Microtubule-like Inclusions in Isolates of the Blue-green Bacteria Anabaena and Nostoc

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Although cytoplasmic microtubules have been reported widely and studied extensively in eukaryotic cells, only a few reports of such structures have been made for prokaryotic cells. Microtubular structures have been reported in several bacteria, where the diameter of the tubules varied from 10 to 25 nm (Petitprez and Beerens 1967, Van Iterson et al. 1967, Schmidt-Lorenz and Kuhlwein 1968, Corfield and Smith 1968, MacRae and McCurdy 1975, D’Aoust and Kushner 1976, Margulis and Chase 1978). In the blue-green bacteria Bailey-Watts et al. (1968) reported a tubular structure 15 nm in diameter and at least 300 nm long in an undescribed species of the genus *Synechococcus*. Jensen and Bowen (1970) and more recently Jensen and Ayala (1976a) have described striated microtubules in *Anabaena minutissima* and a microplate-microtubule array and polyhedral body associated microtubules in several species of *Anabaena* (Jensen and Ayala 1976b).

Thus the reports of microtubular structures in prokaryotes are scattered, and no one has surveyed a group to establish if they are commonly present. In this study we examined all of the isolates of *Anabaena* and *Nostoc* available from the Indiana University Culture Collection (Starr 1964, Starr 1971) and found what we at present interpret to be two new kinds of microtubular structures.

Methods and materials

(1627), *N. calicola* (B-382), *N. foliaceum* (1624), *Nostoc* sp. (B-388), *Nostoc* sp. (588), *Nostoc* sp. (B-587), *Nostoc* sp. (B-586), *Nostoc* sp. (389), *Nostoc* sp. (1597), *Nostoc* sp. (1544), *Nostoc* sp. (756), and *Nostoc* sp. (387). A culture of *A. cylindrica* was also kindly provided by Dr. C. Peter Wolk, MUS/AEC Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48823. This culture is an axenic isolate of *A. cylindrica* from the Indiana University Culture Collection.

Figs. 1–2. 1, light micrograph of *Nostoc* sp. (389) showing various diameters of cells can be observed. The variable diameter microtubule-like inclusions occur only in the small diameter cells such as those at the arrow. ×2,000. 2, thin section of a portion of a filament of *Nostoc* sp. (389). Note the cuboidal shape of the cells. Several polyhedral bodies (Pb), the peripheral thylakoids (t) and a small group of variable diameter microtubule-like inclusions (vm) can be seen. ×20,000.
Figs. 3-4. 3, enlarged view of a single cell of *Nostoc* sp. (389). Visible in the cell are polyhedral bodies (Pb), thylakoids (t), B-granules (l), two tri-lamellar bodies (tl) (Jensen and Ayala 1976c): and two groups of tubules (vm). ×43,000. 4, enlarged view of a variable diameter microtubule-like inclusion in *Nostoc* sp. (389) showing gentle bends (arrow). Note the constant diameter along the 1.1 µm length of the tubule. ×70,000.
A portion of each culture was fixed in osmium tetroxide shortly after it was received (Pankratz and Bowen 1963). Transfers were then made from the cultures into Zender and Gorham's modification of Fitzgerald's medium with or without 2% agar (Fitzgerald et al. 1952, Zender and Gorham 1960). The cultures were allowed to grow for 14 days at 25°C and 5300 lux illumination with a 12 h alternating light-dark cycle. Cells were washed in distilled water, fixed in osmium with subsequent serial dehydration in ethanol and propylene oxide, followed by embedding in Epon (Luft 1961).

Some cultures were also treated in the following ways: 1) fixed in 3% glutaraldehyde (0.2 M cacodylate buffer, pH 6.2), followed by post fixation in osmium, 2) fixed in 3% aqueous KMnO₄, and 3) treated with 0.1 M or 0.001 M aqueous colchicine at 25°C for 3 h or 12 h followed by fixation in osmium.

Sections cut with diamond knives were stained with lead citrate (Reynolds 1963) or uranyl acetate (Stempak and Ward 1964) either alone or in combination and observed in a Hitachi HU-11E electron microscope operating at 75 KV.

Results

In *Anabaena* sp. (B-378), *N. zetterstedtie*, *N. ellipsosporum* (B-1623), *N. punctiforme* (1629), *Nostoc* sp. (380), and *Nostoc* sp. (387) the cultures contained chains of cells of various diameters and shapes (Fig. 1). The smaller cells were cuboidal in shape and about 3 μm in diameter. When grown on agar solidified medium more of the smaller cells were observed. Other cells varied from this diameter to 12.5 μm and were generally barrel-shaped (Fig. 1). Generally a filament was composed of cells of the same size and morphology although variation within filaments occurs (Fig. 1).

