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Polyphosphate Bodies Aligned Along the Anterior Axoneme of the Cryptomonad *Rhodomonas lacustris*: Possible Relevance to Symbiotic Origin of Undulipodia

MIKAL HELDAL
Department of Microbiology, University of Bergen, Jahnebakken 5, 5020 Bergen, Norway, Tel. +47-55-584675, Fax. +47-55-323962, E-mail: mikal.heldal@im.uib.no

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Abstract

The more active, anterior undulipodium of the cryptomonad *Rhodomonas lacustris* (Pascher and Ruttner), taken from a natural lake water sample, Lake Myravatn, Norway, contained 30–40 polyphosphate bodies aligned at regular intervals along the axoneme. The unilateral row of polyphosphate bodies was limited to the distal 3 μm of the 7.5 μm long organelle. The posterior shorter (6.8 μm) undulipodium lacked polyphosphate bodies even though mastigonemes were present on both. The evolutionary origin of the undulipodium (9(3)+0 microtubules where centriole-kinetosomes underlie the 9(2)+2 axonemal shaft) from some free-living motile bacteria (e.g., spirochetes) has been argued. Definitive evidence for the symbiotic origin of undulipodia is still unavailable. Comparable to volutin granules of bacteria, which they strikingly resemble, these polyphosphate bodies may be legacies of their origin that serve as an energy and/or phosphorus store.

Keywords: Polyphosphate, undulipodia, cryptomonads, symbiotic origin

1. Introduction

Intracellular polyphosphate storage inclusions, so-called volutin or polyphosphate granules are common in a wide range of bacteria from all major

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groups. Such structures have been described in bacteria cultured in artificial media (Harold 1966 and Kornberg, 1995; and refs. therein), and in bacteria directly collected from natural aquatic microbial communities (unpublished observations). Polyphosphate bodies are seen by transmission electron microscope (TEM) as electron dense structures in unfixed and unstained preparations (Nissen et al., 1987). Under high intensity electron beam polyphosphate bodies will partly be evaporized and appear as perforated structures, and this has been used for diagnostic purposes (Drews, 1960). Here the relation between polyphosphate bodies in a motile but photosynthetic protist and those known from bacteria are compared.

2. Materials and Methods

Water samples were collected from the epilimnion of the Lake Myravaten, a small eutrophic lake in Bergen, Norway (August 1983). The water samples were brought to the laboratory and prepared for the transmission electron microscope (TEM) within 2 hours.

Cells of the unicellular alga (cryptomonads) were harvested by centrifugation directly onto Nylon grids (Agar Scientific, Herts, England) supported with a carbon coated formvar film. The grids were mounted in a specially-made grid-holder placed in the centrifuge tube. Cells and particles were harvested onto the grids from a water column of 10–20 mm above them after 15 min centrifugation in a swing-out bucket rotor at 1200 g, and 25°C.

After centrifugation excess water was removed from the grids by blotting with filter paper, and the unfixed and unstained cells were air dried and examined in a JEOL CX-100 Transmission Electron Microscope, equipped with a Kevex X-ray energy-dispersive detector and an ASID-scanning attachment. For X-ray microanalysis, the microscope was operated at an accelerating voltage of 80 kV, and a take-off angle of 38°. *Rhodomonas lacustris* (Pascher and Ruttner) was identified in mixed population from lake water by Dr. Dag Klaveness, University of Oslo, Norway. For taxonomic details see Klaveness (1981).

3. Results and Discussion

In the 5–10 different cells studied the longer anterior undulipodium of *Rhodomonas lacustris* had 30–40 electron dense inclusions (Figs. 1a–d). Most appeared in a unilateral row on the distal portion (Figs. 1a and b). These bodies had a high phosphorus content as determined by X-ray microanalysis. At high electron beam intensity they volatilized with a typical appearance of

