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Polyphosphate Hydrolysis within Acidic Vacuoles in Response to Amine-Induced Alkaline Stress in the Halotolerant Alga *Dunaliella salina*

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ABSTRACT

The location and mobilization of polyphosphates in response to an amine-induced alkaline stress were studied in the halotolerant alga *Dunaliella salina*. The following observations suggest that polyphosphates accumulate in acidic vacuoles: (a) Accumulation of large amounts of polyphosphates is manifested as intravacuolar dense osmiophilic bodies in electron micrographs. (b) Uptake of amines into the vacuoles induces massive hydrolysis of polyphosphates, demonstrated by *in vivo* ^{31}P-nuclear magnetic resonance, and by analysis of hydrolytic products on thin layer chromatograms. The analysis indicates that: (a) Polyphosphate hydrolysis is kinetically correlated with amine accumulation and with the recovery of cytoplasmic pH. (b) The major hydrolytic product is tripolyphosphate. (c) The peak position of the tripolyphosphate terminal phosphate in nuclear magnetic resonance spectra is progressively shifted as the cells recover, indicating that the pH inside the vacuoles increases while the pH in the cytoplasm decreases. (d) In lysed cell preparations, in which vacuoles become exposed to the external pH, mild alkalinization in the absence of amines induces polyphosphate hydrolysis to tripolyphosphates. It is suggested that amine accumulation within vacuoles activates a specific phosphatase, which hydrolyzes long-chain polyphosphates to tripolyphosphates. The hydrolysis increases the capacity of the vacuoles to sequester amines from the cytoplasm probably by releasing protons required to buffer the amine, and leads to recovery of cytoplasmic pH. Thus, polyphosphate hydrolysis provides a high-capacity buffering system that sustains amine compartmentation into vacuoles and protects cytoplasmic pH.

Many microorganisms, including bacteria, yeast, algae, and cyanobacteria, accumulate large amounts of polyphosphates (11, 20). For example, yeast have been reported to contain polyphosphates up to 20% of their dry weight (12). The location of polyphosphates in cells of eucaryotes is not clear, but there are reports that in yeast most of the polyphosphates accumulate in acidic vacuoles, although different locations have also been reported (11).

Because both the uptake of inorganic phosphate and its conversion to polyphosphates requires investment of metabolic energy, it should be expected that the accumulation of huge amounts of polyphosphates, which exceed by far the phosphate requirements of the cell, may have an important physiological role. However, the role of polyphosphates is still not clear. It has been suggested that polyphosphates may serve as an energy reservoir for ATP formation through ADP polyphosphate kinase, which has been identified in bacteria and yeast (10, 21) and was recently purified (1). However, the current available information does not support this possibility (7).

The halotolerant unicellular alga *Dunaliella* accumulates large amounts of polyphosphates equivalent to 0.5 to 1 M inorganic phosphate. The polyphosphates in *Dunaliella* appear to be associated with K^+ (16) and Mg^{2+} (9), but their localization or physiological role are not known. ^{31}P-NMR experiments have demonstrated that osmotic changes induce mobilization of polyphosphates in *Dunaliella,* which might indicate a role in osmotic adaptation (2). In a preliminary recent work, we have demonstrated that ammonia at alkaline pH induces alkalinization of the cells and leads to massive hydrolysis of polyphosphates, which is manifested in ^{31}P-NMR spectra and by accumulation of large amounts of tripolyphosphate in the cells (17). The correlation between polyphosphate hydrolysis and recovery of cytoplasmic pH suggested a possible connection to pH homeostasis in this alga. However, this work did not address the location of polyphosphates in the cell, the mechanism of recovery from alkaline stress, and whether the response of *Dunaliella* is specific to ammonia. In the preceding work, we show that ammonia as well as other amines accumulate in acidic vacuoles in *Dunaliella* and that cytoplasmic pH homeostasis involves the compartmentations of amines into the vacuoles (18). In this work, we demonstrate that polyphosphates seem to be located in the same vacuoles and that various amines induce their hydrolysis as a consequence of intracellular pH changes.

