Mechanism of Fluorinated Anthranilate-Induced Growth Inhibition in *Mycobacterium tuberculosis*

Supporting Information

M. Nurul Islam, Reese Hitchings, Santosh Kumar, Fabio L. Fontes, J. Shaun Lott[1*], Nicole A. Kruh-Garcia and Dean C. Crick*

Mycobacteria Research Laboratories, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, CO 80523, United States

[1]Current address: School of Biological Sciences & Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Private Bag 92019, Auckland, New Zealand.

* Corresponding authors: dean.crick@colostate.edu, s.lott@auckland.ac.nz

5 Pages

1 Table

1 Figure

Methods

Trypsin Digestion of recombinant PruA

Briefly, 300 µg of protein was dissolved in 25 mM ammonium bicarbonate buffer. Protein was reduced with 10 mM DTT at room temperature for 1 hour, followed by heating at 100 °C for 5 min; cysteine residues were subsequently alkylated with 25 mM iodoacetamide at room temperature for 1 hour in the dark. Protein digestion was carried out with trypsin (1:20; w/w) at 37 °C in a water bath for overnight.

Selected reaction monitoring mass spectrometry (SRM-MS)

Skyline-Daily (64-bit) was used to build and optimize the SRM assays.[1] The FASTA-formatted sequence of the *M. tuberculosis* PruA (Rv1187) protein was used for in silico tryptic (KR|P) digestion with the 6 peptides containing tryptophan selected. Both double and triple charge precursor ions were empirically tested and a minimum of 5 fragment ions (y/b) were selected for each precursor. Peptide m/z values were also calculated to monitor the incorporation of fluorinated tryptophan, monoisotopic mass of +17.990578. One µL of digested recombinant 6-fluoro-PruA were injected at a concentration of roughly 1µg/µL into a LC MS/MS system consisting of a Waters nano ACQUITY UPLC M-class coupled to a Waters TQ-S mass spectrometer fitted with a Trizaic source. The instrument was operated in positive electrospray ionization mode using MassLynx V4.1 SCN905 (Waters). Chromatography was performed on a 150 µm × 50 mm ionKey packed with BEH C18 130 Å, 1.7 µm. Peptides were separated using gradient elution with a stable flow of 3.060 µL/min. The gradient started with 97% solvent A (99.9% water with 0.1% formic acid) and 3% solvent B (99.9% ACN with 0.1% formic acid) followed by a linear increase to 45% A and 55% B which was achieved at 10.0 min. This was followed by a linear increase towards 95% B which was achieved at 10.5 min and maintained until 12.5 min. The system was subsequently switched to 5% B, which was achieved at 13 min and the column was left to equilibrate for 3 min. The column was maintained at 45 °C during analysis, and the samples were kept at 4 °C. The MS was operating in selective reaction mode using electrospray ionization in positive ion mode, with a capillary voltage of 3.4 kV and a source temperature of 100 °C. Cone voltage was static and the collision energies were optimized for each peptide individually. Peak identification was performed using Mass Lynx software version 4.1 and Skyline-Daily. These data are available through Panorama Public[2] at https://panoramaweb.org/vvED9u.url and using the ProteomeXchange ID# PXD010703.

PruA amino acid sequence:

MDAITQVPVPANEPVHDYAPKSPERTRLRTELASLADHPIDLPHVIGGRHRMGDGERIDVVQPHRHAARL
GTLTNATHADAAAAVEAAMSAKSD**W**AALPFDERAAVFLRAADLLAGP**W**REKIAAATMLGQSKSVYQAEID
AVCELIDF**W**RFNVAFARQILEQQPISGPGE**W**NRIDYRPLDGFVYAITPFNFTSIAGNLPTAPALMGNTVI
WKPSITQTLAAYLTMQLLEAAGLPPGVINLVTGDGFAVSDVALADPRLAGIHFTGSTATFGHL**W**Q**W**VGTN
IGRYHSYPRLVGETGGKDFVVAHASARPDVLRTALIRGAFDYQGQKCSAVSRAFIAHSV**W**QRMGDELLAK
AAELRYGDITDLSNYGGALIDQRAFVKNVDAIERAKGAAAVTVAVGGEYDDSEGYFVRPTVLLSDDPTDE
SFVIEYFGPLLSVHVYPDERYEQILDVIDTGSRYALTGAVIADDRQAVLTALDRLRFAAGNFYVNDKPTG
AVVGRQPFGGARGSGTNDKAGSPLNLLR**W**TSARSIKETFVAATDHIYPHMAVD

Theoretical trypsin digest of PruA (only peptides containing W are shown)

Peptide 1 – SD**W**AALPFDER (underlined above)

Peptide 2 - AADLLAGP**W**R

Peptide 3 - SVYQAEIDAVCELIDF**W**R

Peptide 4 - QILEQQPISGPGE**W**NR

Peptide 5 - IDYRPLDGFVYAITPFNFTSIAGNLPTAPALMGNTVI**W**KPSITQTLAAYLTMQLL

EAAGLPPGVINLVTGDGFAVSDVALADPR

Peptide 6 - LAGIHFTGSTATFGHL**W**Q**W**VGTNIGR

Peptide 7 - AFIAHSV**W**QR

Peptide 8 - **W**TSAR

Table S1. Precursor and fragment ions used for SRM-MS of Peptide 1 (above)

