THE ACTION OF LYSOZYME ON SEVERAL BLUE-GREEN ALGAE*

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The extraction of biliproteins from some varieties of blue-green algae is greatly facilitated by treating the algae with lysozyme (Berns, Crespi and Katz, 1962). Fuhs (1958) previously had found lysozyme treatment to cause extensive disintegration of the filaments of Oscillatoria amoena, and envisaged the use of lysozyme to prepare algal protoplasts. In view of our work and Fuhs (1958) we have made systematic studies with lysozyme and have been successful in producing algal protoplasts.

Algal cultures.--Algae were grown autotrophically (5% CO_2 in nitrogen) in 125 and 250 ml culture flasks containing respectively 50 and 125 ml of blue-green algal medium (Table II, Crespi, Conrad, Uphaus and Katz, 1960). The flasks were illuminated at 300 - 400 foot-candles by overhead fluorescent lights with continuous agitation on an Eberbach rotatory shaker. The cultures, unialgal but not sterile, were generally grown at 25-28°C, but Phormidium luridum was grown at 37°C and Synechococcus lividus at 46-48°C. Fremyella diplosiphon, Plectonema calothricoides, Phormidium luridum, Oscillatoria tenuis, and Oscillatoria formosa were obtained from the Indiana University Culture Collection (Starr, 1960); S. lividus was obtained through the courtesy of

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Dr. D. L. Dyer (Dyer and Gafford, 1961); and cultures of Cyanidium caldarium were kindly made available to us by Dr. L. Bogorad.

Lysis.--Algae were collected from actively growing cultures by centrifugation for 5 minutes at the full speed of an International clinical centrifuge. The cells were washed twice with buffer, and suspended at approximately 2% (volume of packed cells/volume of buffer) in the desired medium. Lysozyme (Worthington, twice recrystallized, ex egg white) was added by micropipette. The tubes were stored in dim light and examined periodically under a phase contrast microscope and by photomicrography. Controls without lysozyme were set up in identical media.

Fremyella diplosiphon (Smith, 1950) a filamentous blue-green alga with a well-defined sheath was chosen for detailed investigation. The optimum conditions of buffer and lysozyme concentration, and pH for lysis were: 0.03M sodium phosphate buffer, pH 6.8-6.9, and 0.05% lysozyme. Extensive lysis occurred in 4-6 hours at 25-28°C; within 24 hours the filaments were almost completely lysed with liberation of the biliproteins. Filaments without lysozyme in the buffer at 24 hours showed no visible changes and the supernatant solutions remained colorless. The lysis appeared to follow a more or less well-defined sequence indicated schematically in Figure 1. The sheath is frequently destroyed completely, and green cells that have lost their phycocyanin and phycoerythrin are liberated from the filaments. As the sheaths dissolve, the biliproteins are lost and the constraints on the individual cells are released, so that they markedly increase in volume. Long chains of cells, with the appearance of a chain of beads, often as many as 50 in a row, that appeared to have no sheath remaining were common in a 6 hour preparation. These chains eventually broke into smaller chains and then into single green cells.

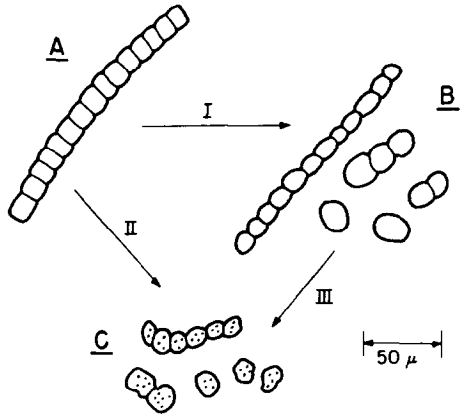

FIGURE 1. The action of lysozyme on Fremyella diplosiphon. A, before treatment; B, blue-gray "protoplasts"; C, green, irregular terminal cells. I, with lysozyme in 0.3M sucrose-Ficoll; II, with lysozyme in 0.03M phosphate buffer; III, dilution to reduce osmotic pressure or vigorous shaking.

The terminal product, under these conditions, is a roughly circular, irregular, warty, green fragment. Under fluorescence microscopy the black spots fluoresce strongly, suggesting localization of chlorophyll in the black dots. These objects appear green because of the loss of biliproteins, and are very stable entities. Experiments with C^{14} indicated that these cells do not take up CO_2, and do not appear to have retained their ability to perform photosynthesis.

The effect of lysozyme on P. calothricoides, O. tenuis and P. luridum was similar to that on F. diplosiphon. The cell membranes in these algae are ruptured more readily by osmotic forces than is the case for F. diplosiphon. While O. tenuis is lysed by 0.05% lysozyme in six hours, O. formosa, in agreement with the observations of Fuhs (1958) on O. amoena, is attacked to only a slight extent in 24 hours. The rapid disintegration of

O. tenuis by lysozyme, in contrast to the resistance of other Oscillatoria sp., raises the possibility that O. tenuis may in fact be a Phormidium sp. and suggests the utilization of lysozyme sensitivity as a basis for taxonomic classification. Synechococcus lividus and Cyanidium caldarium show very little attack by lysozyme. Our results indicate that organisms with distinct mucilaginous sheaths are most easily attacked by lysozyme. Thus, Phormidium sp. which possess sheaths, are readily attacked, whereas Oscillatoria, which lack pronounced sheaths, are relatively resistant. Frank, Leport and Martin (1962) have shown that Phormidium uncinatum cell walls contain the usual substrates for lysozyme action and have muramic acid and muco-polysaccharides as important components. We would infer that distinctly sheathed algae contain relatively greater amounts of these constituents in the cell-walls.

Protoplasts.--When the osmotic forces are balanced, protoplast structures can be prepared in good yield. When F. diplosiphon is treated with 0.005% lysozyme at 35°C in a 0.03M sodium phosphate buffer, pH 6.8, in the presence of 0.3M sucrose and 3% Ficoll (a high-molecular weight polysaccharide, Pharmacia), a 40 to 60% yield of large, blue-gray spherical cells is obtained. These "protoplasts" are very sensitive and easily ruptured. In the formation of these bodies, none of the biliproteins is lost. If this preparation is diluted with buffer, so that the osmotic pressure is lowered, then the protoplast cells immediately swell and burst; the green entities described above are again produced, and phycocyanin and phycocrythrin are evident in the supernatant solution. We have observed that a sucrose-Ficoll mixture inhibits photosynthesis very strongly in ordinary blue-green algae. We have therefore as yet been unable to determine whether the protoplasts are still capable of photosynthetic fixation of $C^{14}O_2$.

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