Ultrastructural Changes in Mollicutes Induced by the Peptide Antibiotic Herbicolin A

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Electron microscopy of negatively stained mycoplasma, ureaplasma, and acholeplasma cells showed ultrastructural changes after 10 min of treatment of the organisms with the peptide antibiotic herbicolin A in concentrations ranging from 10 μg/ml for Mycoplasma capricolum to 600 μg/ml for Ureaplasma urealyticum. The morphological changes were shown to be reversible at low concentrations of the antibiotic but irreversible at high concentrations.

The peptide antibiotic herbicolin A isolated from Erwinia herbicola is known to inhibit the growth of various mollicutes (2). Herbicolin A has been chemically characterized (8) as being a hydrophobic acylated cyclic peptide containing the D and L forms of amino acids and β-hydroxymyristic acid. It was previously shown that the antibiotic acts as a mycoplasmacidal agent rather than as a mycoplasmatastatic agent on Mycoplasma and Ureaplasma species (1).

In the present study we investigated by electron microscopy (EM) the structural changes induced in strains of Mycoplasma, Ureaplasma, and Acholeplasma species by herbicolin A. Marked changes were demonstrated in Mycoplasma capricolum, Ureaplasma urealyticum, and Acholeplasma laidlawii, but not in Mycoplasma gallinarum even at high concentrations of herbicolin A.

M. capricolum California Kid (Fig. 1A through D) showed structural changes faster and at lower concentrations of herbicolin A than did any of the other mollicutes tested. Immediately (i.e., 10 s) after herbicolin A was mixed in a final concentration of 10 μg/ml with a log-phase liquid culture of M. capricolum, some of the mycoplasma cells started to develop filamentous extrusions with a diameter of about 100 nm and a length of up to 2 μm. After incubation at 37°C for 3 min, all cells showed extrusions, some of which apparently connected two or more cell bodies (Fig. 1B). After 1 h of incubation these structures became very straight and apparently rigid with a uniform diameter of 50 nm (Fig. 1C). No internal structure could be seen in the extrusions. The addition of 25 μg of herbicolin A per ml for 10 min at 37°C resulted in the development of similar structures. On longer incubation the filamentous structures predominated and became thinner, and the round cells appeared to be more structureless. At higher concentrations of herbicolin A, the structural changes became much more pronounced, and at 800 μg/ml a total disruption of the cell bodies was seen. The structural changes of M. capricolum is reversible at low concentrations of herbicolin A. Thus, cell suspensions incubated for 30 min at 37°C with 10 μg of the antibiotic per ml, followed by a 1:10 dilution with medium at 37°C for 30 min, showed restoration of the original morphology (Fig. 1D).

Purified membranes of the filamentous structures (Fig. 1D) of M. capricolum prepared by osmotic shock are shown in Fig. 1E. When M. capricolum membranes were incubated with herbicolin A (100 μg/ml), no filamentous structures were seen. Instead, the membranes seemed to fold and disintegrate (Fig. 1F), resembling more or less the membrane of whole cells exposed to high concentrations of the antibiotic.

Production of structural changes in U. urealyticum T-960 serovar VIII required much higher concentrations of herbicolin A (Fig. 1G through I). Normal cells are seen in Fig. 1G. At a concentration of 300 μg of herbicolin A per ml, the cells were still round with an almost normal appearance. Drastic structural changes appeared after incubation with 600 μg/ml for 10 min (Fig. 1H). Bundles of very thin as well as thicker rods were seen. The cells appeared to be more rigid than those resulting from treatment of M. capricolum. The thicker rods may consist of bundles of thin fibers. After exposure to 1,200 μg of herbicolin A per ml for 21 h (Fig. 1I), long rigid rods (20 to 50 nm in diameter and several micrometers in length) were observed. No internal structure of the rods could be detected.

Acholeplasmas do not require sterol for growth, but when supplied in the medium low amounts of free cholesterol were incorporated in the cell membrane of the organisms (5). In our experiments A. laidlawii was more pleomorphic in growth media with 18% horse serum (Fig. 1J) than in serum-free medium. However, whereas the effect of herbicolin A on the ultrastructure of acholeplasmas when grown in serum-free medium was similar to that observed with mycoplasmas and ureaplasmias (Fig. 1L), the addition of serum seemed to prevent the formation of filamentous and rod-shaped structures in A. laidlawii, although the cells got a disintegrated and swollen appearance (Fig. 1K).