MATERIALS AND METHODS

NMR Measurements

Dunaliella salina cells were grown in batch cultures within an illuminated New Brunswick Psychotherm incubator in 0.5 M NaCl medium as previously described (2). For *in vivo* NMR measurements, cells were trapped in 3% agarose beads at a concentration of 6 to 8 × 10^8 cells/mL and perfused continuously during the NMR experiment at a rate of 1 mL/min with a medium containing 0.5 M NaCl, 5 mM $MgCl_2$, 5 mM KCl, 20 mM Tris-Cl, pH 9, saturated with 95% O_2 and 5% CO_2 at 10 to 12°C as previously described (2). Amines were added to the trapped cells through the perfusion medium. NMR measurements were performed in the dark in a Bruker

Table I. *Effect of Growth Conditions on Phosphorous Accumulation in D. salina*

D. salina cells were cultured for 5 to 7 d with the indicated phosphate concentrations containing also ^{32}P (1 μCi/μmol), or starved by transfer of 0.2 mM Pi cells (control medium) for 24 h to Pi-depleted medium. Phosphorous content was estimated in washed cells from the trapped radioactivity.

Conditions	Phosphorous Content
	μeq/10^{10} cells
30 μM Pi	220
0.2 mM Pi	580
2 mM Pi	1190
Pi starvation	290

AM-500 NMR spectrometer. ^{31}P spectra were recorded at 202.5 MH_2 by applying 60° pulses with a repetition time of 15 s. Composite pulse proton decoupling was continuously applied. The spectra were processed using a line broadening of 40 Hz. Cytoplasmic pH was calculated from the resonance position of inorganic phosphate by comparison with calibration curves of inorganic phosphate in pH-buffered solutions containing 20% glycerol, 200 mM KCl, 5 mM $MgCl_2$, which resembles the internal composition of *Dunaliella*. Because the calculated cytoplasmic pH (pHi) values are close to the upper limit of resolution of ^{31}P-NMR (4), recordings were made at low temperatures, integrated every 5 min and averaged for two measurements to improve statistical reliability. Nevertheless, the absolute calculated cytoplasmic pH(pH_i) values above pH 8 may deviate by up to 0.1 pH unit from the real values. Intracellular averaged concentrations of central or terminal phosphates of polyphosphate hydrolytic products were calculated from their corresponding area in the NMR spectra, with reference to the γ-phosphate of ATP control cells (without amine), which was measured independently under the same conditions with the luciferase assay in a Lumac 3M Biocounter.

Extraction and Analysis of Polyphosphates by TLC

The analysis of ^{32}P-labeled *D. salina* extracts was performed essentially as previously described. In brief, cells were cultured for 2 to 3 d with ^{32}Pi orthophosphate (1 mCi/L) and extracted with 2 M formic acid. Lyophilized extracts were separated on PEI[1] cellulose plates (20 × 20 cm) and developed in 1.5 M $KHPO_4$, pH 3.5. Individual components were identified with unlabeled markers, which comigrated with the radioactive extracts, by spraying with a molybdate reagent. Quantitative estimation of individual components was made by cutting out spots and counting with scintillation cocktail (Xylofluor).

Ammonium Uptake

Ammonium uptake was determined by the Nessler procedure on cell samples separated through silicon oil as described in the preceding article (18).

[1] Abbreviations: PEI, polyethyleneimino; TEA, triethylamine; $BzNH_2$, benzylamine; SF-6847, 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalonitrile.

Electron Microscopy

For electron microscopy, cell cultures grown in media containing 2 mM or 30 μM Pi were fixed, sliced, and stained as described in the preceding article (18).

RESULTS

Localization of Polyphosphates in *Dunaliella*

Polyphosphates constitute the major pool of phosphate in *Dunaliella* and their content can be manipulated by the extracellular Pi concentration (9, 16). To get information about the location of polyphosphates in *Dunaliella,* cells were cultured either in phosphate-rich medium or in phosphate-depleted medium.

Electron micrographs of cells cultured in high Pi medium (2 mM), which leads to massive accumulation of polyphosphates (Table I), reveal heavily stained bodies inside vacuolar structures (Fig. 1A, B). Conversely, cells depleted from polyphosphates by phosphate starvation show a significant decrease in the size and number of vacuoles (Fig. 1C, D, ref. 3).

