SUPPLEMENTARY MATERIAL

Identification of Critical Ligand Binding Determinants in *Mycobacterium tuberculosis* APS Reductase

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Running Title: Ligand Recognition in *M. tuberculosis* APS Reductase

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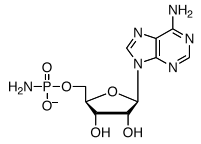
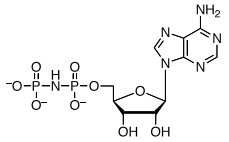
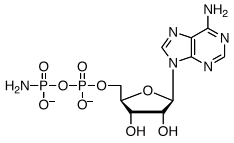
Table S1. Ligand dissociation constants for nitrogen-containing ligands at pH 7.5 and pH 9.0 with APS reductase.[a]

Ligand	Structure	K_d pH 7.5 [μM]	K_d pH 9.0 [μM]	ΔΔG [kcal/mol][b]	p*K*a
5'-AMPN		100	82	-0.16	3.0 (O), 8.15 (N)[c]
AMPNP		257	110	-0.53	7.7 (O), 8.25 (N)[2]
AMPPN		410	56.8	-1.2	3.0 (O), 8.15 (N)[c]

[a] For ligands in this table values of K_i were determined at pH 7.5 or 9.0 under single turnover conditions from the dependence of the observed rate constant at a given inhibitor concentration under conditions of subsaturating APS, such that K_i is equal to the K_d. Each value reflects the average of at least two independent experiments, and the standard deviation was less than 15% of the value of the mean. Kinetic data were nonlinear-least squares fit to a model of competitive inhibition. [b] Energetic difference in affinity of ligand at pH 9.0 relative to pH 7.5, $\Delta\Delta G = -RT\ln(K_d^{9.0}/K_d^{7.5})$. [c] p*K*a estimate approximated from value for phosphoramidic acid[3].

Table S2. Ligand dissociation constants for AMP and ADP with APS reductase in the presence and absence of $MgCl_2$.[a]

Ligand	$MgCl_2$ [mM]	K_d [μM]	ΔΔG [kcal/mol][b]
AMP	0	5.4	N/A
	0.5	21	0.82
	2.0	31	1.0
ADP	0	4.3	N/A
	0.5	7.3	0.32
	2.0	20	0.93

[a] For ligands in this table values of K_i were determined at pH 7.5 and the concentration of $MgCl_2$ indicated under single turnover conditions from the dependence of the observed rate constant at a given inhibitor concentration under conditions of subsaturating APS, such that K_i is equal to the K_d. Each value reflects the average of at least two independent experiments, and the standard deviation was less than 15% of the value of the mean. Kinetic data were nonlinear-least squares fit to a model of competitive inhibition. [b] Energetic difference in affinity for ligand with magnesium relative to without metal ion, $\Delta\Delta G = -RT\ln(K_d^{+MgCl2}/K_d^{-MgCl2})$.

References

1. Jaffe, E. K.; Cohn, M. ^{31}P nuclear magnetic resonance spectra of the thiophosphate analogues of adenine nucleotides; effects of pH and Mg^{2+} binding. *Biochemistry* **1978,** 17, 652-7.

2. Reynolds, M. A.; Gerlt, J. A.; Demou, P. C.; Oppenheimer, N. J.; Kenyon, G. L. N-15 and O-17 NMR-studies of the proton binding-sites in imidodiphosphate, tetraethyl imidodiphosphate, and adenylyl imidodiphosphate. *J. Am. Chem. Soc.* **1983,** 105, 6475-81.

3. Chanley, J. D.; Feageson, E. A study of hydrolysis of phosphoramides .2. Solvolysis of phosphoramidic acid and comparison with phosphate esters. *J. Am. Chem. Soc.* **1963,** 85, 1181-90.

