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Review

Lipid metabolism in mycobacteria—Insights using mass spectrometry-based lipidomics

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ABSTRACT

Diseases including tuberculosis and leprosy are caused by species of the *Mycobacterium* genus and are a huge burden on global health, aggravated by the emergence of drug resistant strains. Mycobacteria have a high lipid content and complex lipid profile including several unique classes of lipid. Recent years have seen a growth in research focused on lipid structures, metabolism and biological functions driven by advances in mass spectrometry techniques and instrumentation, particularly the use of electrospray ionization. Here we review the contributions of lipidomics towards the advancement of our knowledge of lipid metabolism in mycobacterial species.

1. Introduction

The mycobacteria genus is a group of acid-fast species characterized by a lipid-rich waxy cell wall much thicker than that found in most other bacteria. Notable members of the genus include *Mycobacterium tuberculosis*, *Mycobacterium leprae*, and *Mycobacterium ulcerans*, causative agents of tuberculosis (TB), leprosy and Buruli ulcers, respectively. In addition, numerous other non-tuberculosis mycobacteria (NTM) cause various diseases including pulmonary and skin infections.

Lipids in the cell envelope of mycobacteria typically make up 30–60% of the dry weight and are thought to play important biological roles, notably in determining virulence and drug resistance [1]. While the importance of lipids in mycobacterial infections has been known for many years, there has been a recent renaissance in the study of lipids in these bacteria. This can be ascribed to the emergence of the fields of lipidomics and systems biology driven by technical advances in mass spectrometry (MS) instrumentation. In particular, the use of atmospheric pressure ionization (especially electrospray ionization, ESI) and the coupling of liquid chromatography (LC) to MS have allowed the identification and quantitation of ever-increasing numbers of lipid species.

Although lipid metabolism in the whole genus of mycobacteria is of interest, much recent research has been focused on *M. tuberculosis*, and closely-related species, due to the huge burden on global health caused by TB. It is estimated that approximately one third of the human population are latently infected with *M. tuberculosis* despite improvements in public health and the discovery of effective chemotherapeutic treatments [2]. The problem has been aggravated by the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *M. tuberculosis* strains that are increasingly difficult to treat, along with co-incidence of TB with other diseases, including human immunodeficiency virus (HIV) infection.

In this review, we provide an overview of recent investigations into the lipid metabolism of mycobacteria using MS to determine metabolite structures and analyze the biological effects of altered lipid biochemistry.

Abbreviations: ABC, ATP binding cassette; BCG, Bacillus Calmette Guérin; ChIP-Seq, chromatin immunoprecipitation-sequencing; CoA, coenzyme A; DAG, diacylglycerol; DAT, diacyl trehalose; DDM, dideoxymycobactin; ESI, electrospray ionization; FT-ICR, Fourier transform ion cyclotron resonance; GC, gas chromatography; HIV, human immunodeficiency virus; HPLC, high performance liquid chromatography; HRAM, high resolution-accurate mass; LC, liquid chromatography; LIT, linear ion trap; MDR, multidrug-resistant; MF, molecular feature; mmDAG, monomeromycolyl diacylglycerol; MPD, mannosyl β-1-phosphodolichol; MPI, mannosyl β-1-phosphoisoprenoid; MRM, multiple reaction monitoring; MS, mass spectrometry; MscL, mechano-sensitive channel of large conductance; MS^n, mass spectrometry with multistage fragmentation; NMR, nuclear magnetic resonance; NTM, non-tuberculosis mycobacteria; PAT, pentaacyl trehalose; PDIM, phthiocerol dimycocerosate; PGL, phenolic glycolipid; PIM, phosphatidyl-*myo*-inositol mannoside; SGL, sulfated glycolipid; SL, sulfolipid; SPE, solid phase extraction; TAG, triacylglycerol; TAT, triacyl trehalose; TB, tuberculosis; TDM, trehalose dimycolate; THL, tetrahydrolipistatin; TMM, trehalose monomycolate; UV, ultraviolet; XDR, extensively drug-resistant.

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The work described here is not an exhaustive summary of mycobacterial lipidomics, but rather an overview of important advances in the subject in recent years. A summary of the research described is provided in Table 1.

In the first sections, we discuss the targeted identification of individual lipid classes and the use of MS for the quantitation of lipids, structural elucidation by tandem MS, and measurement of lipid biomarkers that may be used to identify an individual species or strain of bacteria. These studies typically use instruments capable of performing tandem MS experiments such as triple quadrupoles and ion traps. We then present global lipidomics and systems biology approaches to the determination of biosynthetic pathways and regulatory networks. Broadly speaking, these are studies that use high resolution MS techniques to analyze multiple lipid classes at the same time. It is important to note that these two lipidomics techniques complement each other, for example novel lipids identified in global approaches can be further investigated by more targeted techniques [3].