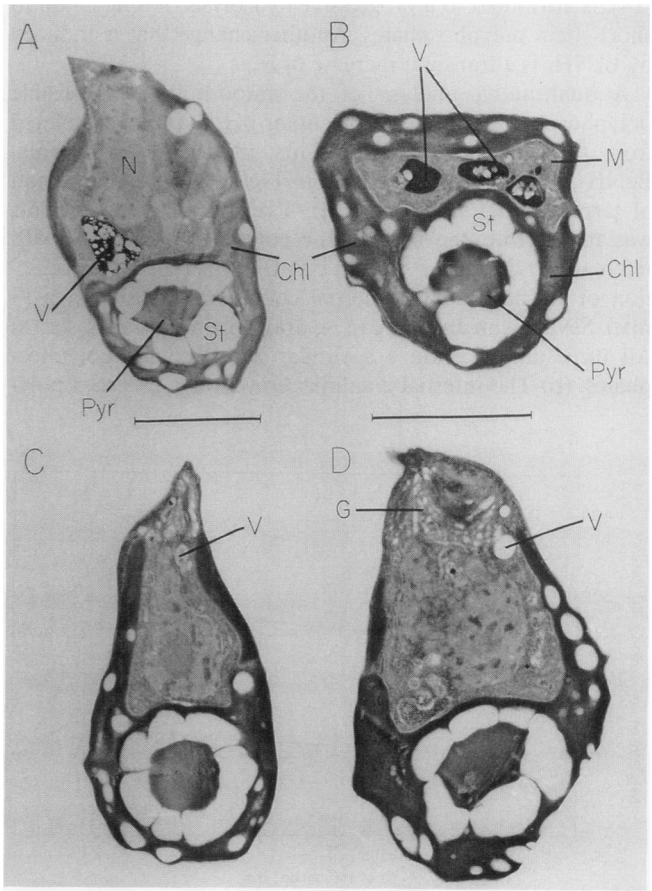

Figure 1. Effect of phosphate nutrition on vacuolar morphology. *D. salina* cells cultured for 1 week in 2 mM phosphate medium (A, B) or with 30 μM phosphate (C, D) were fixed, stained, sliced, and photographed as described in "Materials and Methods." N, nucleus; V, vacuole; Chl, chloroplast; St, starch; Pyr, pyranoid; M, mitochondria; G, golgi. Bars = 5 μm.

These results indicate that polyphosphates accumulate primarily in acidic vacuoles, although localization in other parts of the cell cannot be excluded. Because amines are also suspected to accumulate in acidic vacuoles, it may be expected that polyphosphates will affect the accumulation of amines and vice versa. Phosphate starvation either by 24 h depletion or by prolonged growth in low Pi medium (30 μM) indeed decreases the amount of ammonia uptake (Fig. 2), consistent with a decreased number of vacuoles.

Amine-Induced Polyphosphate Hydrolysis Measured with ^{31}P-NMR

To test whether amines affect polyphosphates, we have utilized ^{31}P-NMR in intact *D. salina* cells immobilized in agarose beads (2). In normal cells, the high mol wt polyphosphates are poorly resolved in the ^{31}P-NMR spectrum due to restricted motion of packed polyphosphate molecules (2). Addition of $BzNH_2$ to the perfusion medium of the immobilized cells induces dramatic changes in the ^{31}P-NMR spectrum, expressed as a large increase in the resonance peaks of terminal (P_1) and central (P_2–P_4) phosphates (Fig. 3). A similar phenomenon, previously observed in other microorganism (14), is attributed to a progressive hydrolysis of long-chain to short-chain polyphosphates. Another change that is induced by $BzNH_2$ is a transient increase of P_i.

A quantitative analysis of the amount of NMR-visible polyphosphates and the intracellular pH changes, calculated from the P_i peak resonance shifts, induced by ammonia, $BzNH_2$, and TEA, is presented in Figure 4. The calculation of terminal (P_1) and central (P_2–P_4) phosphate equivalents was made from the area of the corresponding peaks with reference to the γP of ATP in control cells (before introduction of amines), whose internal concentration is known (3 mM). Several conclusions can be drawn from this analysis: (a) All three amines induce a similar hydrolysis of polyphosphates. (b) The internal alkalinization always precedes polyphosphate hydrolysis. With TEA, both cytoplasmic alkalinization and the initiation of polyphosphate hydrolysis develop slower than with ammonia or $BzNH_2$. In the case of ammonia, polyphosphate hydrolysis is kinetically correlated with the partial recovery of cytoplasmic pH. (c) The appearance of central phosphates precedes appearance of terminal phosphates in agreement with a progressive hydrolysis from longer to shorter polyphosphate chains. (d) Although the experiments did not proceed until a final steady-state had been reached, it can be estimated that the final ratio of central/terminal polyphosphates approached 1. This will correspond to an average size of tetrapolyphosphate. If polyphosphate hydrolytic products indeed accumulate in acidic vacuoles, then they may be used as indicators to follow internal pH changes in the vacuole. Due to lack of information about the ionic strength and Mg concentration that strongly affect the ^{31}P-NMR spectra of polyphosphates (4), it is not possible to derive exact intravacuolar pH values from the data. Nevertheless, the direction and approximate magnitude of the pH change during amine accumulation may be estimated from the shift in the position of the P_1 resonance peak. As is demonstrated in Figure 5, the amines induce a progressive shift in the P_1 peak position toward less negative values, indicative of a progressive alkalinization inside the vacuoles. By assuming a shift of about 2 ppm/pH unit, measured for the γ phosphate of ATP (4), it can be estimated that the overall pH change inside the vacuoles does not exceed 0.5 pH unit during polyphosphate hydrolysis. It has to be noted that a major change in intravacuolar pH may take place before

