Production of Tubules and Bacteriophage-like Particles in Mycobacteria After Bacitracin Treatment

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Received for publication 27 February 1969

Bacitracin-treated mycobacteria liberated tubules and phagelike particles which had no biological activity against selected species. These structures may reflect a state of defective lysogeny.

We have reported that bacitracin causes the disintegration of membrane systems of mycobacteria leading to lysis (3). We now report the isolation, biological activities and morphological characteristics of the bacitracin-induced particles in mycobacteria.

*Mycobacterium tuberculosis* BCG (Instituto Nacional de Tuberculosis, Caracas) and *Mycobacterium* sp. ATCC 607, both of which had never been exposed to any mycobacteriophages in our laboratory, were incubated in a Penassay Broth (Difco) containing 0.04% Tween 80 at 37°C for 18 hr with constant shaking. Bacitracin (Sigma Chemical Co., St. Louis, Mo.), 5 units/ml, was added, and the incubation was continued for 24 hr.

For ultrathin sectioning, cells were centrifuged at 3,000 × g for 10 min and fixed by a modification of the method of Stoeckenius and Rowen (4).

To isolate the bacitracin-induced particles, unbroken cells and large-cell debris were discarded after centrifugation at 3,000 × g for 15 min. The supernatant fluid was further centrifuged at 40,000 × g for 2 hr at 4°C. The pellets were suspended in 0.1 M ammonium acetate and sterilized by membrane filtration (0.45-μm pore size; Millipore Corp., Bedford, Mass.).

The filtered suspension was spotted on Penassay agar plates previously overlaid with exponentially growing indicator mycobacteria, such as *M. bacterium* sp. ATCC 607, *M. tuberculosis* H37Ra and BCG, *M. smegmatis* ATCC 14468, *M. phlei* ATCC 11758, *M. butyricum* ATCC 357, *M. fortuitum* ATCC 6841, and *M. kansasi* ATCC 12478. The same suspension was also examined with an electron microscope by negative staining.

Ultrathin sections of bacitracin-treated cells of both *Mycobacterium* sp. ATCC 607 and BCG showed membranous configurations distributed throughout the cytoplasm (Fig. 1). Negative staining revealed that they were cylindrical tubules, of various lengths, measuring 10 to 17 nm in outer diameter and 3.5 to 4 nm in the inner (Fig. 3 and 4). Occasionally they showed loop ends (Fig. 4). These tubular objects were no longer observed after Pronase treatment (1 mg/ml), suggesting their protein nature.

A roughly hexagonal electron-dense substance enclosed by a membrane was also observed in the cytoplasm (Fig. 2). Figure 5 shows tadpole-shaped particles, their heads measuring 53 nm in width and their tails 180 nm in length and 8 nm in diameter. Morphology of these particles resembles that of mycobacteriophages.

The spot test of the suspensions containing both tubules and phagelike particles showed negative results; i.e., no killing activities were found against nine species of both pathogenic and non-pathogenic mycobacteria. We assume that these bacitracin-induced particles, especially those phagelike ones, represent defective phages.

Rhapsodosomes observed in normal *Proteus* (6, 7) and *Saprospira* (1, 7) and in microtubules in *Proteus* spheroplasts (2) have a morphology almost identical to the tubules in mycobacteria, although the diameter of the cylindrical configuration is different. In addition, the tubules were not found in either cell walls or cytoplasmis of mycobacteria during any stages of growth (7; Imaeda et al., J. Med. Microbiol., in press) nor in phage-infected cells (5). Whereas rhapsodosomes of gram-negative bacteria are believed to reflect the normal bacterial function (7), our evidence suggests that the tubules in mycobacteria may occur as a result of metabolic disturbances caused by bacitracin. However, the occurrence of phagelike particles induced by bacitracin leads us to consider the possibility that the tubules may be
another type of defective phage, the assumption in the case of the defective lysogen of Proteus (2).

This investigation was supported by Public Health Service research grant AI-07888 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

FIG. 3. Negatively stained (2% sodium silicotungstate at pH 7.6) tubules isolated from bacitracin-treated Mycobacterium sp. ATCC 607. Tubules are various in length and diameter. The marker bar represents 100 nm.

FIG. 4. Negatively stained tubules of bacitracin-treated M. tuberculosis BCG. One of them shows the loop end (arrow). The marker bar represents 100 nm.

FIG. 5. Phagelike object induced by bacitracin in Mycobacterium sp. ATCC 607. The marker bar represents 50 nm.

FIG. 6. Phage-tail-like object, showing helical configuration, isolated from Mycobacterium sp. ATCC 607 after bacitracin treatment. The marker bar represents 50 nm.