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Review

Pyridoxal-phosphate dependent mycobacterial cysteine synthases: Structure, mechanism and potential as drug targets ☆

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ABSTRACT

The alarming increase of drug resistance in *Mycobacterium tuberculosis* strains poses a severe threat to human health. Chemotherapy is particularly challenging because *M. tuberculosis* can persist in the lungs of infected individuals; estimates of the WHO indicate that about 1/3 of the world population is infected with latent tuberculosis providing a large reservoir for relapse and subsequent spread of the disease. Persistent *M. tuberculosis* shows considerable tolerance towards conventional antibiotics making treatment particularly difficult. In this phase the bacilli are exposed to oxygen and nitrogen radicals generated as part of the host response and redox-defense mechanisms are thus vital for the survival of the pathogen. Sulfur metabolism and *de novo* cysteine biosynthesis have been shown to be important for the redox homeostasis in persistent *M. tuberculosis* and these pathways could provide promising targets for novel antibiotics for the treatment of the latent form of the disease. Recent research has provided evidence for three *de novo* metabolic routes of cysteine biosynthesis in *M. tuberculosis*, each with a specific PLP dependent cysteine synthase with distinct substrate specificities. In this review we summarize our present understanding of these pathways, with a focus on the advances on functional and mechanistic characterization of mycobacterial PLP dependent cysteine synthases, their role in the various pathways to cysteine, and first attempts to develop specific inhibitors of mycobacterial cysteine biosynthesis. This article is part of a Special Issue entitled: Cofactor-dependent proteins: evolution, chemical diversity and bio-applications.

1. Introduction

Mycobacterium tuberculosis and modern humans share a long coevolutionary history, reaching back to about 70,000 years ago [1]. Unfortunately this common history still continues and, in spite of the availability of chemotherapy, the pathogen causes about 2 million deaths per year (http://www.who.int/tb/en/). The emergence of *M. tuberculosis* strains resistant to most first line defense drugs and of totally drug resistant strains [2] threatens to reverse the advances in the treatment of tuberculosis made in the last century and poses a severe challenge to human health.

In the initial stages of tuberculosis, infected macrophages will release cytokines that lead to the recruitment of immune cells, for instance neutrophils, macrophages, NK cells and T cells to form granuloma. These are structures within the infected tissue, with bacilli-containing macrophages at the center surrounded by other immune cells such as B and T lymphocytes, non-infected macrophages and fibroblasts [3]. The function of the granuloma is not quite clear, but it has been suggested that they contain the bacilli and thus prevent their uncontrolled spread. Within the granuloma, *M. tuberculosis* is exposed to environmental stress conditions, thought to include hypoxia, nutrient depletion and oxidative stress [4]. Under these conditions mycobacteria can enter a persistent phase, characterized by a lack of (or very slow) cell division and substantial changes in metabolism [5]. At this stage the infection is non-contagious and no symptoms are observed. Present estimates of the WHO indicate that about 1/3 of the world population is infected with latent tuberculosis, providing a large reservoir for relapse and subsequent spread of the disease. Treatment of latent tuberculosis is very difficult, if at all possible, because persistent *M. tuberculosis* shows considerable tolerance towards conventional antibiotics [6].

Not least in view of the challenges in chemotherapy of tuberculosis and the alarming increase of antibiotic resistance in *M. tuberculosis*, the elucidation of the regulatory mechanisms for the switch from actively dividing to persistent bacteria, as well as the metabolic processes essential

Abbreviations: OAS, O-acetyl serine; OPS, O-phosphoserine; PLP, pyridoxal-5-phosphate, APS, adenosine 5′phosphosulfate, SAR, structure–activity relationships

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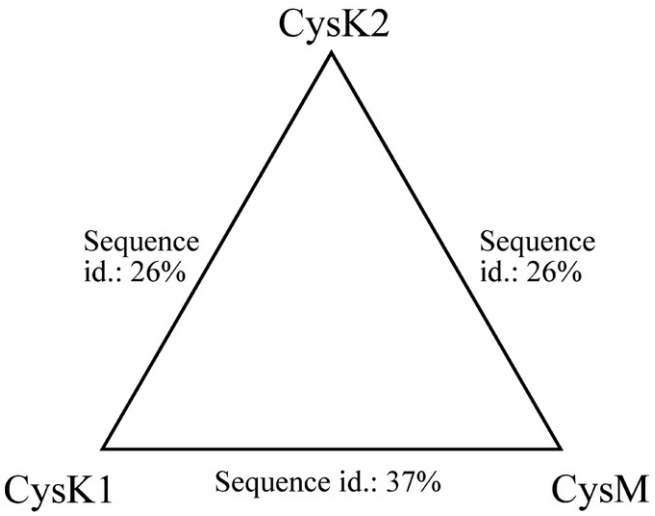

Fig. 1. The three cysteine synthases encoded in the genome of *M. tuberculosis*.

for survival of the bacteria in the host, has attracted considerable interest. Persistent bacilli are exposed to reactive oxygen (ROI) and reactive nitrogen (RNI) intermediates generated as a part of the host response [7]. Redox-defense mechanisms are thus vital for the survival of the pathogen and related pathways are consistently found to be up-regulated in persisters [5,8,9]. An important metabolite in the redox-defense of *M. tuberculosis* is mycothiol, found in millimolar concentration in the cell [10–12]. This compound contains a cysteine-derived sulfhydryl group as active moiety [13] and its biosynthesis requires the availability of cysteine. Also other molecules involved in the defense against oxidative stress in *M. tuberculosis* such as thioredoxins rely on a supply of cysteine, underpinning cysteine biosynthesis as an important cellular process in maintaining redox homeostasis in this pathogen.