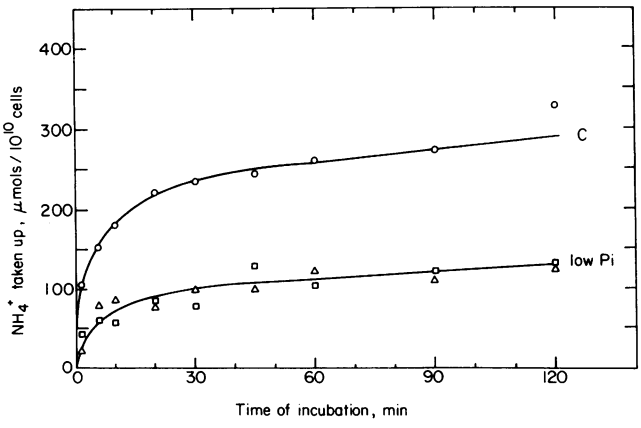

Figure 2. Effect of phosphate starvation on ammonium uptake. *D. salina* cells cultured in standard medium containing 0.2 mM Pi (○), in low-Pi medium containing 30 μM Pi (△) or depleted of Pi for 24 h (□) were suspended in incubation medium containing 20 mM NH_4Cl at 2 to 3 × 10^8 cells/mol. Ammonium uptake was measured as described in "Materials and Methods."

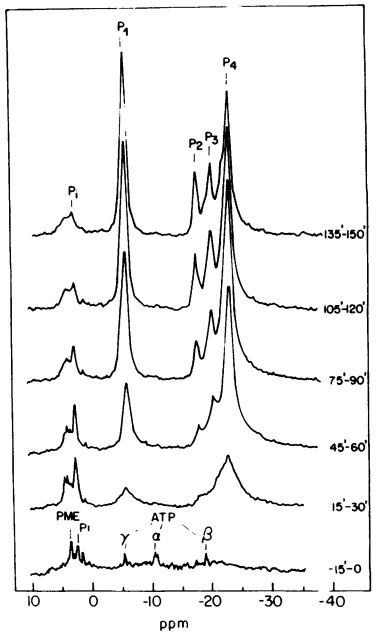

Figure 3. Effect of benzylamine on ^{31}P-NMR spectra. NMR spectra of *D. salina* cells immobilized in agarose beads were measured as described in "Materials and Methods." At zero time, 20 mM benzylamine was added to the perfusion medium. The indicated times are the integration periods after addition of benzylamine. Pi, inorganic phosphate; PME, phosphomonoesters; P_1, terminal phosphate in polyphosphates; P_2–P_4, central phosphates in polyphosphates.

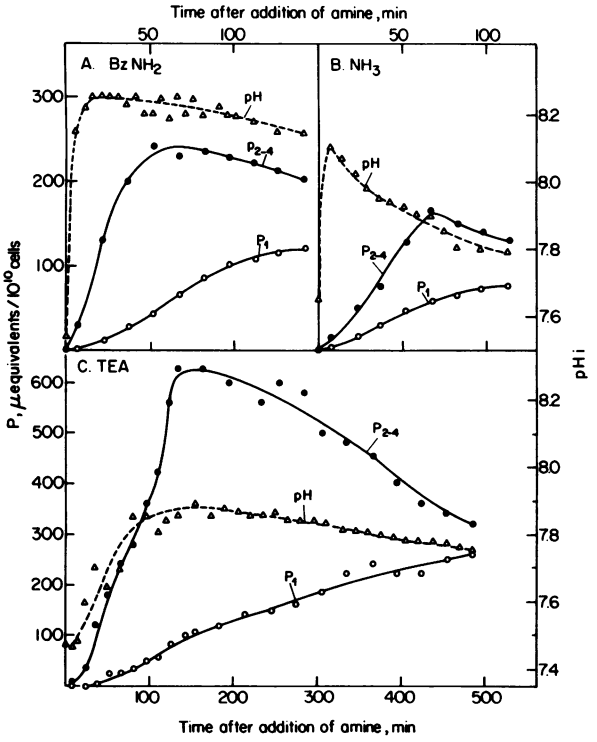

Figure 4. Time course of polyphosphate hydrolysis and intracellular pH changes after addition of amines. ^{31}P-NMR spectra after addition of 20 mM benzylamine (A), 20 mM NH_4Cl (B), or 50 mM TEA through the perfusion medium were recorded as in Figure 3. pH was calculated from the position of the Pi resonance peak at 5 min intervals. Internal contents of terminal (P_1) and central (P_{2-4}) phosphates of polyphosphates in Pi equivalents was calculated from the area of the corresponding peaks, integrated over 15 min as described in "Materials and Methods."

the initiation of polyphosphate hydrolysis, which cannot be detected due to the absence of hydrolyzed polyphosphates. These results are consistent with intravacuolar localization of the hydrolyzed polyphosphates.

