

In summary, the cultivator developed for the NMR study of chemostat cultures was used in conjunction with supernatant analyses to investigate how yeast cells respond to medium alkalinization and anaerobiosis. On the basis of our results and the suggestions of Schuddemat et al. (18), a model for how the effects of alkalinization are counteracted can be proposed. When the polyphosphate content in the total culture is restricted, reacidification occurs primarily by carbon flux being directed to the production of such acids as acetate. When polyphosphate is abundant in the total culture, rapid degradation to tripolyphosphoric acid occurs, which, because of the high H^+ yield, conceivably contributes to neutralization. Once degradation has proceeded to a significant extent, repolymerization via 1,3-diphosphoglycerate–polyphosphate phosphotransferase may occur. In a sense, polyphosphate degradation can be viewed as an initial rapid response while metabolic shifts occur to assist and then finalize the restoration of the intra- and extracellular environments. In contrast, anaerobiosis

induces extensive degradation to P_i, which may establish a new phosphorylation potential that fosters substrate-level ATP formation by glycolysis.

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REFERENCES

1. **Beauvoit, B., M. Rigoulet, B. Guerin, and P. Canioni.** 1989. Polyphosphates as a source of high energy phosphates in yeast mitochondria: a ^{31}P NMR study. FEBS Lett. **252:**17–21.
2. **Cramer, C. L., and H. L. Davis.** 1980. Polyphosphate-cation interaction in the amino acid-containing vacuole of *Neurospora crassa*. J. Biol. Chem. **259:**5152–5157.
3. **den Hollander, J. A., K. Ugurbil, T. R. Brown, and R. G. Shulman.** 1981. Phosphorus-31 nuclear magnetic resonance studies of the effect of oxygen upon glycolysis in yeast. Biochemistry **20:**5871–5880.
4. **den Hollander, J. A., K. Ugurbil, and R. G. Shulman.** 1986. ^{31}P and ^{13}C NMR studies of intermediates of aerobic and anaerobic glycolysis in *Saccharomyces cerevisiae*. Biochemistry **25:**212–219.
5. **Durr, M., K. Urech, T. Boller, A. Wiemkemm, J. Schwenke, and M. Nagy.** 1979. Sequestration of arginine by polyphosphate in vacuoles of yeast (Saccharomyces cerevisiae). Arch. Microbiol. **121:**169–175.
6. **Greenfield, N. J., M. Hussain, and J. Lenard.** 1987. Effects of growth state and amines on cytoplasmic and vacuolar pH, phosphate, and polyphosphate levels in *Saccharomyces cerevisiae*: a 31P nuclear magnetic resonance study. Biochim. Biophys. Acta **926:**205–214.
7. **Klionsky, D. J., P. K. Herman, and S. C. Emr.** 1990. The fungal vacuole: composition, function, and biogenesis. Microbiol. Rev. **54:**266–292.
8. **Kugel, H., A. Mayer, G. O. Kirst, and D. Leibfritz.** 1987. *In vivo* P-31 NMR measurements of phosphate metabolism in *Platymonas subcordiformis* as related to external pH. Eur. Biophys. J. **14:**461–470.
9. **Kulaev, I. S., S. E. Mansurova, and V. M. Vagabov.** 1982. Some aspects of studies of the biological role of inorganic phosphates and pyrophosphate, p. 26–57. *In* V. P. Skulachev (ed.), Biological reviews, vol. 3. Cambridge University Press, London.
10. **Ludwig, J. R., II, S. G. Oliver, and C. S. McLaughlin.** 1977. The effect of amino acids on growth and phosphate metabolism in a prototrophic yeast strain. Biochem. Biophys. Res. Commun. **79:**16–23.
11. **Lusby, E. W., Jr., and C. S. McLaughlin.** 1980. The metabolic properties of acid soluble polyphosphates in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **178:**69–76.
12. **MacDonald, J. C., and M. Mazurek.** 1987. Phosphorus magnetic resonance spectra of open-chain linear polyphosphates. J. Magnet. Reson. **72:**48–60.
13. **Meehan, A. J., C. J. Eskey, A. P. Koretsky, and M. M. Domach.** 1992. Cultivator for NMR studies of suspended cell cultures. Biotechnol. Bioeng. **40:**1359–1366.
14. **Miller, J. J.** 1984. In vitro experiments concerning the state of polyphosphate in the yeast vacuole. Can. J. Microbiol. **30:**236–246.
15. **Navon, G., R. G. Shulman, T. Yamane, T. R. Eccleshall, K.-B. Lam, J. J. Baranofsky, and J. Marmur.** 1979. Phosphorus-31 nuclear magnetic resonance studies of wild-type and glycolytic pathway mutants of Saccharomyces cerevisiae. Biochemistry **18:**4487–4499.
16. **Pick, U., M. Bental, E. Chitlaru, and M. Weiss.** 1990. Polyphosphate hydrolysis—a protective mechanism against alkaline stress? FEBS Lett. **274:**15–18.
17. **Rayner, M. H., P. J. Sadler, and M. D. Scawen.** 1990. NMR studies of a bacterial cell medium LB broth; cyclic nucleosides in yeast extracts. FEMS Microbiol. Lett. **68:**217–222.
18. **Schuddemat, J., R. de Boo, C. C. M. van Leeuwen, P. J. A. van den Broek, and J. van Steveninck.** 1989. Polyphosphate synthesis in yeast. Biochim. Biophys. Acta **1010:**191–198.
19. **Shanks, J. V., and J. E. Bailey.** 1988. Estimation of intracellular sugar phosphate concentrations in *Saccharomyces cerevisiae* using 31P nuclear magnetic resonance spectroscopy. Biotechnol. Bioeng. **32:**1138.
20. **Shanks, J. V., and J. E. Bailey.** 1990. Elucidation of the cytoplasmic and vacuolar components in the inorganic phosphate region in the 31P NMR spectrum of yeast. Biotechnol. Bioeng. **35:**1102–1110.
21. **Van Ween, H. W., T. Abee, G. J. S. Kortsbee, H. Pereira, W. N. Konings, and A. J. B. Zehnder.** 1994. Generation of a proton motive force by the excretion of metal-phosphate accumulating *Acinetobacter johnsonii* strain 210A. J. Biol. Chem. **269:**29509–29514.