SUPPLEMENTAL INFORMATION

In *Salmonella enterica*, the Gcn5-related acetyltransferase MddA (formerly YncA) acetylates methionine sulfone and methionine sulfoximine, blocking their toxic effects

Kristy L. Hentchel and Jorge C. Escalante-Semerena*

[1]*Department of Microbiology, University of Georgia, Athens, GA, 30602*

*To whom correspondence should be addressed: E-mail: jcescala@uga.edu; Phone: (+1) 706-542-2651; Department of Microbiology, 212C Biological Sciences Building, Athens GA 30602-2605

Running title: MddA acetylates oxidized methionine in *S. enterica*

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Supplementary tables

Table S1. Strains and plasmids used in this study.

Strain	**Relevant genotype**	**Reference/source**[a]
JE10079	*ara-9 mddA*$^+$	Laboratory strain
Derivatives of JE10079		
JE18333	*mddA1*::*cat*$^+$	
JE18543	*mddA1*::*cat*$^+$ / pNK972[b]	
JE18622	Δ*mddA2*	
JE18955	pMDD8	
JE18961	*mddA1*::*cat*$^+$ / pMDD8	
JE19029	*mddA1*::*cat*$^+$ / pMDD11	
JE19583	*metNI2703*::*kan*$^+$	
JE19730	Δ*mddA2 metNI2703*::*kan*$^+$	
JE20027	Δ*mddA2 glnP1561*::Tn*10*d(*tet*$^+$)[c]	
JE20064	*glnPQ1562*::*cat*$^+$	
JE20065	Δ*mddA2 glnPQ1562*::*cat*$^+$	
JE20067	Δ*mddA2 metNI2703*::*kan*$^+$ *glnPQ1562*::*cat*$^+$	
JE20073	Δ*mddA2 glnPQ1562*::*cat*$^+$ / pGLN2	
JE20329	Δ*mddA2 metNI2703*::*kan*$^+$ / pMETN1	
JE6583	*metE205 ara-9*	K. Sanderson via J. Roth
***E. coli* strains**		
E. coli C41(lDE3)	*ompT hsdS* (r_B m_B) *gal* λ (DE3) including at least one non-characterized mutation	(1, 2)
Plasmids		
pMDD7	*mddA*$^+$ cloned into pKLD66[d]	

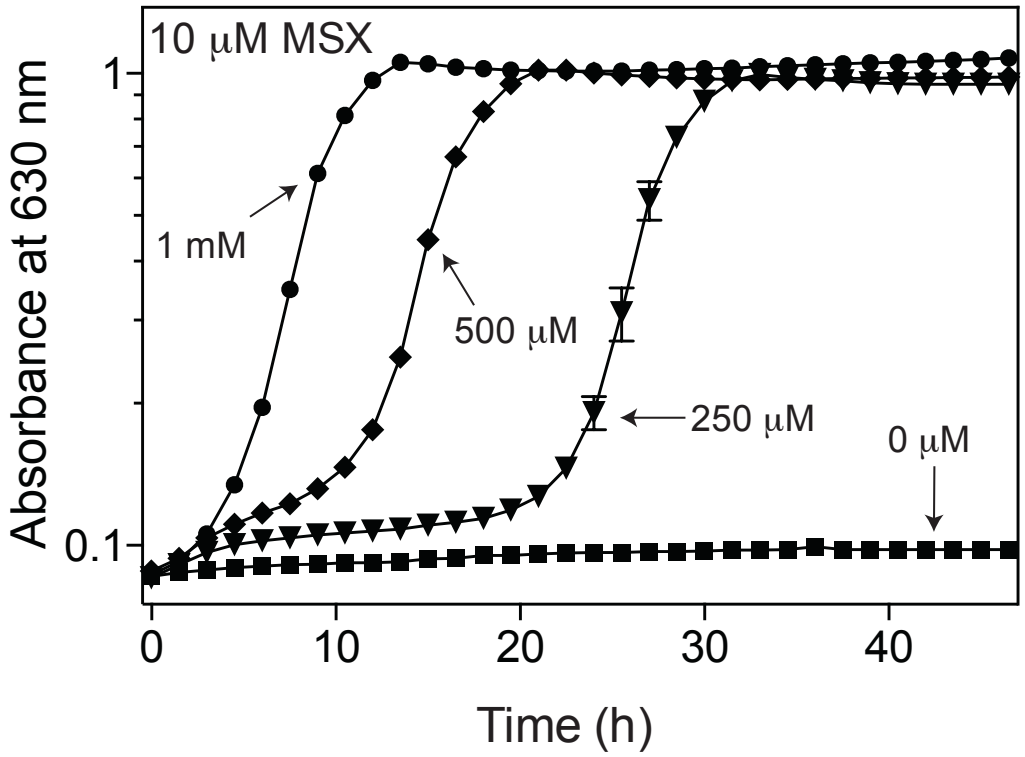
pMDD8	*mddA*$^{+}$ cloned into pBAD24[e]	
pMDD10	*mddA3* cloned into pKLD66[d] (encodes MddA^{E82Q})	
pMDD11	*mddA3* cloned into pBAD24[e], (encodes MddA^{E82Q})	
pGLN2	*glnPQ*$^{+}$ cloned into pBAD24[e]	
pMETN1	*metNI*$^{+}$ cloned into pBAD24[e]	
pNK972	*tpn*$^{+}$ *bla*$^{+}$	(3)