When the small cells are observed ultrastructurally they exhibit the usual inclusions observed in blue-green bacteria with the thylakoids arranged in several layers around the periphery of the cell (Figs. 2, 3, 5a). However, in addition to these inclusions, microtubule-like structures are also observed (Figs. 2–8). They are generally located in the central area of the cell although occasionally they are observed in a peripheral location (Fig. 7). The microtubule-like structures lie at various angles in the cell (Fig. 3) and generally occur in groups of up to 5 (Fig. 2–7), but also may exist singly (Fig. 8). They are of undetermined length but when viewed in longitudinal sections can be traced for up to 1 μm (Fig. 4). The structures are generally straight (Figs. 2–3, 4), but some appear to have gentle bends (Fig. 4). The diameter of the tubules varies from 9.5 to 22 nm, but generally a single tubule will be the same diameter along its observable length.

In cross section the different diameters of the tubules are easily observed (Figs. 5a–8). Among 50 randomly selected tubules the following diameters were observed: 6% were 15 nm, 40% were 14 nm, 38% were 13 nm, 12% were 11 nm, and 3% were 10 nm. The tubules may be very close together and they have a wall approximately 2.5 nm in thickness (Figs. 5a, 5b, 6, 7, 8). No substructure was observed in the wall. Dense bodies 7.2 nm in diameter with a slightly electron transparent core, are sometimes observed associated with the tubules (Fig. 6).
No effect on the tubules was seen in colchicine treated material and KMnO₄ did not preserve them.

In *N. ellipsosporum* (B-383) and *N. punctiforme* (1629) microtubule-like...
structures were found associated with the plasma membrane (Fig. 9–14). These tubules had a constant diameter of approximately 15 nm with an electron dense wall about 4 nm in thickness (Fig. 10–12). They occurred either singly or in groups of up to 4, surrounded by an electron transparent zone of about 4 nm. In longitudinal section these tubules were seen close to the plasma membrane, always oriented perpendicular to it (Figs. 13 and 14), and measured up to 0.2 μm in length. No substructure was observable in the wall of the tubules and they were not affected by colchicine or preserved by KMnO₄.

Fig. 9. Micrograph of *N. elliposporum* (B-383) showing several plasma membrane associated microtubule-like structures (PM). Note that the cell has been cut tangentially just inside the plasma membrane. ×48,006.
Figs. 10–12. 10, section of a cell of *N. ellipsosporum* (B-383) cut tangentially. Note the plasma membrane associated tubules (PM). The top one is near a cyanophycin granule (C). ×83,000. 11, high magnification of section of *N. ellipsosporum* (B-383) showing several plasma membrane associated microtubule-like inclusions (PM). Note the clear zone around the lower tubules. ×100,000. 12, high magnification of *N. ellipsosporum* (B-383) showing several groups of plasma membrane associated tubules (PM). Note the two in the lower portion of the micrograph are in apparent contact. ×100,100.
Discussion

The microtubule-like structures described here are not easily observed in thin sections. The variable diameter microtubule-like inclusions are seldom observed.
because they are only found in the small cuboidal cells which may make up only a small portion of a culture. They must also be sectioned in a near cross or longitudinal plane or they are not visible in the section. They are also present in different numbers in the cells. *Anabaena* sp. (B-378), *Nostoc* sp. (387), *Nostoc* sp. (389) and *N. punctiforme* (1629) contain many tubules per cell while only a few were observed in *N. ellipsosporum* (B-1623) and *N. zetterstedtie*. Thus a single examination of the culture will not necessarily reveal these structures. This will be discussed to a greater extent below.

The plasma membrane associated microtubule-like inclusions are even more difficult to observe. They are found only in actively growing cultures. Thus examination of older cultures or those growing slowly may not reveal their presence. Because of their length and location, they can only be observed in cross section, in tangential sections of the cells. The cytoplasm of the cells is also very dense and in areas where the tubules are located there are ribosomes, glycogen and lipid inclusions, which make observation difficult. It is especially easy to mistake a tubule sectioned longitudinally for a thylakoid cut in cross section. Thus we have seen these structures in large numbers in only two species but they may well be present in the other species and not observed because the cells examined were not growing rapidly or only a few of the tubules are present and thus may not be recognized.

