Mycoside G, a Specific Glycolipid in *Mycobacterium marinum* (Balnei)

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Received for publication 13 March 1965

Abstract

Navalkar, R. G. (University of Wisconsin, Madison), E. Wiegashaus, E. Kondo, H. K. Kim, and D. W. Smith. Mycoside G, a specific glycolipid in *Mycobacterium marinum* (Balnei). J. Bacteriol. 90:262-265. 1965.—A new specific glycolipid in extracts prepared from strains designated *Mycobacterium marinum* and *M. balnei* has been demonstrated by use of the techniques of column chromatography and infrared spectroscopy. Since there is now agreement among many workers that *M. marinum* and *M. balnei* are identical, the demonstration of the same specific glycolipid in both species is not surprising. This substance, which we have designated mycoside G, is chemically similar to mycosides A and B, and apparently differs only in the sugar moiety. In addition, the lipids extracted from these cultures contain phthiocerol dimycoceirosate, a wax component found also in *M. tuberculosis* and *M. bovis.

Previous publications from this laboratory (for references to earlier papers, see Randall and Smith, 1964) described a series of glycolipid and glycolipid-peptide compounds, called mycosides, unique in their distribution among various species of mycobacteria. These studies have been extended to a consideration of cultures of *Mycobacterium marinum* and *M. balnei*, and the purpose of this paper is to report the recognition of a new mycoside in lipids extracted from these cultures.

Materials and Methods

Cultures of *M. balnei* strains 688, 695, 1163, 2311, 2313, 2314, and 2237 were obtained from E. Runyon, Veterans Administration Hospital, Salt Lake City, Utah. A culture of *M. balnei* from a patient (J. M.) was obtained from W. Schaefer, National Jewish Hospital, Denver, Colo. A culture of *M. marinum* 611 was obtained from M. Magnusson, Statens Serum Institut, Copenhagen, Denmark, and cultures of *M. marinum* strains 687 and 2558 (ATCC 927) were obtained from E. Runyon.

The procedures used in this study are essentially those described earlier (Fregnan, Smith, and Randall, 1961), with the following modifications. All cultures were incubated at 31°C, and mass cultures grown under static conditions were prepared on Sauton medium. The distribution of compounds in the ethyl alcohol-ether extracted lipids was determined by examining infrared spectra of the fractions eluted from chromatographic columns.

Results

Colony studies. As in previous studies, all cultures were plated on Dubos oleic acid albumin agar (OAA; Difco) before mass culture to determine the homogeneity of the inoculum. Preliminary to harvest of each mass culture, colony studies were repeated to establish the fact that shifts in the bacterial population had not taken place. The colony form seen most commonly in cultures of *M. marinum* and *M. balnei* is shown in Fig. 1. Growth was first evident on OAA plates in 1 week, and the colonies were fully developed in 2 to 3 weeks.

Distribution of specific lipids. Examination of the infrared spectra of the ethyl alcohol-ether extracted lipids, after separation by column chromatography, revealed the presence of two specific lipid compounds, phthiocerol dimycoceirosate (DIM) and a glycolipid to be called mycoside G. DIM is also found in the ethyl alcohol-ether extracted lipids of *M. tuberculosis* and *M. bovis*, whereas mycoside G has been demonstrated only in the lipids extracted from cultures of *M. marinum* and *M. balnei*.

Mycoside G. Infrared spectra shown in Fig. 2 demonstrate the close relationship of mycoside G to mycoside A (*M. kansasii*) and mycoside B (*M. bovis*). The infrared spectra of the three mycosides are similar in the region from 2 to 8 μ, whereas the characteristic infrared absorption

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bands that permit a differentiation of these three compounds are in the region from 9.5 to 12 μ.
Two characteristic bands are indicated by dotted lines drawn on each spectrum: in mycoside A at 10.27 and 10.35 μ, in mycoside B at 9.72 and 9.95 μ, and in mycoside G at 9.79 and 10.12 μ.

Purification and hydrolysis of mycoside G. Pooled eluates of Florisil columns [usually benzene-ether (50:50, v/v) and ether-methanol

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Fig. 1. Photograph of colony of Mycobacterium balnei strain 1168 on oleic acid albumin agar. Culture 3 weeks old. X24.

Fig. 2. Infrared spectra of mycosides A, B, and G.

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Fig. 3. Flow diagram of the steps in the purification of mycoside G.
Thin-layer chromatography of the pools from Florisil columns showed traces of components not containing sugar when the chromatograms were sprayed with anthrone-sulfuric acid and heated.

The pool of mycoside G was extracted as a solid (330 mg) three times with 10 ml of hot methanol. The methanol-soluble fraction was discarded, and the remaining lipid was dissolved in 40 ml of a mixture of methanol-diethyl ether (1:1, v/v) and allowed to stand at 5 °C overnight. A precipitate that formed was filtered off and discarded, and the solvent was evaporated. To decrease the possibility of free fatty acid contaminants, the lipid was dissolved in heptane and partitioned with an equal volume of methanol containing a few drops of phenolphthalein. Methanol potassium hydroxide was added until the methanol layer remained a slightly pink color. The layers were quickly separated, the methanol layer was discarded, and the heptane was evaporated. The material in the heptane layer was shown to be homogeneous by thin-layer chromatography and was identified as mycoside G by its infrared spectrum (yield, 269 mg).

The sugar component liberated by acid hydrolysis of mycoside G was shown by thin-layer chromatography to be different from the sugars of mycosides A and B. Alkaline hydrolysis of the acid-hydrolyzed mycoside G liberates several components, predominantly mycocerosic acid and an alcohol spectroscopically similar to the alcohol liberated from mycoside B under similar conditions.

DIM. The presence of DIM in column eluates was recognized by comparison of the infrared spectra of the eluates with the spectrum of a standard sample of DIM obtained from M. tuberculosis.

Discussion

Examination, by means of column chromatography and infrared spectroscopy, of the lipids extracted from cultures of M. marinum and M. balnei has revealed the presence of a new glycolipid compound called mycoside G. The glycolipid nature of the substance and its restricted distribution in lipids of mycobacteria (limited to M. marinum and M. balnei) is sufficient evidence to permit the use of the term mycoside (Smith et al., 1960a). Previously reported mycosides are: mycoside B, present in M. bovis (formerly called Gb; Smith, Harrell, and Randall, 1954); mycoside A, present in M. kansasii (formerly Ga; Smith et al., 1957); mycoside Cm (formerly JAV), present in M. avium and group III unclassified mycobacteria; mycoside D (formerly JAT), present in group II unclassified mycobacteria (Smith et al., 1960b); and mycoside F, present in M. fortuitum (Fregnan, Smith, and Randall, 1961).

Clark and Shepard (1963) called attention to the confusion in the nomenclature of M. marinum, isolated by Aronson (1926), and M. balnei, described by Linell and Norden (1954). Clark and Shepard (1963) reviewed earlier work which suggests that the two species are similar in their growth pattern in HeLa cells, immunoelectrophoretic patterns, infectivity for mice, and optimal temperature for growth. Similar observations have been reported by Juhlin (1960) and Bönike (1961) with amidase tests and by Lind and Norlin (1963) by use of immunodiffusion techniques. Clark and Shepard concluded that there appears to be no basis for separating M. marinum and M. balnei as two distinct species. The occurrence of mycoside G in both types of cultures lends additional support to this proposal. The value of lipid distribution studies in the classification of mycobacteria is further demonstrated by these studies.

Acknowledgment

This work was supported by Public Health Service research grant AI-00646 from the National Institute of Allergy and Infectious Diseases.

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