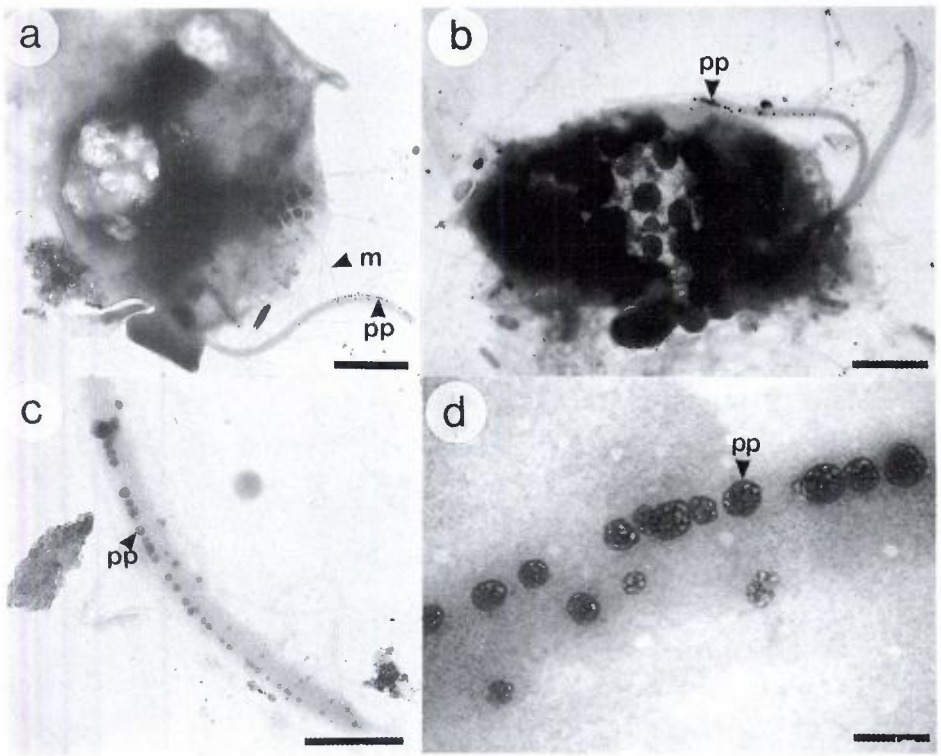

Figure 1. Transmission electron microscope micrographs from air dried preparations of unfixed and unstained cells of *Rhodomonas lacustris*. a) The longer anterior undulipoium with two rows of mastigonemes (m) and a row of polyphosphate bodies (pp) (bar = 2 μm). b) Cell with both anterior and posterior (shorter) undulipodia: the posterior undulipodium with polyphosphate bodies (pp) (bar = 2 μm). c) Distal part of an anterior undulipodium with about 40 polyphosphate bodies (pp) (bar = 1 μm). d) Structure of polyphosphate bodies (pp) which have volatilized under the electron beam (bar = 0.2 μm).

polyphosphate bodies (Figs. 1c and d). The polyphosphate bodies, 60–110 nm in diameter, closely resemble polyphosphate bodies of the same size and the same tendency to volatilize leaving holes in a dish as do those of bacteria (Fig. 2), see also Jensen (1969) and Nissen et al. (1987). The posterior 6.8 μm undulipodium on all cells lacked any evidence of polyphosphate bodies. Both anterior and posterior undulipodia bore mastigonemes: two rows on the longer and one row on the shorter one (Figs. 1a and b). No relation between the mastigonemes and polyphosphate bodies was observed. The undulipodia are

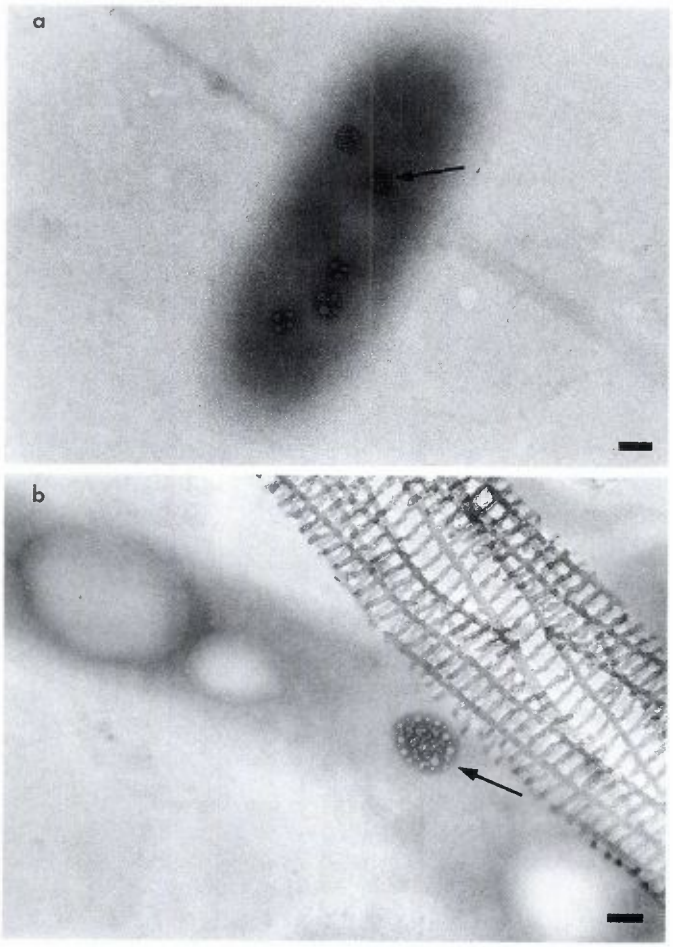

Figure 2. Transmission electron microscope micrographs from air dried preparations of unfixed and unstained bacteria containing polyphosphate bodies which have volatilized under high intensity electron beam. a) bacterium from Lake Nesjøvatnet (69°8'N, 11°50'E, 1981) with five polyphosphate bodies. The appearance of the polyphosphate bodies may change slightly due to the intensity of the electron beam applied as seen from the two upper polyphosphate bodies (arrow) compared to the lower ones. b) Polyphosphate body (arrow) from a marine filamentous bacterium Hylsfjorden (59°32'N, 06°29'E, 1995). Bar 100 nm.