The microtubules described here may be microtubules similar in function to eukaryotic microtubules but of a more primitive nature than eukaryotic microtubules. Their failure to break down when exposed to colchicine may be explained on this basis. In some eukaryotic lower plants colchicine also fails to break down all the cytoplasmic microtubules (Heath 1975, Schnepf and Deichgrachgraber 1976). Recently Margulis et al. (1978) have reported on the composition of microtubules in the spirochete, *Pillotina* sp., *Diplocalyx* sp., *Hollandina* sp. and an unidentified gliding bacteria from *Pterotermes occidentis*. The first three prokaryotes are symbiotic in the hindguts of dry-wood and subterranean termites. On the basis of electron microscopy, staining with fluorescent antibodies to tubulin and comigration with authentic tubulin on acrylamide gels they suggest these bacteria contain microtubules composed of tubulin. They also report variation in the diameter of the microtubules from 15 to 21 nm. Further work should be in this direction in regard to the microtubule-like inclusions described here.

Lazaroff (1973) has shown that in certain *Nostoc* species a developmental cycle occurs, one part of which consists of motile filaments composed of small cells. Other blue-greens have been shown to possess similar morphological variation (Evans et al. 1976). It is therefore possible that many other blue-green bacteria including species examined in this study may, under the right environmental conditions, produce small motile hormogonia and that these cells will also possess the variable diameter microtubule-like inclusions. It is interesting to note that a culture of *N. ellipsosporum* (B-1623) fixed and embedded in 1962 but not examined until the present study also has the tubules. Another possibility is that they could represent a virus or a mutant form of virus. There is no evidence,
however, of plaque formation when the algae are grown on solid media and the structures are found only in healthy actively growing cultures. It is also possible that they represent a cellular response to a viral infection. The production of tubules has been reported in potato-mop-top virus infected plants (Fraser 1976) and pinwheel structured inclusions have been reported in other virus infected plants (Edwardson 1966a, Edwardson 1966b, Kamei et al. 1969, Kim and Fulton 1969, Krass and Ford 1969, Harrison and Roberts 1971). The tubules could also be a pigment which has been produced in excess. Recently Bryant et al. (1976) have isolated phycobilins from an *Anabaena* sp. and examined them in negatively stained preparation. They observed 12 nm tubules with cross striations. However, no structures were observed in thin sections of the cells. Blue-green algae have often been compared to plastids and it has been suggested that this may have been the origin of chloroplasts in eukaryotic cells. Similar structures, to those described here, have been reported in chloroplasts. Some of these structures are tubular membranes (Ponzi and Pizzolongo 1973, Falk 1976) while others are reported as microtubules (Brandoa and Salema 1974). The non-preservation of the tubules reported in the present study by KMnO₄ strongly suggests that they are not membranous. In chromoplasts of *Trapaemaum majus* L. tubular structures 15 to 20 nm in diameter are observed which are shown, when analyzed, to consist of a core of carotenoids and their esters coated by a monolayer of acyl lipids and proteins (Winkenbach et al. 1976). It is possible that the structures reported here may be related to these reported plastid structures. The tubules reported here could be inclusions which have a specific function such as the gas vacuoles (Bowen and Jensen 1964). They would then be present only under conditions in which the inclusion would function.

This present study reports on two new kinds of microtubular structures in blue-green algal cells. Further work will be needed to establish how wide spread these inclusions are and what their function is.

### Summary

Ultrastructural observation of isolates of *Anabaena* and *Nostoc* revealed the presence of variable diameter microtubule-like inclusions in *Anabaena* sp. (B-378), *N. zetterstedtii*, *N. ellipsoïdum* (B-1623), *N. punctiforme* (1629), *Nostoc* sp. (389) and *Nostoc* sp. (387) and plasma membrane associated microtubule-like inclusions in *N. ellipsoïdum* (B-383) and *N. punctiforme* (1629).

Variable diameter microtubule-like inclusions were found mainly in groups primarily in the central cytoplasm. They varied in diameter from 9.5 to 22 nm and measured up to 1 μm in length. They were found only in the small cuboidal cells of the various cell sizes and shapes observed in the cultures.

Plasma membrane associated microtubule-like structures were always oriented with the long axis perpendicular to the plasma membrane, and measured 15 nm in diameter and up to 0.2 μm in length.

Neither kind of microtubule-like structure is depolymerized by colchicine or preserved by potassium permanganate fixation. The structures are compared
to similar structures in other cells.

References


