Reconstitution of a new Cysteine biosynthetic pathway in *Mycobacterium tuberculosis*

Kristin E. Burns, Sabine Baumgart, Pieter C. Dorrestein, Huili Zhai, Fred W. McLafferty, Tadhg P. Begley*

Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853

Supporting Information

Experimental

General Information: All reagents were purchased from Sigma-Aldrich.

Cloning, Expression and Purification of CysO, CysM: *M. tuberculosis* Rv1335 and Rv1336 were cloned into pET16b vectors, overexpressed in *E. coli* in LB at reduced temperature, and purified by Ni-Nta chromatography. The proteins were desalted and concentrated prior to use.

Cloning, Expression and Purification of CysO-thiocarboxylate: The CysO-intein construct was cloned according to manufacturer's instructions with a pTYB1 vector. It was overexpressed in *E. coli* at reduced temperature and purified using Chitin resin. Following elution with $(NH_4)S$ for 40 hours at 4^oC, the protein was desalted and concentrated.

Cloning, Expression and Purification of Mec^+: The plasmid pET-MAL-HT (6 histidine residues at the carboxy terminus of maltose binding protein) was used to clone Rv1334. It was overexpressed in *E. coli* at reduced temperature and purified by Ni-Nta chromatography. The protein was desalted and concentrated prior to use.

Cloning, Expression and Purification of His_{10}-CysO-cys: A cysteine residue was added to the carboxy terminus of CysO by adding TGC bases to the primer before the stop codon. The construct was expressed and purified similarly to CysO.

CysO-cysteine formation by ESI-MS: 13mM O-acetylserine, 300μg CysO-thiocarboxylate, and 6.7mM DTT were incubated with 480μg CysM in 50mM Tris-HCl (pH 8.0). After incubation at 37^oC for certain time points, the samples were frozen at -80^oC. The samples were analyzed by ESI-FTMS.

Cleavage of His_{10}-CysO-cys by Mec^+: 255μg of His_{10}-CysO-cysteine, 10mM DTT, 10μM $ZnCl_2$, and 45μg Mec^+ were incubated in 50mM Tris, pH 8 for 1 hour, then frozen. Samples were analyzed by ESI-FTMS.

Ninhydrin assay for cysteine: Briefly, 6mM O-acetylserine, 10mM DTT, 250μg CysO-thiocarboxylate, 100μg CysM, 0.1mM Zn^{2+}, and 140μg Mec^{+} were incubated in 100mM Phosphate buffer, pH 8.0. Samples were withdrawn at appropriate times, ninhydrin was added, boiled for 10 minutes, cooled, and ethanol added. Absorbance at 560nm was measured.

Copurification of CysO and MoeZ: CysO and MoeZ were expressed in *E. coli* at reduced temperature. The cell pellets were combined in lysis buffer and sonicated. Purification proceeded according to Ni-Nta protocol. The sample was desalted and concentrated prior to analysis by ESI-FTMS.