typical: a central pair of single microtubules and 9 outer doublet microtubule pairs as has already been reported in cryptomonads (Gantt, 1980). The polyphosphate bodies in *R. lacustris* undulipodia appear to be located at

regular intervals along the axoneme between the outer doublet microtubules and the plasma membrane (Figs. 1c and d). This suggests a highly specific course of synthesis and development of inorganic polyphosphate as a linear polymer of tens – to hundreds of orthophosphate, Pi, residues linked by high-energy phosphoanhydride bonds (Kornberg, 1995). Thin sectioning and TEM studies will be required to confirm this inference.

Although polyphosphate bodies have been observed in many algae (Kulaev and Vagabov, 1983; and refs. therein), I am not aware of any previous reports of a specific axonemal distribution as seen here.

The setae of the marine diatom *Chaetoceros gracilis* (Bacillariophyceae) are four co-planar siliceous projections which grow as valve projections after cell divisions at new frustule production (Round and Crawford, 1990). In the silicifying setae a microtubule-like axial structure, elongated as the setae grow at rates of 0.2–0.38 μm min^{-1}, many 30-nm polyphosphate bodies were located along this microtubule, inside the surrounding double membrane (Rogerson et al., 1986). These polyphosphate bodies were postulated to be an *in situ* energy source, an ATP substitute, because the lumina of the setae are too small to accommodate mitochondria (Rogerson et al., 1986). Polyphospate bodies along the setae of *C. gracilis* were also observed in DAPI (4',6-diamidino-2-phenylindole) stained preparations from a coastal area south of Bergen, Norway, in the summer 1995 (F. Thingstad, personal communication).

In many species of pavlovalian prymnesiophytes the longer undulipodia have small dense bodies outside the axonemal membrane (Green and Manton, 1970), but the composition and function of these bodies remain uncertain. The prymnesiophyte extracellular bodies bear little resemblance to the polyphosphate bodies reported here.

The formation and function of polyphosphate bodies in cells are still uncertain. Since polyphosphate is seen in TEM only when it is condensed in granules, we suspect soluble fractions of polyphosphate are more common (Kjeldstad et al., 1991). The formation of polyphosphate bodies in some bacteria has been shown by Nissen et al. (1987) to be a transient phenomenon related to rapid uptake of external phosphate. Polyphosphate in bacteria is generally regarded as an endogenous pool of phosphorus (Harold, 1966). In *Helicobacter pylori* polyphosphate bodies in the size range of 50–200 nm were identified in the cytoplasm, while smaller polyphosphate bodies (20 nm) were seen near the flagellar pole (Bode et al., 1993). This specialized localization of polyphosphate bodies may point on different function for polyphosphate within a cell.

Inorganic polyphosphate with tens to hundreds Pi residues is better considered as a "molecule for many reasons": and storage of energy for metabolism and of phosphate for nucleic acid synthesis, metal chelation of

Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, etc, and as a buffer against alkali (Kornberg, 1995). Polyphosphate inclusions in *R. lacustris* are likely to serve as an energy source for motility or as a phosphorus storage for cell growth. The longer undulipoda of these algae have been observed to be more active in motion of the cells (Pringsheim, 1968). The stiff and inactive shorter one has less of a requirement for energy at the location of the bend. These observations, consistent with the suggestion of the origin of undulipodia from motile polyphosphate utilizing bacteria (Margulis, 1992), suggest that a direct comparison between polyphosphate inclusions and their use in motile bacteria and in axonemes may be undertaken.

The evolutionary role of polyphosphate bodies should be explored as the energy of the anhydride-bond and the phosphate of polyphosphate are likely to have been early sources of nucleoside triphosphates (Kornberg, 1995). Until recently methods of detection of polyphosphate bodies have been elusive, but by combining X-ray microanalysis in TEM, NMR-analysis and new staining techniques the analytical possibilities will greatly improve. We expect to find polyphosphate in most bacteria, and predict that it is related to growth and in aspects of the movement of some spirochetes. How polyphosphate is regulated in protists and its possible evolutionary link to the ATP-energy conversion system is still unknown but offers a promising field for further research.

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