Figure Legends

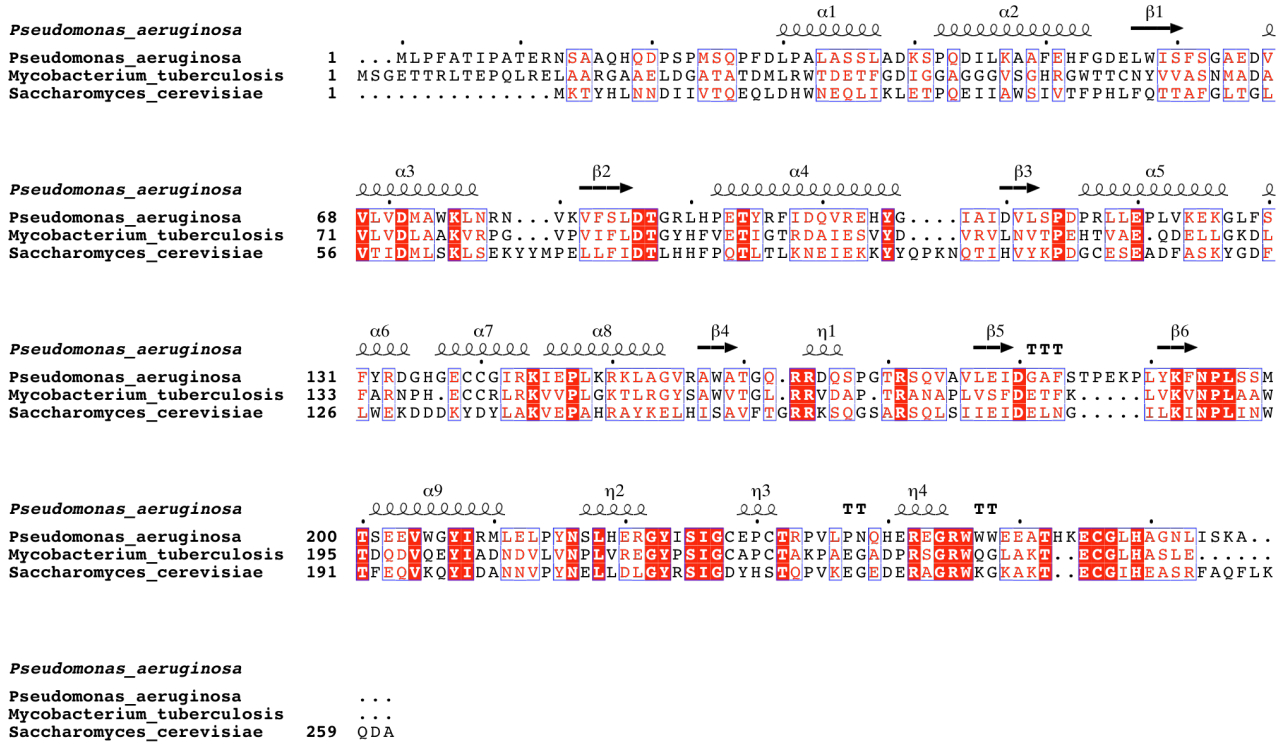
Figure S1. Structure based sequence alignment of APS reductases from *Pseudomonas aeruginosa, Mycobacterium tuberculosis* and *Saccharomyces cerevisiae*. The ClustalW Multiple Sequence Alignment program was used. Strictly conserved residues are outlined in red, red letters indicate conserved residues and conserved regions are boxed in blue. Alignment picture was rendered with the server ESPript 2.2 (http://espript.ibcp.fr).

Figure S2. The apparent affinity, $K_{1/2}$, of APS reductase in single turnover experiment. The affinity of APS reductase (E) for APS was determined from the dependence of the observed rate constant for *S*-sulfocysteine formation on protein concentration according to: $K_{obs} = K_{max} \times \left(\frac{[E]}{K_{1/2} + [E]} \right)$. In this equation, k_{obs} is the observed rate constant at a particular protein concentration, k_{max} is the maximal rate constant with saturating protein, and $K_{1/2}$ is the protein concentration that provides half the maximal rate. Because the chemical step is rate-determining for *S*-sulfocysteine formation, k_{max} is equal to the rate constant for the reaction of the E•APS complex, and $K_{1/2}$ is equal to the dissociation constant (K_d) of APS for APS reductase. The concentration of active protein was determined by direct titration with a high concentration of APS (*i.e.* [APS] >> K_d).

Figure S3. pH dependence for ADPβS (A) and AMPS (B) binding. The association equilibrium constant, ($K_a = 1/K_d$) is plotted as a function of pH. Values of K_d were determined by inhibition of APS reduction (pH 6.0-9.5). See methods for details. (A) The pH dependence for ADPβS binding. Nonlinear-least-squares fit of the data to a model for a single ionization gave pK_a

values of 5.8 ± 0.15. The pK_a of ADPβS in solution is 5.2[1]. (B) The pH dependence for AMPS binding. The dashed line represents the best fit of a model for a single ionization and yields a pK_a of 7.7 ± 0.15. The pKa of AMPS in solution is 5.3[1].

