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Elemental composition of the polyphosphate bodies in microbial cells from a small lake

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With 3 figures in the text

Abstract

Polyphosphate bodies (PPBs) were observed with the electron microscope in air dried preparations of bacteria-sized plankton recovered from lake water. Analysis of the bodies was carried out using the scanning transmission mode (STEM) of the transmission electron microscope (TEM) in conjunction with an energy dispersive X-ray spectrometer (EDX). The bodies in most cells were composed of Mg, P, K and Ca. Some bodies also possessed S, Cl, Na and Fe. In PPBs from most cells the K peak was low and in some cells it was absent. The PPBs in three different morphotypes also possessed Al. The numerous other cell types did not have Al as a part of their PPBs. The Al containing cell types consisted of a small rod shaped organism 0.3 by 0.6 μm, a larger roughly spherical organism 1×1.5 μm and a large boat shaped organism 3×7 μm. The presence of Al in the PPBs of these organisms, as well as the other elements, demonstrates that metals can be selectively taken up by different species in nature. The movement of metals in the food web would be at least in part dependent on the consumption of these specific organisms by the next trophic level.

Introduction

Polyphosphate bodies (PPBs) are common cellular inclusions in bacteria, algae and fungi (Harold 1966, Beevers & Burns 1980, Kulaev & Vagabov 1983). Early studies have shown that they are composed of the elements Mg, P, K and Ca as well as an organic component (Friedberg & Avigad 1968, Jones & Chambers 1975, Doonan et al. 1979, Baxter & Jensen 1980 b).

Previously several groups, especially Jensen and co-workers and Sicko-Goad and her associates, have demonstrated that polyphosphate bodies bioconcentrate certain heavy metals. They have shown that a variety of axenic cultures of cyanobacteria and eukaryotic algae can concentrate Ba, Cd, Co, Ca, Hg, Mg, Mn, Ni, Pb, Ti, Sr, Cu and Zn in their PPBs (Crang & Jensen 1975,

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Sicko-Goad & Stoermer 1979, Baxter & Jensen 1980 a, Sicko-Goad 1982, Rachlin et al. 1982 a, Rachlin et al. 1982 b, Jensen et al. 1982 a, Jensen et al. 1982 b, Rachlin et al. 1984, Rachlin et al. 1985, Rai et al. 1990). In addition Petterson et al. (1985) have shown that Al will also accumulate in these bodies under laboratory conditions.

No work has been carried out on the elemental composition of PPBs in native populations of microbes from aquatic environments. Pedersen et al. (1981) have analyzed *whole cells* from polluted and non-polluted environments. In polluted environments whole cells contained the metals Ti, Mn, Cu, Ni, Zn and Al. In this present study we have utilized a method which allowed us to determine the elemental composition of individual cell components. We report here for the first time on the elemental composition of PPBs in cells of a biomass fraction concentrated from natural environments.

Materials and methods

Samples were collected from Lake Arthur which is one of a small group of lakes situated in the Black Rock Forest near Cornwall, N.Y. All but one of the seven lakes serve as a reservoir system supplying freshwater to several towns in the region. Pollution is minimal and the nutrient level of the waters is quite low (Adirondack Lake Survey, 1987). The lakes are considered oligotrophic (Corpe & Jensen 1992). Three liter water samples were collected from Arthur's Lake once a month from May through November 1990. Surface samples were by bucket. An open Niskin type bottle was closed at a depth of 13 feet, one foot above the sediment, to provide a deep sample.

Single surface collections were done at Sutherland Lake and Alec Meadow Lake in July 1990 for comparative purposes. Each sample was handled in the same manner as the Lake Arthur samples.

Cells were collected by passing 3 liters of water through a 20 μm Nitex screen and then centrifuging the filtrate at 14,000 × g for 2 hrs (Johnson & Sieburth 1979). A portion of the concentrated biomass was then placed on formvar-coated Cu or Ni grids, and they were either air dried for 12 hrs or dried in a 35 °C oven for 4 hrs. Samples were also fixed in 3 % glutaraldehyde followed by staining with o_sO_4 (Pankratz & Bowen 1963) and then embedded in Epon (Luft 1961). Thin sections cut with a diamond knife were post-stained in uranyl acetate (Stempak & Ward 1964) and then examined in a TEM (Hitachi H-7000) operating at 75 kV.