The origin of inorganic phosphate, which increases following amine addition, is not clear. The evidence for intravacuolar Pi in *Dunaliella* is vague, because the peak located 1.3 ppm upfield to the cytoplasmic Pi peak (Fig. 3, bottom trace), which could fit an acidic Pi pool, has been identified as glycerolphosphoglycerol (5). Nevertheless, it is possible that a small intravacuolar Pi pool exists, which is masked by the glycerolphosphoglycerol peak, and coalesces with the cytoplasmic Pi peak after amine administration due to vacuolar alkalinization. Although such a possibility cannot be excluded, it appears unlikely for two reasons: (a) The continuing massive accumulation of amines within vacuoles (18) indicates that a significant pH difference between vacuoles and cytoplasm is maintained. Therefore, a vacuolar Pi pool should appear as a distinct peak in the NMR spectrum, for which we find no good evidence. (b) The transient increase in Pi following addition of ammonia and TEA is fairly well correlated with the drop in ATP (determined under the same conditions by the luciferase technique) as indicated in Figure 6. For $BzNH_2$, a large Pi increase that cannot be fully accounted for by ATP hydrolysis is observed, indicative of additional phosphorolytic activities. These results suggest that most of the Pi increase induced by amines originates from ATP and not from polyphosphate hydrolysis.

Identification of Polyphosphate Hydrolytic Products

In a previous preliminary report, we have demonstrated that ammonia at alkaline pH induces hydrolysis of polyphosphate in *D. salina* mainly to tripolyphosphate (17). As is demonstrated in Figure 7, the hydrolysis of polyphosphates induced by $BzNH_2$ and TEA also results in formation of several species of short-chain polyphosphates, mostly tripolyphosphate, and smaller quantities of pyrophosphate, tetrapolyphosphate, and longer chain polyphosphates. The pattern of polyphosphate products is very similar for all three amines. The quantities of tripolyphosphate in the cell extracts are at least 10-fold higher than the cellular ATP content (Fig. 8), and the kinetic analysis of tripolyphosphate formation indicates that the production of tripolyphosphates is initiated after the drop in ATP and the intracellular alkalinization (compare Fig. 8 with Fig. 4). The production of tripolyphosphate induced by TEA is slower than with NH_3 or $BzNH_2$, and the overall production of tripolyphosphate with NH_3 is lower, and saturates faster than with TEA or $BzNH_2$, consistent with the ^{31}P-NMR experiments (Fig. 4). The rate of production of different polyphosphate species following addition of ammonia is very similar (Fig. 9).

What Triggers Polyphosphate Hydrolysis?

Because amines induce internal alkalinization in the cytoplasm and inside the vacuole, it appears likely that the pH changes in one of these compartments triggers the hydrolysis of polyphosphates. Two approaches were taken to test whether intracellular pH changes are responsible for triggering polyphosphate hydrolysis. A comparison between the effect of amines on polyphosphate hydrolysis at pH 9 and pH 7 was made. At pH 7, the rate and amount of amine influx is greatly

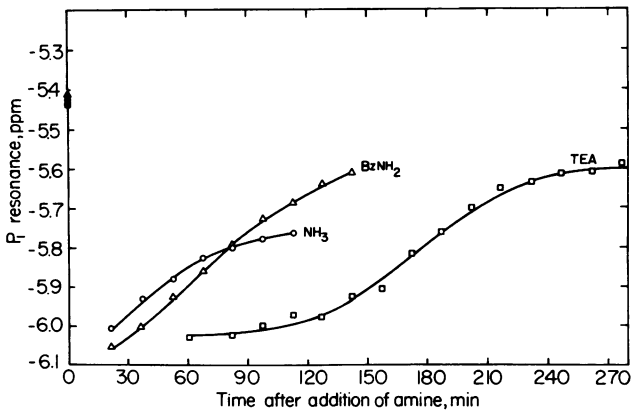

Figure 5. Shift in the resonance peak of P_1. The position of the P_1 resonance peak was obtained from the NMR spectra after addition of amines (see Fig. 3). Solid symbols (0 time) represent positions of the ATP γ-phosphate.