2. Mycolic acids

Mycolic acids are a class of fatty acid unique to mycobacteria, and closely-related species, characterized by a long beta-hydroxy chain with a shorter alpha side chain (Fig. 1A). In the past, gas chromatography (GC)-MS has been widely used for the analysis of mycolic acids [4]. However, GC-MS typically relies on electron ionization (EI) which leads to complex fragmentation patterns that are difficult to interpret. In addition, derivatization of mycolic acids before analysis is necessary to enhance volatility. Mass spectrometry with multistage fragmentation (MS^n) using ESI can overcome these problems—mycolic acids form intense ions in negative ion mode MS and collision-induced dissociated

Table 1
Studies using atmospheric pressure ionization techniques for the analysis of lipids from mycobacteria and related species, reviewed in this work. APCI, atmospheric pressure chemical ionization; BCG, Bacillus Calmette Guérin; ESI, electrospray ionization; FT-ICR, Fourier transform-ion cyclotron resonance; HPLC, high performance liquid chromatography; MALDI, matrix assisted laser desorption/ionization; MS, mass spectrometry MS^n, mass spectrometry with multistage fragmentation; NMR, nuclear magnetic resonance; NTM, non-tuberculosis mycobacteria; Q-TOF, quadrupole-time of flight; Q-trap, quadrupole-ion trap; QqQ, triple quadrupole. Definitions of lipid abbreviations are given in the text and in Fig. 1.

Lipids analyzed	Reference	Organisms	Instruments	Comments
Mycolic acids	[5]	*M. bovis* BCG *M. tuberculosis*	ESI-Q-TOF	First ESI analysis of MAs
	[6]	*M. tuberculosis* Various NTM	ESI-QqQ	*M. tuberculosis* differentiated from NTM
	[7]	*R. equi* *Rhodococcus rhodnii*	ESI-ion trap ESI-QqQ	Deuteration used to confirm structures
	[8]	*Rhodococcus erythropolis*	ESI-QqQ	Makeup of MAs varies with carbon source
	[9]	*C. glutamicum* (5 strains)	ESI-Q-TOF	Makeup of MAs varies with growth temperature
	[10]	*M. bovis* BCG	ESI-Q-trap	Knockout of ethA–ethR alters MA makeup
	[12]	*M. bovis* BCG (12 strains)	APCI-ion trap	MS used to confirm HPLC studies
	[13]	*M. bovis* BCG *M. tuberculosis* Various NTM	ESI-QqQ ESI-Q-trap	Sputum samples analyzed. Detailed MA profile of 33 species.
	[14]	*M. tuberculosis*	ESI-Q-trap	Sputum samples analyzed
	[15]	*M. tuberculosis*	ESI-Q-trap	MAs identified in archeological sample
Glycolipids	[19]	*M. bovis* BCG	ESI-ion trap MALDI-TOF	MS^n used to identify structures of PIMs
	[20]	*M. bovis* BCG	ESI-ion trap MALDI-TOF	MS^n used to identify structures of PIMs
	[21]	*M. simiae*	ESI-ion trap	NMR and MS. TMM and TDM structures.
	[22]	*R. equi*	ESI-ion trap ESI-Orbitrap	Determination of TMM and TDM structures
	[23]	*C. glutamicum*	ESI-Q-trap	Acetylation is required for TMM transport
	[25]	*M. marinum*	ESI-Q-TOF	*tesA* mutant deficient in PDIMs and PGLs
	[26]	*M. tuberculosis* *M. bovis* BCG	ESI-ion trap	MPD lipid product of *pks12* activates T-cells
Sulfolipids	[27]	*M. tuberculosis* *M. smegmatis*	ESI-FT-ICR	Discovery of sulphated lipids using ^{32}S and ^{34}S
	[28]	*M. tuberculosis*	ESI-Q-TOF	Analysis of mutant lacking transporter
	[29]	*M. tuberculosis*	ESI-ion trap ESI-Orbitrap ESI-FT-ICR	Reassignment of structure of SL-II
Triacylglycerols and related lipids	[31]	*M. tuberculosis*	ESI-quad	Beijing strains accumulate TAGs
	[32]	*M. bovis* BCG	ESI-Q-trap	TAGs measured across growth curve
	[33]	*M. bovis* BCG	ESI-Orbitrap	TAG metabolism affected by tetrahydrolipistatin
	[34]	*M. smegmatis*	ESI-Orbitrap	Structures of TAGs and mmDAGs
Phthiocerol dimycocerosates	[35]	*M. tuberculosis*	GC-MS	PDIM components detected in sputum
	[36]	*M. tuberculosis*	GC-MS	PDIM components detected in bison skeleton
Global analysis	[37]	*M. tuberculosis*	ESI-FT-ICR	Software package for data analysis (FAAT)
	[38]	*M. tuberculosis*	ESI-FT-ICR	Link between MM-CoA and SL-I/PDIMs
	[39]	*M. tuberculosis*	ESI-Q-TOF	Revised mycobactin biosynthesis pathway
	[40]	*M. tuberculosis*	ESI/APCI-TOF	Setup of *Mtb* LipidDB database
	[43]	*M. tuberculosis*	ESI-Orbitrap	Search tool for use with *Mtb* LipidDB
	[44]	*M. tuberculosis* *M. smegmatis*	ESI-Q-TOF	Setup of MycoMass and MycoMap databases
	[46]	*M. tuberculosis* *M. bovis* BCG	ESI-Q-TOF	Novel tuberculosinyl nucleoside identified
Systems biology	[47]	*M. tuberculosis*	ESI-Q-TOF	Lipidomics with transcriptomics for regulatory network during hypoxia
	[48]	*M. tuberculosis* (35 strains)	ESI-QqQ	Lipidomics with genomics to investigate MA metabolism