The Mycoside Capsule of *Mycobacterium avium* 357

By P. DRAPER

National Institute for Medical Research, Mill Hill, London NW7 1AA

(Received 25 January 1974)

INTRODUCTION

Draper & Rees (1973) have shown that *Mycobacterium lepraemurium*, an obligate intracellular parasite of small rodents, surrounds itself, inside the phagocytic cells of the infected host, with a capsule of peptidoglycolipid having a fibrillar appearance in the electron microscope and chemically resembling the main mycoside of *M. avium* (Voiland, Bruneteau & Michel, 1971). It seemed of interest to discover whether the latter substance had a similar ultrastructure and location.

METHODS AND RESULTS

Mycobacterium avium 357 (kindly supplied by Dr J. Stanford) was grown in Sauton's (1912) medium, unshaken, for 7 weeks at 37 °C. Cultures were sterilized with gamma radiation (2.5 Mrad from ⁶⁰Co) before handling.

Bacteria were harvested by centrifuging at 10000 *g* for 10 min; the supernatant medium contained no fibrillar material. The bacteria were homogenized in 0.3 M-sucrose, buffered with 2.5 mM-potassium EDTA to pH 7.2 (conditions used by Draper, 1971, for mouse livers infected by *M. lepraemurium*), centrifuged at 10000 *g* for 10 min to collect bacteria and then at 30000 *g* for 1 h. The high-speed sediment was suspended in water for electron microscopy, then freeze-dried, dissolved in chloroform, mixed with an equal volume of methanol, centrifuged (to remove a small residue of bacteria), evaporated and weighed.

Homogenizing removed 2.4 mg from 385 mg of cells (0.62 %) of material soluble in chloroform (0.66 % in a second experiment). Negatively stained suspensions had a fibrillar structure, with individual fibrils arranged in parallel bands (Fig. 1*a*). The fibrils had a diameter of 6.8 nm. Even after homogenizing, the bacteria possessed a capsule of similar material (Fig. 1*b*).

Thin-layer chromatography (technique of Draper & Rees, 1973) showed the presence of one major and a number of minor glycolipids. The range of mobilities was similar to that of the glycolipids from the capsule of *M. lepraemurium*, but no individual spot had the same mobility as a component of *M. lepraemurium*.

The fibrillar material from *M. avium* contained 6-deoxyhexose, equimolar amounts of alanine, threonine and alaninol, and smaller amounts of phenylalanine, leucine and valine (analyses by the methods of Draper & Rees, 1973).

Homogenized bacteria were freeze-dried, weighed and extracted with ethanol-ether (1:1 by vol.) for 1 week (4 changes). The evaporated lipid extracts were treated with ether, and the ether-insoluble portion (authentic mycoside; Voiland *et al.* 1971) was collected and weighed; 21.5 mg was obtained from 386 mg of bacteria (5.6 %), and consisted of the same substances in the same relative proportions as the fibrillar material removed mechanically, judged by thin-layer chromatography.

Fig. 1. (a) Electron micrograph of fibrillar material (mycoside) removed from *Mycobacterium avium* by homogenization. Negatively stained with sodium silicotungstate. (b) Electron micrograph of cells of *Mycobacterium avium* showing fibrillar material still adhering after homogenization. Negatively stained with uranyl acetate.

DISCUSSION

The chemistry of the major mycoside of *M. avium* has been thoroughly studied (Jollès, Bigler, Gendre & Lederer, 1961; MacLennan, 1962; Chaput, Michel & Lederer, 1963; Vilkas, 1966; Bruneteau, Michel & Guilluy, 1968; Voiland *et al.* 1971). Our results showed

that material with the same chromatographic properties as authentic mycoside, and containing the expected sugars and amino acids, could be removed from the bacteria by homogenizing them under conditions that are known to remove the mycoside from cells of *M. lepraemurium*, in spite of the difference in culture methods. Such homogenization removed only about 10 % of the mycoside of *M. avium*. No particulate glycolipid occurred in the medium in which *M. avium* had been grown, but fibrillar material could be seen adhering to the cells after homogenizing. Evidently the mycoside capsule of *M. avium* was more firmly attached than that of *M. lepraemurium* (which may perhaps have been loosened by the action of the host cell containing the bacteria).