[a] All strains and plasmids were constructed during the course of this work, unless otherwise stated
[b] pNK972 is a pBR332 derivative carrying the IS10transposase gene described in (3)
[c] Tn*10*d(*tet*$^{+}$) is an abbreviation of Tn*10*Δ*16*Δ*17* (4)
[d] pKLD66 is an overexpression vector described in (2)
[e] pBAD24 is a cloning vector described in (5)

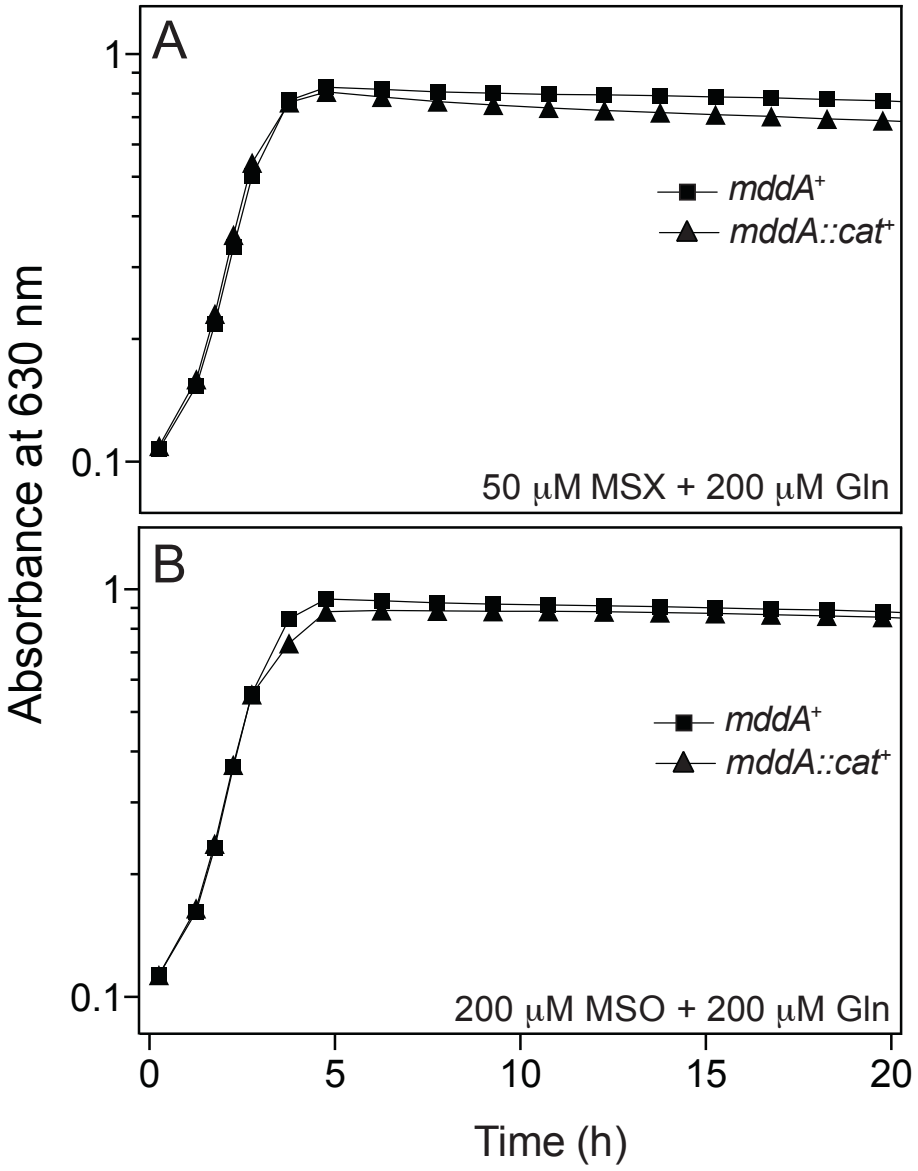
Table S2. Primers used in this study.	
Primer Name	**Primer Sequence**
Cloning	
5' *glnPQ* pBAD24	NNGCTCTTCNTTCATGCAGTTTGACTGGAGCGCCATCT
3' *glnPQ* pBAD24	NNGCTCTTCNTTATCAGGAGACGTGCTGTAAAAATTCC
5' *metNI* pBAD24	NNGCTCTTCNTTCATGATTAAACTTTCGAATATTACCA
3' *metNI* pBAD24	NNGCTCTTCNTTATTACTTATGCGTGACAGCCCTGACG
5' *mddA* pBAD24	NNGCTCTTCNTTCATGACGATTCGCTTTGCCGATAAAG
