BIOCHEMISTRY
including biophysical chemistry & molecular biology
Rapid Report

Biochemistry **2009,** *48,* 3819–3821 3819
DOI:10.1021/bi9005418

Cholesterol Metabolism Increases the Metabolic Pool of Propionate in *Mycobacterium tuberculosis*[†]

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Received March 30, 2009; Revised Manuscript Received April 10, 2009

ABSTRACT: *Mycobacterium tuberculosis* can metabolize cholesterol to both acetate and propionate. The mass of isolated phthiocerol dimycoserate, a methyl-branched fatty acylated polyketide, was used as a reporter for intracellular propionate metabolic flux. When *M. tuberculosis* is grown using cholesterol as the only source of carbon, a 42 amu increase in average phthiocerol dimycoserate molecular weight is observed, consistent with the cellular pool of propionate and, thus, methylmalonyl CoA increasing upon cholesterol metabolism. In contrast, no shift in phthiocerol dimycoserate molecular weight is observed upon supplementation of medium containing glycerol and glucose with cholesterol. We conclude that cholesterol is a significant source of propionate only in the absence of sugar carbon sources.

Mycobacterium tuberculosis (*M. tb*), the etiologic agent of tuberculosis (TB) in humans, is responsible for the majority of deaths caused by bacterial infections. Although it is estimated that one-third of the world's population is infected with the pathogen, only 10% of immuno-competent individuals carrying the active form of *M. tb* develop the disease (*1*), and upon suppression of the immune system, *M. tb* bacilli may become active. Consequently, TB is responsible for the majority of HIV-related deaths worldwide. The emergence of multidrug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) has made the successful treatment of the disease cumbersome, often resulting in death of individuals infected with these strains. Therefore, we have undertaken studies to investigate the metabolic state of *M. tb* that ensues upon switching to a lipid-based metabolism in the host, to better develop new therapies.

Unlike most pathogenic bacteria, *M. tb* lacks common virulence factors like capsules, endotoxins, and exotoxins. Its success as a pathogen lies in its ability to elude the host's immune response and persist within the harsh milieu of the macrophage (*2*, *3*). Several distinct modes for virulence have been proposed to play important roles in the survival of *M. tb* within the phagosome, including inhibition of phagosome−lysosome fusion (*4*−*6*), preventing maturation of the phagosome (*7*−*11*), disruption of IFN-γ-mediated signaling (*12*, *13*), adaptation to the host nutritional supply (*2*, *14*, *15*), and alteration of lipids comprising the mycobacterial cell wall (*16*−*18*); however, the exact mechanisms by which these processes occur remains enigmatic.

M. tb adapts to the intracellular host environment by shifting to a lipid metabolism in the glucose-deficient milieu of the macrophage (*19*, *20*). Catabolism of lipids via β-oxidation in conjunction with the anaplerotic glyoxalate cycle supports energy production. However, β-oxidation of branched and odd chain fatty acids and lipids like cholesterol yields propionyl CoA in addition to acetyl CoA (*21*−*25*). [Propionate is also produced from degradation of branched amino acids (*26*).] The accumulation of propionate is toxic, and it is shunted into the methyl citrate cycle and the methylmalonyl pathway. In addition to anapleuorosis of succinate, the methylmalonyl pathway provides methylmalonyl CoA for the biosynthesis of methyl-branched fatty acids. These methyl-branched fatty acids are in turn incorporated into complex bacterial lipids (Scheme 1).

Cholesterol is abundant in the intracellular environment, and survival of *M. tb* in the host requires the Mce4 lipid transporter which can function to take up cholesterol (*27*). Furthermore, metabolic labeling studies with *M. tb* demonstrated that cholesterol degradation products can be converted to both CO_2 and to phthiocerol dimycoserate (PDIM) (*27*). The CO_2 is generated during ATP formation in the TCA cycle. The label in PDIM is thought to derive from propionyl CoA that is converted to methylmalonyl CoA in a reaction catalyzed by pyruvate carboxylase.

PDIM is one of several lipids found on the surface of the bacterial cell. Although not essential for viability, some of them are proposed to play a role in downregulation of the host response (*28*). In addition to PDIM, they include acyltrehaloses, sulfolipids, liparabinomannan, diacyl- and polyacyltrehaloses, di- and trimycolates, and phenolic glycolipids. They contain methyl-branched long chain fatty acids, like mycocerosic, mycolipenic, and mycolipanoic acids. These fatty acids are synthesized by individual fatty acid synthase complexes from malonyl and methylmalonyl CoA, and their biosynthesis requires a metabolic pool of acetyl and propionyl CoA.

[†]This work was supported by the NIH [AI065251 (N.S.S.), AI065987 (I.S.), and DK007521, (N.M.N.) and S10RR025072 (mass spec)] and the NYSTAR Program [FDP C040076 (N.S.S.)].

