The Effect of Lysozyme on Cell Wall Morphology in a Blue-green Alga, *Cylindrospermum* sp.

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SUMMARY

Electron micrographs of thin sections of the wall of a *Cylindrospermum* sp. treated with lysozyme show that layer 2 (intermediate layer) of the wall is removed. Hence this layer is composed of mucopolymer. This observation, and the previous reports that this layer is thickened in mature spores of a *Cylindrospermum* sp. and in vegetative cells of *Oscillatoria princeps*, suggest that mucopolymer determines wall strength and maintains cell shape.

INTRODUCTION

Various terms have been used to describe wall structure in blue-green algae. Pankratz & Bowen (1963) describe the wall as three-layered while Jost (1965) considers it to be four-layered. In this paper the terminology of Jost (1965) will be used. Using this terminology the wall of blue-green algae, starting with the innermost layer and proceeding outward, consists of: an electron-transparent layer (1) immediately outside the plasma membrane; an electron-dense layer (2); an electron-transparent layer (3); and an outer electron-dense layer (4). Which of these layers contains the mucopolymer described in walls of blue-green algae (Frank, Lefort & Martin, 1962*a, b*; Drews & Meyer, 1964; Hock, Martin & Kandler, 1965) has not been conclusively demonstrated. Frank *et al.* (1962*b*) suggests that it is the electron-transparent layer (layer 1) while others (Allen, 1968; Lamont, 1969) suggest that it is layer 2. We therefore decided to use lysozyme, which is known specifically to remove the mucopolymer, to determine more precisely the location of this wall component. A number of investigators have described the effects of lysozyme on blue-green algae in producing spheroplasts (Fuhs, 1958; Crespi, Mandeville & Katz, 1962; Fulco, Karfunkel & Aaronson, 1967; Vance & Ward, 1969) but these studies were confined to light microscopic observations.

METHODS

Cylindrospermum sp. Indiana University Culture Collection no. LB 942 (Starr, 1964) was grown in Chu's medium 10 with soil extract (Chu, 1942). Cultures were maintained at 21° under 500 ft candles of illumination with a 12 h. alternating light and dark period.

Samples were fixed in 3 % glutaraldehyde in 0.1 M-phosphate buffer at pH 7.2 for 1 h. at 4° and then thoroughly rinsed in buffer. For lysozyme treatment the method of Murray, Steed & Elson (1965) was followed: the algae were suspended in 0.3 M-tris buffer at pH 8.0 containing 134 µg. EDTA/ml., 68 µg. lysozyme/ml. (Worthington, twice crystallized) and 10 % w/v sucrose. After 8 h., MgSO₄·7H₂O was added to 0.05 M; 1/2 vol. 40 % sucrose was then added. The samples, after rinsing in distilled water, were placed in Michaelis buffer

Fig. 1. Portion of the wall of untreated *Cyindrospermum* sp. showing the successive layers. Immediately outside the plasma membrane (PM) is layer 1. This is followed by the electron-dense layer 2, by the electron-transparent layer 3, and the outer layer 4 which shows its double-track membrane nature and characteristic undulations. Bar = 100 nm.

Fig. 2. Portion of the wall of *Cyindrospermum* sp. treated with lysozyme. Portions of layer 2 still remain. The outer layer is continuous over the missing segments. Bar = 100 nm.

Fig. 3. Portion of the wall treated with lysozyme. One portion of the layer 2 still remains. The outer layer is continuous. Bar = 100 nm.

at pH 6.1 overnight. The samples were then fixed in OsO_4 (1 % w/v) according to the method of Pankratz & Bowen (1963).

After fixation the samples were dehydrated rapidly in a graded ethanol series, treated with propylene oxide and embedded in Epon 812 (Luft, 1961). Sections cut with a diamond knife on an LKB Ultratome were stained with uranyl salts and examined with an electron microscope.

Fig. 4. Portion of the cell wall of *Cyindrospermum* sp. treated with lysozyme in which layer 2 is no longer visible. The outer layer and the plasma membrane (PM) are continuous. Bar = 100 nm.

Fig. 5. Portion of the cell wall of *Cyindrospermum* sp. treated with lysozyme showing the invagination of the outer layer through a missing segment in layer 2. Bar = 100 nm.

OBSERVATIONS

Fig. 1 shows the four layers of the wall of *Cyindrospermum* sp. The electron-transparent layer 1, about 3 nm. thick, surrounds the plasma membrane; in an outward direction it is followed by the electron-dense layer 2, about 10 nm. thick, another electron-transparent layer 3, about 3 nm. thick and the outer layer 4, consisting of a double-track membrane about 8 nm. thick and with characteristic undulations.

In material treated with lysozyme for 8 h. portions of layer 2 were missing in some cells (Fig. 2, 3), while in others the layer was completely absent (Fig. 4). The outer layer 4 was

continuous across these missing segments of layer 2. In sections of material in which most of layer 2 had been removed much distortion was observed.

Digestion by lysozyme appeared to start in one area with the formation of a small 'hole' through layer 2. Fig. 5 shows the outer layer 4 drawn into an area from which layer 2 had been removed. This movement of the outer layer into the cell was probably caused by some plasmolysis when the sample was placed in the last sucrose solution.

DISCUSSION

Murray *et al.* (1965) have shown that lysozyme digestion removes the layer 2 (intermediate) from walls of Gram-negative bacteria. Our results show that this layer is also removed from the walls of a blue-green alga, *Cylindrospermum* sp., exposed to lysozyme. Thus in Gram-negative bacteria and in blue-green algae the mucopolymer seems to be concentrated in layer 2.

Murray *et al.* (1965) have suggested that this layer determines the strength of the wall and thus aids in maintaining the shape of the organism. The previous report by Jensen & Clark (1969), that in mature spores of a *Cylindrospermum* sp. layer 2 is thickened, supports this concept. The vegetative cells in this organism are approximately 4 to 5 μm . in diameter by 4 to 7 μm . long while the spore is approximately 8 to 10 μm . wide by 14 to 18 μm . long. With an increase in cell size one would expect an increase in the material which aids in maintaining its shape. This concept is also supported by the fact that in the blue-green alga *Oscillatoria princeps*, whose cell diameter is about 40 μm ., this layer is also thicker. Layer 2 is up to 0.4 μm . thick compared to the average thickness of about 10 nm. reported for other vegetative cells of the blue-green algae (Jensen, 1965). It therefore appears that in this case also the necessity of increased strength in the wall to maintain the shape of the larger cell is solved by increasing the thickness of the mucopolymer layer.

These observations provide evidence for our proposal that layer 2 is composed of mucopolymer. The composition of the other layers found in blue-green algal cell walls must await further analysis.

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