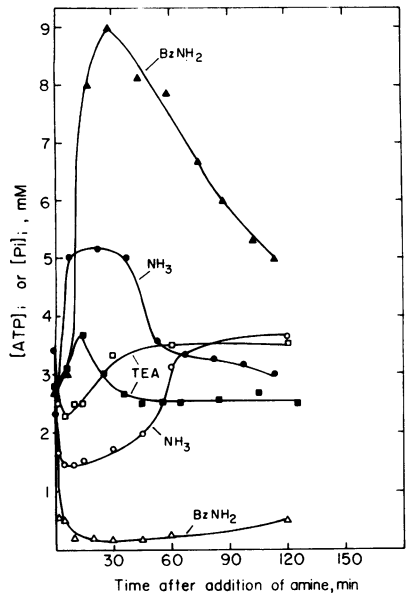

Figure 6. Time course of ATP and P_i intracellular changes after addition of amines. Inorganic phosphate concentration, after addition of amines, was calculated from the area of the P_i peak in ^{31}P-NMR spectra as in Figures 3 and 4. ATP was determined separately under similar conditions (11°C, 3×10^8 cells/mL, pH 9, the same amine concentrations) by the luciferase technique. Other details are described in "Materials and Methods." ATP, open symbols; Pi, closed symbols.

reduced, although a significant release of trapped atebrin indicates that the amine is compartmentalized into the vacuoles (18). Therefore, at pH 7 the effect of amines on cytoplasmic pH is expected to be small, yet the vacuolar pH is expected to increase. As is demonstrated in Fig. 7, ammonia and $BzNH_2$ induce a pronounced hydrolysis of polyphosphates also at pH 7, and even TEA, whose permeability at pH 7 is extremely low (18), induces a significant increase in tripolyphosphate content. Conversely, the protonophore SF-6847, which permeabilizes the outer cell membranes to protons and induces cytoplasmic alkalinization (19), does not induce polyphosphate hydrolysis. These results indicate that intravacuolar and not cytoplasmic alkalinization triggers the activation of the vacuolar phosphatase.

If indeed intravacuolar pH changes activate polyphosphate hydrolysis, then it may be possible to induce it also by alkalinization of vacuolar preparations in the absence of amines. In the accompanying work, we have shown that gentle lysis of *D. salina* cells by a Yeda press treatment permeabilizes the plasma membrane, but maintains the capacity of vacuoles to accumulate amines in the presence of external ATP. Incubation of such lysed cells at pH 8 induced a significant hydrolysis of polyphosphates to tripolyphosphate that is not dependent on amines or ATP (Table II), indicating that the pH itself activates the polyphosphate phosphatase.

DISCUSSION

Unequivocal localization of polyphosphates in *Dunaliella* requires purification of intact vacuoles, which has not yet been achieved. However, several observations suggest that polyphosphates in *Dunaliella* are located in acidic vacuoles:

(a) The dense osmiophilic bodies observed in electron micrographs of polyphosphate-rich cells, which disappear upon accumulation of amines (Fig. 5 in ref. 18). Similar structures were identified before as Ca-polyphosphates by x-ray microanalysis (7).

(b) The observation that phosphate depletion decreases ammonium accumulation in acidic vacuoles (Figure 2) as well as their number (3).

(c) The correlation between polyphosphate hydrolysis and accumulation of amines in the vacuoles.

In the accompanying work (18), it was demonstrated that the accumulation of amines in acidic vacuoles is correlated with the recovery of cytoplasmic pH and depends on the capacity of the acidic vacuoles to accumulate the amines. Several lines of evidence suggest that the hydrolysis of polyphosphates provides this capacity in *Dunaliella*.

(a) The time course of cytoplasmic pH recovery is correlated with the time course of polyphosphate hydrolysis, except for $BzNH_2$, which appears to be toxic to the cells.