3' *mddA* pBAD24	NNGCTCTTCNTTATCAGCAGGCGTCCGGCGCGGCGTGT
5' *mddA* pTEV16	NNGCTCTTCNAGCATGACGATTCGCTTTGCCGATAAAG
3' *mddA* pTEV16	NNGCTCTTCNTTATCAGCAGGCGTCCGGCGCGGCGTGT
Mutagenesis	
5' *mddA* G244C	GTTTTCGCTATACCGTCCAGCACTCGGTTTATGTTC
3' *mddA* G244C	GAACATAAACCGAGTGCTGGACGGTATAGCGAAAAC
Strain deletions	
5' *glnPQ* DEL	TGACTATTTACACCACGGTAACAGGAACGACATATGGTGTAGGCTGG AGCTGCTTC
3' *glnPQ* DEL	CCCTTCCTGCCGCAGGGCTGGAAGGGCGATATCTCACATATGAATAT CCTCCTTAG
5' *metNI* DEL	CTCCGCTCATTTCATTACGATAATAAAGAATCAATGGTGTAGGCTGG AGCTGCTTC
3' *metNI* DEL	TTTCCTTAATGAGTATTTGTGTTGTGTTTAACGTTACATATGAATATCC TCCTTAG
5' *mddA* DEL	TATCGTAAACATTCCCTGGGGTTCCCTATGGTGTAGGCTGGAGCTGCT TC
3' *mddA* DEL	AAAAGATGAGCGTC GCGACTGGTTCATCACATATGAATATCCTCCTTAG