For X-ray analysis air-dried cells of interest were first located using the TEM mode. The microscope was then switched to the STEM mode. Analysis of cell components was carried out in the spot mode (75 kV) of the STEM. The spot diameter at 100,000 × is approximately 20 nm. The objective lens current was adjusted so a total X-ray count of about 600 to 1,000 cps was attained. Count time was 100 sec. Spectra were collected on a PGT System 4 Plus energy dispersive X-ray spectrometer (EDX). Spectra were collected from PPBs, cytoplasmic areas, wall areas and from the unoccupied formvar surface. All spectra presented have been background subtracted (counts from the formvar next to the cell with the same settings on the EDX system). No other manipulations of the spectra were done. Approximately 600 polyphosphate bodies were analyzed along with other cellular areas.

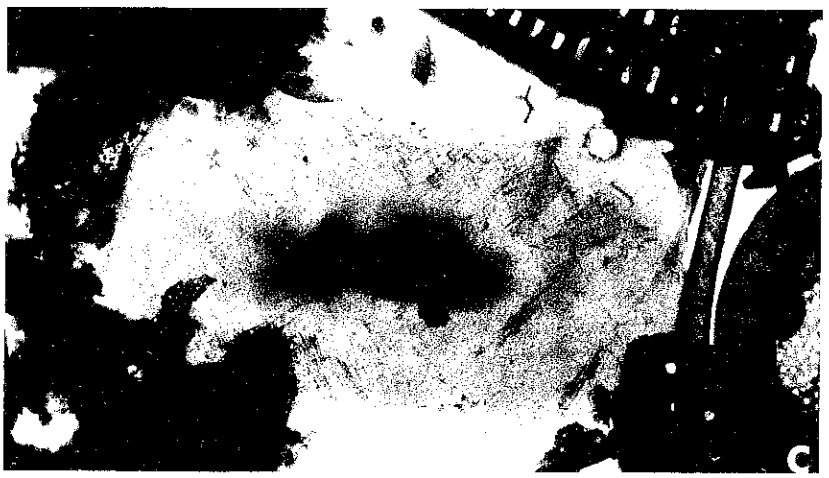
Results

Most of the microbial cells observed in the samples possessed PPBs. Cells from the deep sample generally possessed more polyphosphate bodies. The same cell morphotypes were found in both samples. The spectra generated by the polyphosphate bodies in these morphotypes are identical and only one is shown Fig. 3 A. Fig. 1 shows images of cells in the TEM mode. Analysis of the bodies showed that they contained the elements P, Mg, Ca and K. The Cu peaks are generated from the Cu grid and are not present when Ni grids are used. All polyphosphate bodies also possess detectable C and O emissions. In most cells the P and Ca peaks were high and the Mg and K peaks low (Fig. 2 B and 3 A). In some of the bodies no K was detected. In about 25 % of the bodies a significant S (Fig. 3 A) and Fe (Fig. 2 B) peak was also present. In about 50 % of the PPBs Cl (Fig. 3 A) and Na (Fig. 3 A) peaks were also evident. For most of the cell morphotypes the PPBs contained the elements as described above. However in 3 cell morphotypes shown in Fig. 1 a significant Al peak is also present (Fig. 2 B). The cell type in Fig. 1 A is $1 \times 1.5\,\mu$m in size and roughly spherical. Based on the observation of cells in thin sections we feel it is a cyanobacterial cell. All cells possessed numerous PPBs which were spherical and about 0.8 to 0.18 μm in diameter. In this cell type (Fig. 1 A) Mg, Al, P, S, Cl and Ca were the common components (Fig. 3 A). A K signal was detected in about 50 % of the bodies and was always very small. The cell type in Fig. 1 B is about $0.3 \times 0.6\,\mu$m. Cells of this morphotype were numerous. Each cell generally contained more than one spherical PPB with diameters between .08 μm to 0.1 μm. In this cell type Na, Mg, Al, P, S, Cl, K, Ca and Fe were the common components (Fig. 2 B). The third cell type shown in Fig. 1 C is roughly boat shaped and about $3 \times 7\,\mu$m in size. The PPBs are located near the midline of the cell and the area in which they are located is slightly more electron dense (Fig. 1 C and 2 A). The bodies are between 0.06 and 0.3 μm in diameter. The cytoplasmic area is nearly electron transparent. However on the surface of the cell a pattern of lines can be seen (Fig. 2 C). These electron dense striations average 33 nm in width and spaced 66 nm apart. We were unable to make a correlation between this cell type and cells observed in thin sections in spite of the fact that over a 1,000 micrographs of the material were prepared. Observation with the light microscope was difficult and the cells could not be located unless first identified in the TEM with appropriate markers. We suggest it is an eukaryotic cell because few prokaryotes have diameters larger than 2 μm. Analysis of the line patterns shows that this material is high in S and Ca (Fig. 2 D). In this cell type Mg, Al, P, Ca and Fe were the common elements detected in the PPBs (Fig. 2 B). In a few of the bodies small S and K peaks were found (Fig. 3 A). Fe was found in 70 % of the PPBs. Cl was observed in 30 % of the bodies and Na in 10 %.