M. gallinarum PG16 differed markedly from the other strains examined in this study in that no structural changes were induced by herbicolin A even at a concentration of 1,200 μg/ml for 21 h at 37°C. EM of uninoculated medium-herbicolin controls revealed no formed elements.

The selection of strains subjected to EM following exposure to herbicolin A was guided by the different susceptibility to the antibiotic shown by different species of the mollicutes (1). Thus, in that study the final MIC of herbicolin A was found to be 50 μg/ml for M. capricolum California Kid and 6.25 μg/ml for U. urealyticum T-960. Moreover, herbicolin A was shown to have a cidal effect on both organisms, with cell death being dependent on the antibiotic concentration as well as on the incubation time. U. urealyticum was killed at a low concentration (12.5 μg/ml) after 24 h, but the number of viable cells remained stationary during incubation for 2 h at concentrations of up to 100 μg/ml (1). M. gallinarum PG16 differed from a number of other

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Mycoplasma species tested in that it exhibited a remarkably low susceptibility to the antibiotic, with the MIC determined by Freundt and Winkelmann (2) being 50 to 100 μg/ml. The MIC of herbicolin A against A. laidlawii in liquid medium has not been determined, but in a plate diffusion test on media containing 10 to 18% horse serum, the organism was not inhibited by 100 μg per disk, nor were the type strains of five other Acholeplasma species that were tested. However, on media devoid of cholesterol, A. laidlawii PG8 was moderately inhibited by the antibiotic at 100 μg/ml (2).

The variability of the structural changes induced in different species of the mollicutes when exposed for various periods of time to various concentrations of herbicolin A is generally in fair accordance with the observations of the growth-inhibiting and mycoplasmaloidal effect of the antibiotic summarized above, although this is not invariably so. It is surprising, for example, to find that drastic structural changes develop in M. capricolum in the presence of low concentrations (10 to 25 μg/ml) of herbicolin A, although much higher concentrations are required to induce similar changes in U. urealyticum. The absence of any structural changes in M. gallinarum during prolonged incubation at very high concentrations of herbicolin A is in agreement with the relatively high degree of resistance of this organism (2). A. laidlawii seems to be partially protected by the addition of serum (and thereby cholesterol and other lipids) to the growth medium, as concurrently indicated by growth inhibition tests and observations by EM.

At present we have no explanation for the nature of the aberrant structures induced by herbicolin A. Although the curved filamentous structures seen at low concentrations of the antibiotic apparently do not differ essentially from the filaments seen in several mycoplasmas under normal conditions, the straight and apparently rigid rods that develop in all strains tested, except for M. gallinarum, at higher concentrations do not seem to have their exact counterparts in any other mycoplasmal structures described in the literature. They do have a superficial resemblance to the rho forms that have been demonstrated especially frequently in freshly isolated strains of M. mycoides subsp. mycoides and at least occasionally in M. capricolum California goat (4, 6). However, the transversal banding that is very typical for the rho forms could not be demonstrated in the rodlike structures induced by herbicolin A.

The biochemical basis for the structural changes induced by herbicolin A is unexplained at present. However, because the reaction is concentration dependent and occurs almost instantly at high concentrations, it seems reasonable to propose that herbicolin A reacts with components of the cell membrane, resulting in a change of their configuration. Because herbicolin A is hydrophobic, it may very well interfere with the lipid-protein interaction in the membrane. Mollicutes reveal a wide diversity in the lipids present in the cell membrane (3), which might explain the varying susceptibility of different species to the antibiotic.

To the best of our knowledge, very few other studies by EM of antibiotic-produced ultrastructural changes in mollicutes have been published. Polyene filipin has been shown by freeze-etch EM to alter the structure of the membrane of A. laidlawii, whereas amphotericin B produced no visible structural changes (7). Preliminary studies of A. laidlawii by the EM freeze-etch technique did show similar rodlike structures when incubated with herbicolin A, an observation which we hope may contribute to the elucidation of the nature of the ultrastructural changes induced in susceptible mycoplasmas.

LITERATURE CITED