(b) The calculated ammonium content that is accumulated

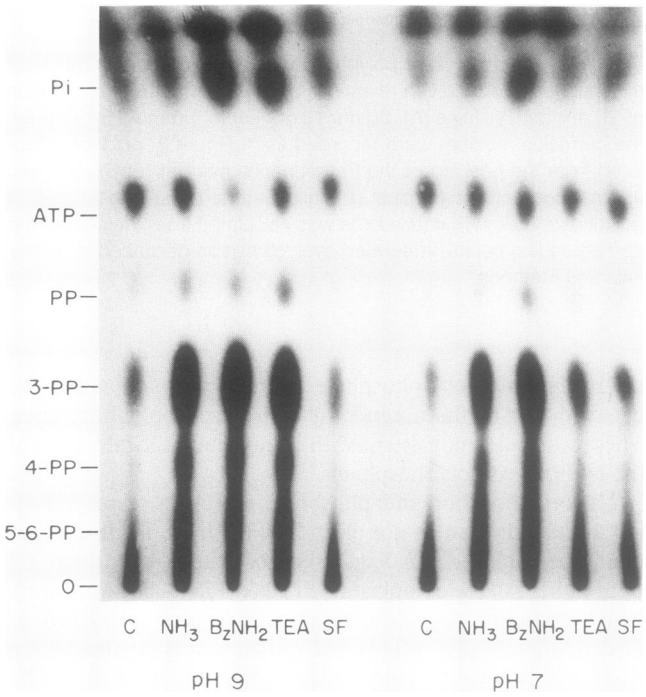

Figure 7. Analysis of polyphosphate hydrolytic products induced by amines at pH 9 or pH 7. *D. salina* cells cultured for 2 d with [^{32}P] orthophosphate were incubated in the suspension buffer used for NMR measurements (see "Materials and Methods") containing either 20 mM Na-Hepes, pH 7, or 20 mM Tris-Cl, pH 9, and 5×10^6 cells/mL. Ammonium chloride (20 mM) or benzylamine (20 mM) or TEA (50 mM) or SF-6847 (5 μM) were added, and the cells were incubated for 1 h at 23°C. Forty microliter samples were extracted at 0°C in 2 M formic acid, and the extracts were separated on PEI-cellulose plates and analyzed by autoradiography. PP, 3-PP, 4-PP, 5–6 PP denote polyphosphates containing linear chains of 2, 3, 4, and 5 or 6 phosphates.

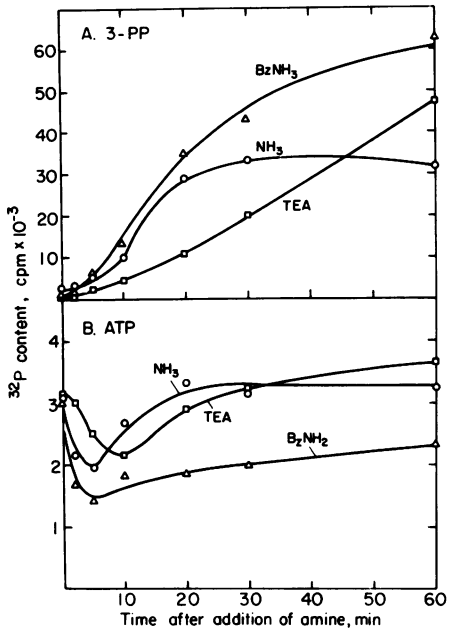

Figure 8. Time course of tripolyphosphate formation and ATP changes. ^{32}P-labeled *D. salina* cells were incubated for the indicated times with NH_3, $BzNH_2$, or TEA at pH 9 and extracted as described in Figure 7. Spots corresponding to tripolyphosphate (3-PP, A) and to ATP (B) were cut out and counted as described in "Materials and Methods."

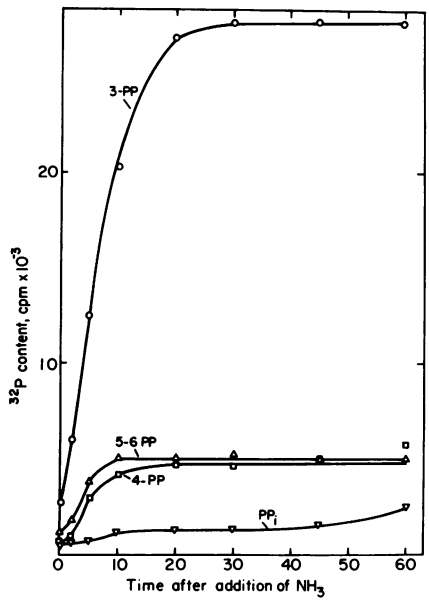

Figure 9. Time course of formation of different polyphosphate hydrolytic products after addition of ammonia. ^{32}P-labeled cells were extracted at the indicated times after the addition of 20 mM NH_4Cl at pH 9 buffered suspension media. Lyophylized extracts were separated on PEI-cellulose plates and analyzed as in Figure 8.

Table II. *Induction of Polyphosphate Hydrolysis in Lysed Cells by Alkaline pH*

D. salina cells were cultured for 3 d with ^{32}Pi and lysed by Yeda press treatment as described in the preceding paper (18). The lysed cells were centrifuged (10 min × 800 *g* and resuspended in suspension medium containing 20 mM Na-Mes, pH 6, or Na-Hepes, pH 7 or pH 8. The cells were incubated for 1 h at 23°C with or without 1 mM ATP and 30 mM NH_4Cl and extracted with formic acid. Extracts were separated on PEI-cellulose, and tripolyphosphate spots were cut out and counted.