Analysis of cytoplasmic and wall areas did not reveal an Al peak.

A

B



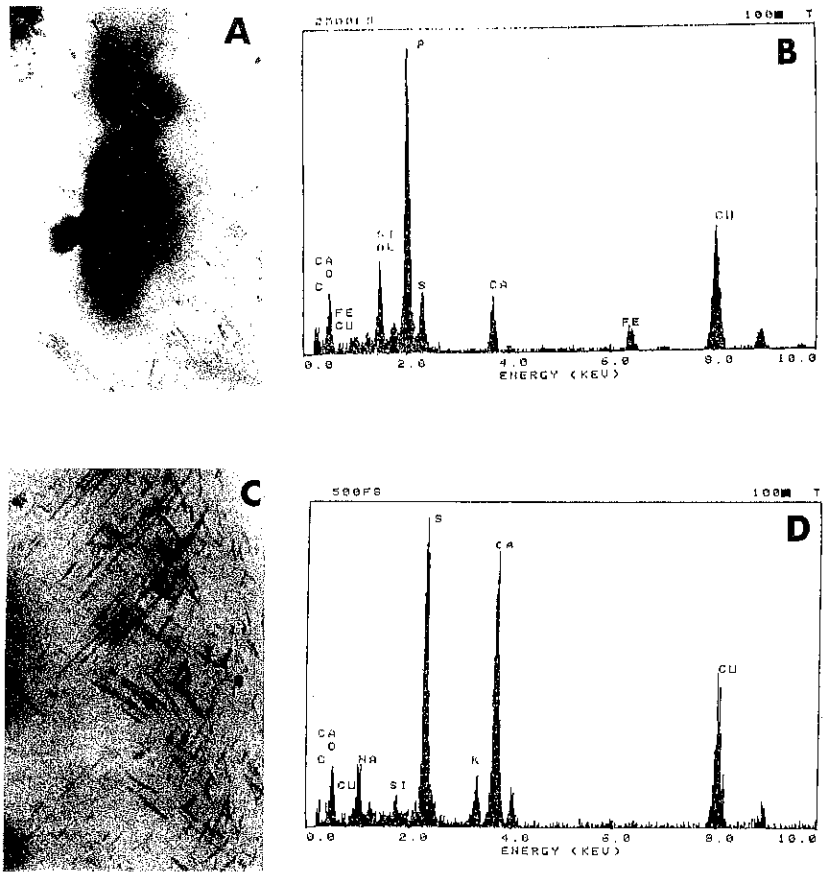


Fig. 2. A. High magnification of polyphosphate bodies from the unidentified organism shown in Fig. 1 C. 12,700×. B. Spectrum obtained from analysis of the polyphosphate bodies shown in Fig. 2 A. C. Striations visible on the surface of the organisms shown in Fig. 1 C. 9300×. D. Spectrum generated when probe is placed on the dense portion of the striations shown in Fig. 2C.

Discussion

In this study we have used whole air dried cells prepared from natural assemblages of the nanoplanktonic biomass to study the elemental composition

Fig. 1. Whole air dried cells of the three organisms which had Al as a constituent of their polyphosphate bodies. A. Organism which is probably a coccoid cyanobacterium. Numerous electron dense polyphosphate bodies are present in the cell. The body (arrow) with the electron lucent area was analyzed before the negative was taken. The electron beam volatilizes the polyphosphate. 36,000×. B. Bacterial cell showing six electron dense polyphosphate bodies. 40,500×. C. Unidentified cell with numerous electron dense polyphosphate bodies 13,500×.