Research during the last decade has painted a rather complex picture of the *de novo* biosynthesis of cysteine in *M. tuberculosis*. In this review we will summarize our present understanding of these pathways, with a focus on the mycobacterial pyridoxal-5-phosphate (PLP) dependent cysteine synthases. A previous review [14] highlighted the insights obtained from structural studies of enzymes from sulfur metabolism. Here we focus on the advances on functional and mechanistic characterization of these enzymes, in particular the role of PLP dependent cysteine synthases in the various pathways to cysteine, and first attempts to develop specific inhibitors.

2. The *de novo* cysteine biosynthetic pathways in *M. tuberculosis*

2.1. The M. tuberculosis genome encodes three cysteine synthases

The genome of *M. tuberculosis* H37Rv contains three genes that had been annotated to encode putative O-acetylserine sulfhydrylases, Rv2334, Rv0848 and Rv1336. The corresponding proteins are denoted CysK1, CysK2 and CysM (Fig. 1). The three proteins are related in amino acid sequence, with CysK1 and CysM displaying 37% sequence identity. CysK2 is more distantly related to the other two enzymes, with only about 26% identity to CysK1 and CysM, respectively. Unfortunately the nomenclature of cysteine synthases varies between different organisms and is confusing. Most bacteria contain two cysteine synthases, usually denoted OASS A (or CysK) and OASS B (or CysM). In mycobacteria, OASS A is denoted CysK1, whereas OASS B corresponds to CysK2. Mycobacterial CysM is rather unique in its properties and is only found in actinobacteria, including Mycobacteria, Streptomyces and Corynebacteria. In the following we shall use the mycobacterial naming convention unless stated otherwise.

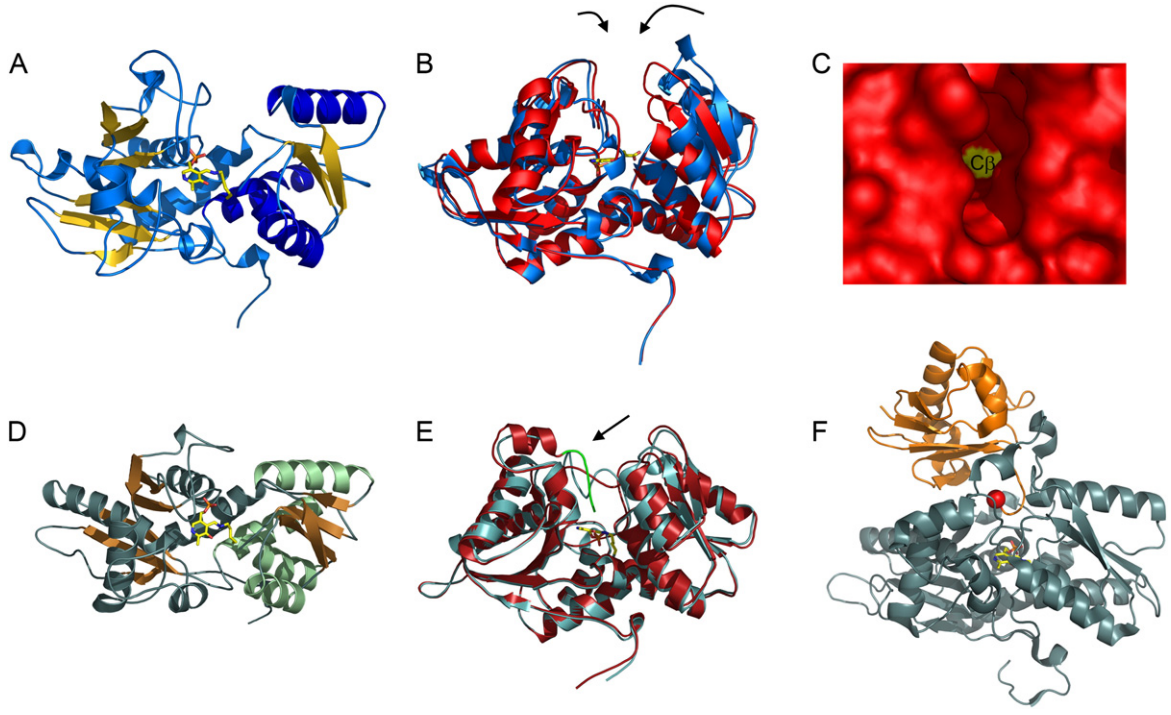

Fig. 2. Structural enzymology of cysteine biosynthesis in *M. tuberculosis*. A: Cartoon of the three-dimensional structure of CysK1 [15]. The N-terminal domain is indicated by α-helices in dark blue and the C-terminal domain by helices in lighter blue color. The PLP cofactor is shown as stick model. B: Conformational changes leading to partial active site closure upon formation of the α-aminoacrylate intermediate in CysK1. The open form of the enzyme is shown in blue, and the closed form in red. The arrows indicate the domain movement leading to active site closure. C: Surface view of the CysK1-aminoacrylate complex, showing the narrow channel leading to the Cβ carbon atom of the intermediate, indicated in yellow. D: Cartoon of the three-dimensional structure of CysM, with the N-terminal and C-terminal domains shown in light and dark green, respectively. PLP is shown as a stick model. E: Superposition of the open (red) and closed (blue) form of CysM [17,28]. The C-terminal residues, which insert into the active site cleft in the closed form, are highlighted by an arrow. F: Cartoon of the CysM–CysO complex [16]. CysM is shown in green and CysO in orange. The position of the C-terminus of CysO, carrying the thiocarboxylate, is indicated by a red sphere.