Additions	Tripolyphosphates		
	pH 6	pH 7	pH 8
		cpm	
	1054	2158	3466
ATP	1276	1626	3032
ATP + NH_4Cl	1118	2692	3390
NH_4Cl	1630	2398	3328

in *Dunaliella* at steady-state at pH 9 in the presence of 20 mM NH_4Cl is around 200 $\mu eq/10^{10}$ cells, about twice the estimated number of hydrolyzed anhydride bonds. Because part of the amine may not be completely protonated, part may be bound or assimilated in other compartments, and since the visibility of the hydrolyzed polyphosphates in the NMR spectrum may be incomplete, the amount of amine in the vacuoles approximately equals the number of hydrolyzed anhydride bonds (17).

(c) The progressive shift of the peak resonance position of P_i indicates that the pH inside the vacuoles increases while the pH in the cytoplasm decreases. These pH changes are consistent with a progressive transfer of free amine from the cytoplasm into the vacuoles.

The massive hydrolysis of polyphosphates may facilitate the compartmentation of amines into acidic vacuoles in several ways. The formation of short polyphosphate oligomers from long-chain polymers should increase the osmotic potential inside the vacuole and thus contribute to osmotic swelling. More importantly, the hydrolysis of anhydride bonds produces protons, and thus can serve as a high-capacity pH buffering system to preserve the low pH inside the vacuole relative to cytoplasm, which is required to protonate and trap large quantities of amines. The relatively small increase estimated in vacuolar pH during polyphosphate hydrolysis (Figure 5) is consistent with this hypothesis.

The trapping of amines in acidic vacuoles should have a major impact on cytoplasmic pH homeostasis; in the absence of such a mechanism, diffusion of free amines into the cells would lead to rapid cytoplasmic alkalinization, as was indeed observed, which may interfere in essential physiological functions. Compartmentation and protonation of the amine inside acidic vacuoles would relieve the cytoplasmic pH stress and provide an effective pH buffering system. This idea is presented in Figure 10.

The nature of the intravacuolar phosphatase, which is responsible for polyphosphate hydrolysis in *Dunaliella,* can be predicted from the results described herein. The phosphatase appears to be activated at neutral or mild alkaline pH and repressed at the physiological intravacuolar acidic pH. Be-

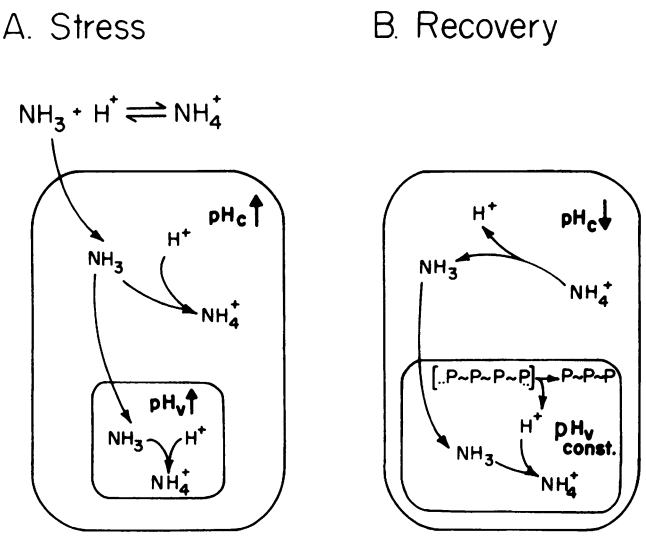

Figure 10. Proposed mechanism of the role of intravacular polyphosphate hydrolysis in amine compartmentation and pH homeostasis.

cause it appears to clip tripolyphosphate units, it should be defined as an exophosphatase and not as an endophosphatase. The ^{31}P-NMR observations of progressive increase in the terminal/central phosphate ratio is inconsistent with this mechanism and may indicate that an additional endophosphatase is operative. The discrepancy may result from the presence of longer chain polyphosphate intermediates, which are not resolved by the acid extraction, but show up in the NMR spectrum. Previous reports suggest that in yeast, amines and basic amino acids also induced hydrolysis of polyphosphates and accumulation of tripolyphosphate (6, 13, 14). These observations suggest that hydrolysis of long-chain polyphosphates to tripolyphosphate may provide a general protective mechanism to counterbalance alkaline stress and to accumulate amines in different classes of microorganisms.

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