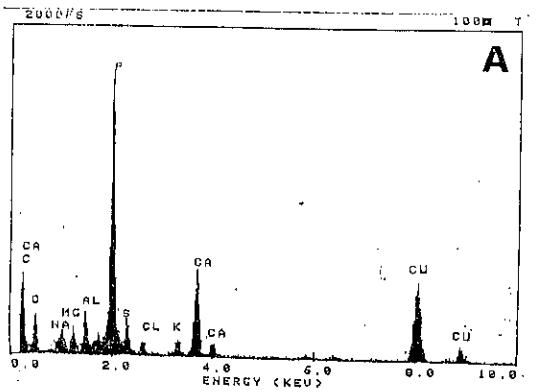


Fig. 3. Spectrum generated from polyphosphate body showing K, S, Cl and Na in addition to the usual elements found in these bodies.

of PPBs with the TEM. Previous studies, of pure cultures, had shown the method suitable for detecting metal in whole cells and PPBs (BAXTER & JENSEN 1980 b).

In analysis of PPBs from whole cells which have been air dried, care must be taken to select areas which do not have other particulate material either on top of or under the bodies. Particles containing Si are very often associated with whole cells collected from nature (JENSEN & CORPE 1991). In any case where this element is detected we assume that a small particle, presumably a small diatom fragment has contaminated the area. The probe at 100,000× is about 20 nm in diameter and has about 20 nm spacial resolution. Therefore when care is taken in placement of the probe reliable and representative results are obtained.

In the laboratory, we and others have shown, that cells can take up a wide variety of toxic heavy metals which are sequestered by PPBs. However little is known about this phenomenon in the natural environment. In laboratory studies PPBs generally have the elements Mg, P, K and Ca. In some cases S is also present (BAXTER & JENSEN 1980 b). The abundance of K as suggested by peak height varies greatly in cells from natural environments and in many cases it is not present or present in very small amounts. All PPBs from nature have Mg, P and Ca as the minimal elemental makeup. The biosynthesis of polyphosphate and PPBs and their role in the life of the cell has been reviewed often and well. Laboratory studies indicate that they function as a phosphorus reserve, an energy source, as a pool for essential ions and as a detoxification mechanism (JENSEN et al. 1982 a, KULAEV & VAGOBOV 1983, PREISS 1989).

We showed in the present study the abundance of PPBs in microbes from nature which reveals for the first time that a variety of elements can be sequestered in PPBs in natural habitats. We also found that the bodies did not have a

common composition except that they all possessed Mg, P and Ca. The bodies varied with regard to the presence of Cl, Al, S, Na, K and Fe. We suggest that as PPBs are investigated from other habitats, other elements will likely be detected in them. Based on this present study it appears that the elements which are part of the PPBs may be species dependent. Of the several dozen different organisms in which we analyzed PPBs only 3 had Al as part of the body. The other elements we detected seemed to be common in many of the PPBs in the different microbes. Variation in elemental composition of PPBs has been reported. COLEMAN et al. (1973) showed that phosphorus containing refractive bodies in *Amoeba proteus* also varied in regard to whether they possessed the elements Mg, S, K and Ca. In phosphorus-rich granules isolated from barnacles, variation in the presence and amount of the elements Zn, Fe, Ca, K, Cu, Mn, Pb, Hg and Cd has also been reported (PULLEN & RAINBOW 1991). A function of PPBs suggested by our work is their role in chelation of metals acting either in the regulation of homeostatics of metals within cells and/or in neutralizing the toxic effects of heavy metals (BAXTER & JENSEN 1980 a, WOOD & CLARK 1988). The fact that composition of PPBs varies with species in the same sample indicates that environmental differences are not alone responsible. FIXTER & SHERWANI (1991) point out that there are two polyphosphate pools in some organisms one of which can serve as an energy saver and the other as a simple phosphate store and concludes that generalizations about the function of this compound are difficult without detailed study of strains concerned.

The presence of elements such as Al and Fe in planktonic bacterial PPBs is of interest since they may be moved to the next trophic level when the prokaryotic cells are consumed by zooplankton.

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