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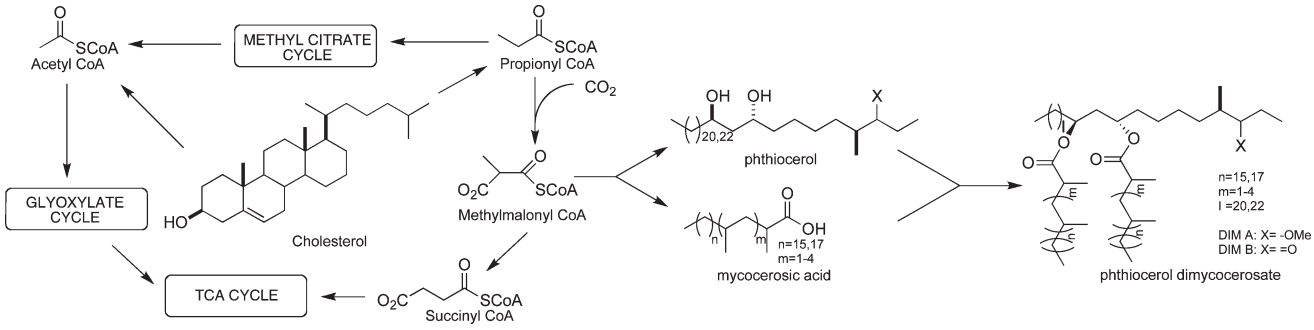
Published on Web 4/13/2009

Scheme 1: Flux of Metabolites from Cholesterol Catabolism



The average mass of PDIM increases ~52 amu when the in vitro culture propionate concentration is increased from 0 to 1000 μM (*21*). That is, the chain length of the methyl-branched mycocerosic acid increases with increasing concentrations of propionate. Growth on valerate, the β-oxidation of which yields one acetate and one propionate, elicits a similar mass shift. Growth on butyrate, the β-oxidation of which yields two acetates, does not result in an increase in PDIM molecular weight. The higher-molecular weight PDIMs observed upon growth on propionate in vitro are also observed in bacteria infecting the mouse lung (*21*). Cholesterol may serve as a source of propionate in vivo, and we asked whether catabolism of cholesterol results in a sufficient intracellular pool of propionate to increase the rate of methyl-branched fatty acid biosynthesis by *M. tb*. Specifically, we investigated whether sufficient propionyl CoA can be derived from *M. tb* growth on cholesterol as a sole carbon source or as an additive to standard medium to increase cellular flux into the methylmalonyl CoA pathway with a consequent alteration of PDIM molecular weight.

The degradation pathway of cholesterol in *M. tb* is partially elucidated, although genes have not been definitively assigned to all steps (*29*−*32*). β-Oxidation of the side chain yields at least one propionyl CoA and one acetyl CoA per molecule of cholesterol. Nonenzymatic cleavage of the C22 carboxylic acid to yield testosterone and propionyl CoA has been proposed (*33*). However, it is unlikely that there is an uncatalyzed step in catabolism, and α-oxidation may occur. Ring cleavage can yield at least one more propionyl CoA molecule per molecule of cholesterol. The exact ratio of acetate and propionate obtained from cholesterol degradation depends on the identity of the ultimate products that have not been fully identified in *M. tb*.

We prepared apolar lipid extracts for mass spectral analysis of PDIM from wild-type H37Rv *M. tb* grown on standard medium (7H9 salts supplemented with glycerol, albumin, and dextrose), grown on standard medium supplemented with cholesterol, and grown on 7H9 salts supplemented with only cholesterol. The extracts were analyzed by high-resolution mass spectrometry using electrospray ionization on an LTQ Orbitrap. PDIM was a mixture of DIM A (methoxy ether) and DIM B (ketone) in the wild-type *M. tb* grown in the standard medium (Figure 1A and Figure S1A of the Supporting Information). DIM A is heavier by 16 mass units than DIM B with the same lipid and polyketide chains. Both contain mycocerosic acid, a methyl-branched long chain fatty acid that is esterified to phthiocerol or phenylphthiocerol. Characteristic of fatty acids, a family of peaks separated by 14 mass units is observed at 1318, 1332, 1346, 1360, 1374, 1388, 1402, 1416, 1430, 1444, 1458, and 1472 for DIM B. A similar series for DIM A is observed at 1334, 1362, 1376, 1390, 1404,