PruA containing 6-fW			**PruA**	
Peptide	**Sequence**	**m/z**	**Sequence**	**m/z**
Precursor	SD(**6-fW**)AALPFDER	662.802^{++}	SD**W**AALPFDER	653.8086^{++}
y10	D(**6-fW**)AALPFDER	1237.5648^{+}	D**W**AALPFDER	1219.5742^{+}
y9	**(6-fW**)AALPFDER	1122.5378^{+}	**W**AALPFDER	1104.5473^{+}
y8	AALPFDER	918.4680^{+}	AALPFDER	918.4680^{+}
y7	ALPFDER	847.4308^{+}	ALPFDER	847.4308^{+}
y6	LPFDER	776.3937^{+}	LPFDER	776.3937^{+}
y5	PFDER	663.3097^{+}	PFDER	663.3097^{+}

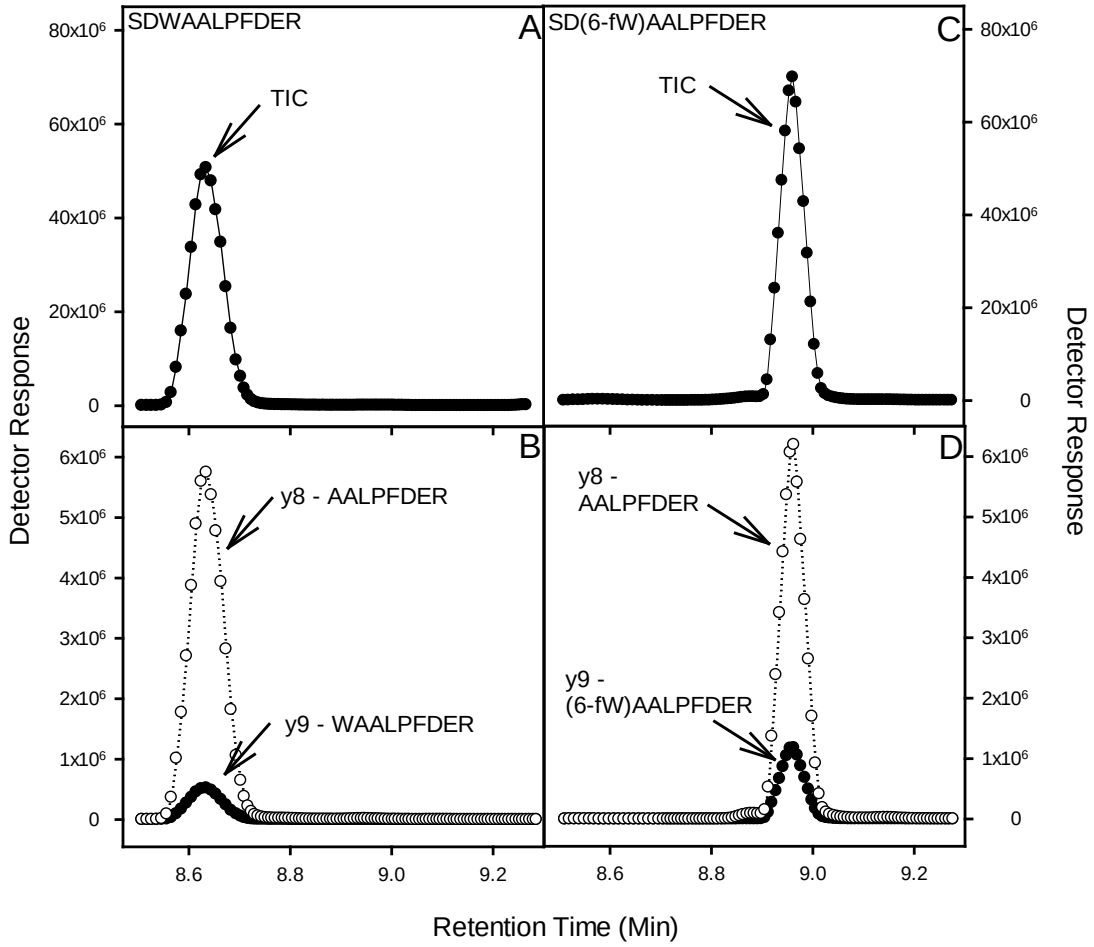

Figure S1. Results of SRM-MS experiments on trypsin digested peptide-1 from recombinant PruA isolated from *M. smegmatis* bacilli treated with 6-fluorotryptophan for 24 hours during induction (see manuscript for experimental details). Panels A and B show total ion chromatogram (TIC) and selected diagnostic transitions for peptide-1 without incorporated 6-fluorotryptophan. Panels C and D show total ion chromatogram (TIC) and selected diagnostic transitions for peptide-1 with incorporated 6-fluorotryptophan. Similar results were seen for the other tryptic peptides. Results clearly indicate that 6-fluorotryptophan is incorporated into PruA under the conditions tested.

References:

(1) MacLean, B.; Tomazela, D. M.; Shulman, N.; Chambers, M.; Finney, G. L.; Frewen, B.; Kern, R.; Tabb, D. L.; Liebler, D. C.; MacCoss, M. J. Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. *Bioinformatics* **2010**, *26* (7), 966–968, DOI: 10.1093/bioinformatics/btq054.

(2) Sharma, V.; Eckels, J.; Taylor, G. K.; Shulman, N. J.; Stergachis, A. B.; Joyner, S. A.; Yan, P.; Whiteaker, J. R.; Halusa, G. N.; Schilling, B.; Gibson, B. W.; Colangelo, C. M.; Paulovich, A. G.; Carr, S. A.; Jaffe, J. D.; MacCoss, M. J.; MacLean, B. Panorama: a targeted proteomics knowledge base. *J. Proteome Res.* **2014**, *13* (9), 4205–4210, DOI: 10.1021/pr5006636.