Morphologically the mycosides of *Mycobacterium avium* and *M. lepraemurium* were similar. The diameters of the individual fibrils, 6·8 and 7 nm respectively, were probably identical within the uncertainties of measurements from electron micrographs.

Mycobacterium avium and *M. lepraemurium* are severe pathogens in their respective hosts and the 'natural' environment of both species is the phagolysosome of the host cell (Hart, Armstrong, Bron & Draper, 1972; Draper, unpublished results). There is immunological evidence, both from serology (Kwapinsky & Kwapinsky, 1973; Stanford, 1973) and immunization experiments (I. N. Brown, unpublished results) that the species are closely related. Apparently they protect themselves from the environment with similar layers of mycoside.

I am grateful to Dr R. J. W. Rees for arranging the supply of *M. avium*.

REFERENCES

- BRUNETEAU, M., MICHEL, G. & GUILLUY, R. (1968). Isolement et identification du di-O-méthyl-3,4 L-rhamnoside d'alaninol à partir du mycoside C₂. *Comptes rendus hebdomadaires des séances de l'Académie des sciences C* **267**, 745-748.
- CHAPUT, M., MICHEL, G. & LEDERER, E. (1963). Structure du mycoside C₂ de *Mycobacterium avium*. *Biochimica et biophysica acta* **78**, 329-341.
- DRAPER, P. (1971). The walls of *Mycobacterium lepraemurium*: chemistry and ultrastructure. *Journal of General Microbiology* **69**, 313-324.
- DRAPER, P. & REES, R. J. W. (1973). The nature of the electron-transparent zone that surrounds *Mycobacterium lepraemurium* inside host cells. *Journal of General Microbiology* **77**, 79-87.
- HART, P. D'A., ARMSTRONG, J. A., BROWN, C. A. & DRAPER, P. (1972). Ultrastructural study of the behaviour of macrophages towards parasitic mycobacteria. *Infection and Immunity* **5**, 803-807.
- JOLLÈS, P., BIGLER, F., GENDRE, T. & LEDERER, E. (1961). Sur la structure chimique du 'mycoside C', peptido-glycolipide de *Mycobacterium avium*. *Bulletin de la Société de chimie biologique* **43**, 177-192.
- KWAPINSKY, J. B. G. & KWAPINSKY, E. H. (1973). Immunological reactions of *Mycobacterium leprae* and *Mycobacterium lepraemurium* grown in cayman. *Canadian Journal of Microbiology* **19**, 764-766.
- MACLENNAN, A. P. (1962). The monosaccharide units in specific glycolipids of *Mycobacterium avium*. *Biochemical Journal* **82**, 394-400.
- SAUTON, B. (1912). Sur la nutrition minérale du bacille tuberculeux. *Comptes rendus hebdomadaires des séances de l'Académie des sciences C* **155**, 860-861.
- STANFORD, J. S. (1973). An immunodiffusion analysis of *Mycobacterium lepraemurium* Marchoux and Sorel. *Journal of Medical Microbiology* **6**, 435-439.
- VILKAS, E. (1966). Analogie des structures des mycosides C₂ de *Mycobacterium avium* et C₁ de *Mycobacterium butyricum*. *Comptes rendus hebdomadaires des séances de l'Académie des sciences C* **262**, 786-788.
- VOILAND, A., BRUNETEAU, M. & MICHEL, G. (1971). Étude du mycoside C₂ de *Mycobacterium avium*. Détermination de la structure. *European Journal of Biochemistry* **21**, 285-291.