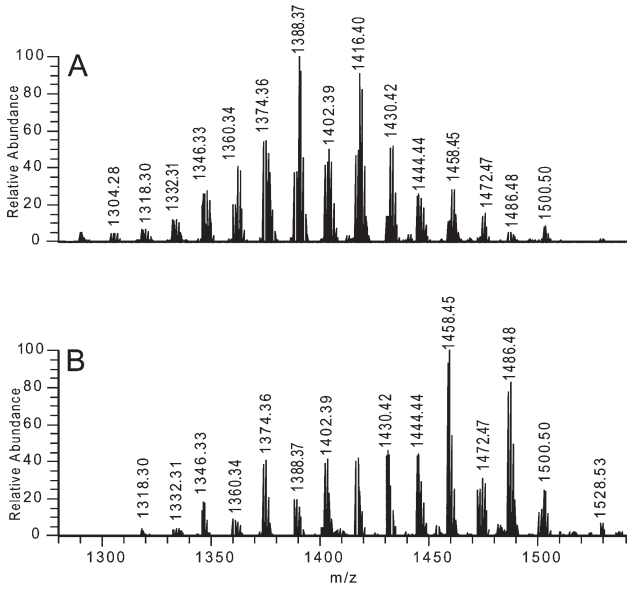


FIGURE 1: Mass spectrometric analysis of apolar lipids of wild-type H37Rv *M. tb*. (A) *M. tb* grown in standard medium and (B) *M. tb* grown using 2.6 mM cholesterol as the sole carbon source solubilized with tyloxapol (5%, w/v).

1418, 1432, 1446, 1460, 1474, and 1488. The molecular weight distribution and DIM A/B content were unchanged in wild-type *M. tb* grown on standard medium that is supplemented with 2.6 mM cholesterol (Figure S1B of the Supporting Information). In contrast, when cholesterol is the sole carbon source, only DIM B is observed and its average molecular weight is shifted 42 amu higher (Figure 1B). This increase in weight was due to the incorporation of longer mycoserosic acids into PDIM as determined by MS^2 fragmentation (Figure 2). These data support the hypothesis that propionyl CoA is formed during the catabolism of cholesterol and that the intracellular propionate concentration is elevated sufficiently to increase the metabolic flux into methylmalonyl CoA biosynthesis. The MWs observed are similar to those seen upon growth on 500−1000 μM propionate (*21*). However, the presence of cholesterol is insufficient to alter the metabolic flux when alternative sugar-based carbon sources, e.g., glycerol or dextrose, are present.

Just as was observed upon growth on 1000 μM propionate and in infected mouse lungs (*21*), when wild-type *M. tb* was grown using cholesterol as the sole carbon source only DIM B is detected (Figure 1). This abundance of the ketone form of PDIM suggests that the cellular pool of NADH is reduced upon cholesterol catabolism. Alternatively, the ketoreductase (Rv2951c) that catalyzes the conversion of the ketone DIM B form to the alcohol precursor of DIM A (*34*) may be inhibited or

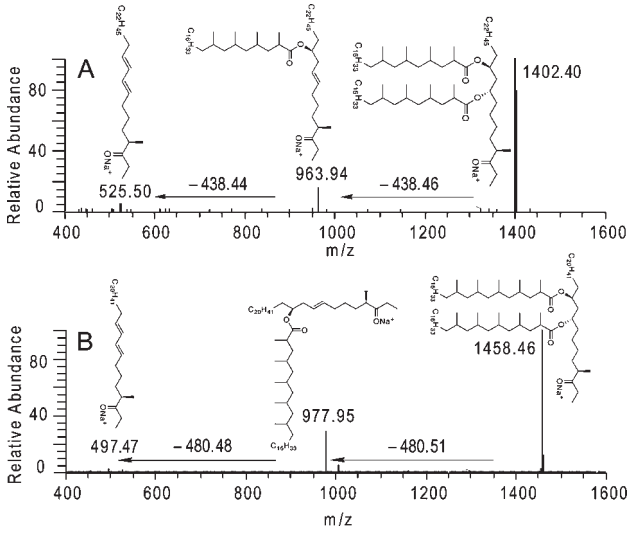


FIGURE 2: Mass spectrometric fragmentation analysis of apolar lipids of wild-type H37Rv *M. tb*. (A) *M. tb* grown in standard medium. The parent ion at 1402.4 in Figure 1A was selected for fragmentation. (B) *M. tb* grown using cholesterol as the sole carbon source. The parent ion at 1458.46 in Figure 1B was selected for fragmentation.

downregulated. However, the loss of DIM A does not affect virulence (*34*).

In conclusion, we have used high-resolution mass spectral analysis of a complex lipid as a reporter for the intracellular formation of propionate upon sterol catabolism. The mass shifts in the PDIM molecular weight distribution observed upon growth on cholesterol as a sole carbon source are analogous to those seen upon growth on 500−1000 μM propionate. Importantly, this same mass shift is not observed upon supplementation of sugar carbon sources with cholesterol. Thus, the metabolic pool of propionate is increased only when the availability of acetate-forming carbon sources is limited. This observation and the previously observed higher-molecular weight PDIMs in mouse lungs (*21*) suggest that in the host, methyl-branched lipid carbon sources are the primary source of nutrition for *M. tb*.

ACKNOWLEDGMENT

We thank Dr. Antonius Koller (SBU Proteomics Center) for assistance with mass spectrometry.

SUPPORTING INFORMATION AVAILABLE

Experimental protocols and Figure S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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