Supplementary figures

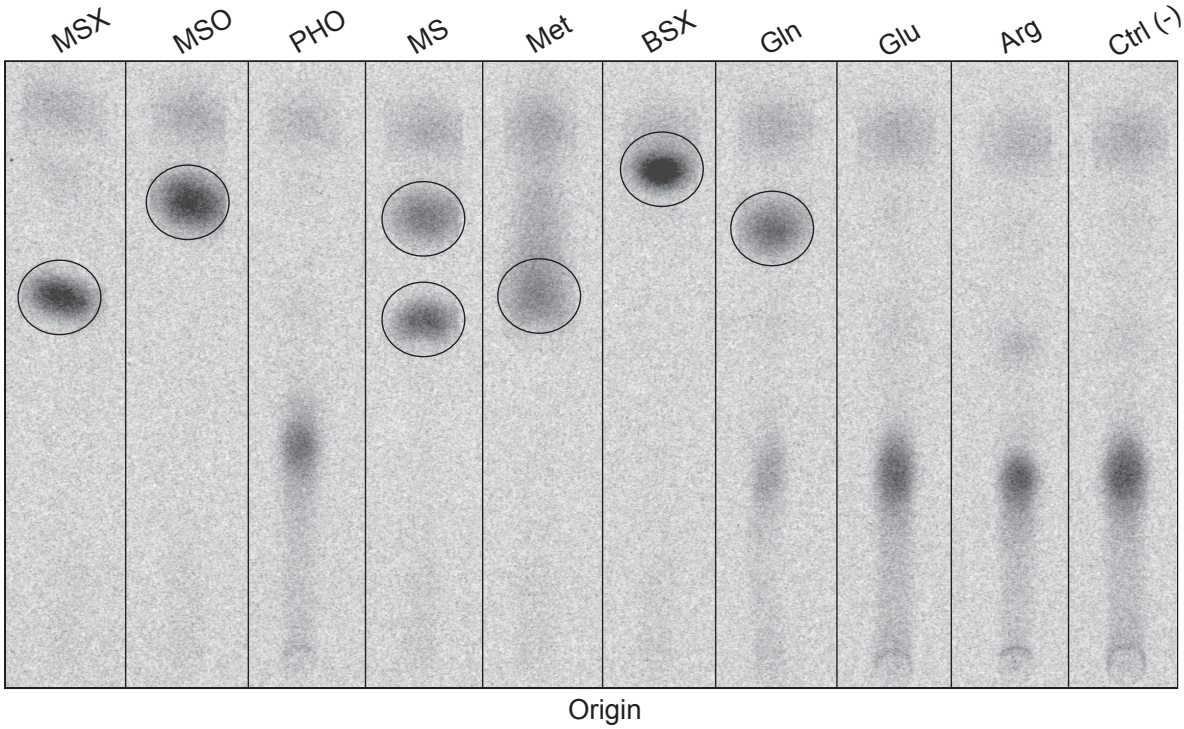
Figure S1. High levels of MddA^{E82Q} variant allow an *mddA1*::*cat*$^{+}$ strain to grow in the presence of MSX. Growth of the *S. enterica* strain *mddA1*::*cat*$^{+}$ / pMDD11 *(*encodes MddA^{E82Q}) in NCE minimal medium (glycerol, 22 mM) was examined in the presence of MSX (10 μM) and increasing concentrations of inducer (L-(+)-arabinose; 250 μM, 500 μM, and 1000 μM), as indicated. Growth curves were performed using a microplate reader (Bio-Tek Instruments) as described under *Materials and Methods*. The strain analyzed was JE19029 (*mddA1*::*cat*$^{+}$ / pMDD11 *mddA3*$^{+}$, which encodes MddA^{E82Q}). Error bars represent standard deviation.



Supplemental Figure 2. Addition of glutamine fully restores growth of an *mddA* strain exposed to MSX and MSO in rich medium. Growth of the *S. enterica* strains *mddA*$^+$ and *mddA1*::*cat*$^+$ was examined with (A) MSX (50 μM) and (B) MSO (100 μM), with the addition of glutamine (200 μM). The medium used was nutrient broth. Growth curves were performed using a microplate reader (Bio-Tek Instruments) as described under *Materials and Methods*. The following strains were analyzed: *mddA*$^+$ (JE10079) and *mddA1*::*cat*$^+$ (JE18333). Error bars represent standard deviation.



Supplemental Figure 3. *Se*MddA^{WT} acetylates methionine derivatives. The substrate specificity of *Se*MddA^{WT} was examined using thin layer chromatography. Reactions included 1 µg of *Se*MddA^{WT} or *Se*MddA^{E82Q} (neg. ctrl), [1-^{14}C]-acetyl-CoA, and 0.5 mM of substrate. After exposure of the TLC plate to a phosphor screen the resulting image was detected using a Typhoon Trio+ Variable Mode Imager (GE Healthcare).



Supplementary references

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