Figure S4. The radioactive assay for APS reductase. (A) The reaction progress curve for APS reductase. Under the subsaturating concentration of substrate, the reaction is described by the apparent second-order rate constant, k_{cat}/K_m, which under the conditions of this assay is ~2×10^6 $M^{-1}s^{-1}$. (B) AMP inhibits APS reductase activity. Nonlinear least squares fit to a model of competitive inhibition gives a K_d value of 5.4 μM.


Pseudomonas_aeruginosa
Pseudomonas_aeruginosa 1 ...MLPFATIPATERNSAAQHQDPSPMSQPFDLPALASSLADKSPQDILKAAFEHFGDELWISFSGAEDV
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Saccharomyces_cerevisiae 1 .....MKTYHLNNDIIVTQEQLDHWNEQLIKLETPQEIIAWSIVTFPHLFQTTAFGLTGL
Pseudomonas_aeruginosa 68 VLVDMAWKLNRN...VKVFSLDTGRLHPETYRFIDQVREHVG....IAIDVLSPDPRLLEPLVKEKGLFS
Mycobacterium_tuberculosis 71 VLVDLAAKVRPG...VPVIFLDTGYHFVETIGTRDAIESVYD....VRVLNVTPEHTVAE.QDELLGKDL
Saccharomyces_cerevisiae 56 VTIDMLSKLSEKYYMPELLFIDTLHHFPQTLTLKNEIEKKYYQPKNQTIHVYKPDGCESEADFASKYGDF
Pseudomonas_aeruginosa 131 FYRDGHGECCGIRKIEPLKRKLAGVRAWATGQ.RRDQSPGTRSQVAVLEIDGAFSTPEKPLYKFNPLSSM
Mycobacterium_tuberculosis 133 FARNPH.ECCRLRKVVPLGKTLRGYSAWVTGL.RRVDAP.TRANAPLVSFDETFK.....LVKVNPLAAW
Saccharomyces_cerevisiae 126 LWEKDDDKYDYLAKVEPAHRAYKELHISAVFTGRRKSQGSARSQLSIIEIDELNG.....ILKINPLINW
Pseudomonas_aeruginosa 200 TSEEVWGYIRMLELPYNSLHERGYISIGCEPCTRPVLPNQHEREGRWWEEATHKECGLHAGNLISKA..
Mycobacterium_tuberculosis 195 TDQDVQEYIADNDVLVNPLVREGYPSIGCAPCTAKPAEGADPRSGRWQGLAKT..ECGLHASLE.....
Saccharomyces_cerevisiae 191 TFEQVKQYIDANNVPYNELLDLGYRSIGDYHSTQPVKEGEDERAGRWKGKAKT..ECGIHEASRFAQFLK
Pseudomonas_aeruginosa
Pseudomonas_aeruginosa ...
Mycobacterium_tuberculosis ...
Saccharomyces_cerevisiae 259 QDA

Figure S1

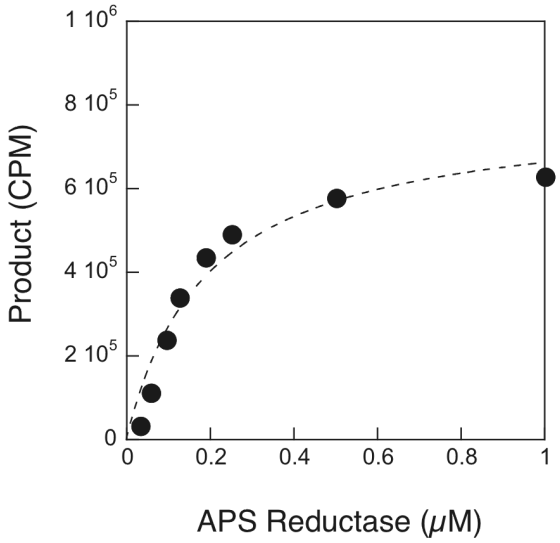
1 10^6
8 10^5
6 10^5
4 10^5
2 10^5
0
Product (CPM)
0
0.2
0.4
0.6
0.8
1
APS Reductase (μM)

Figure S2

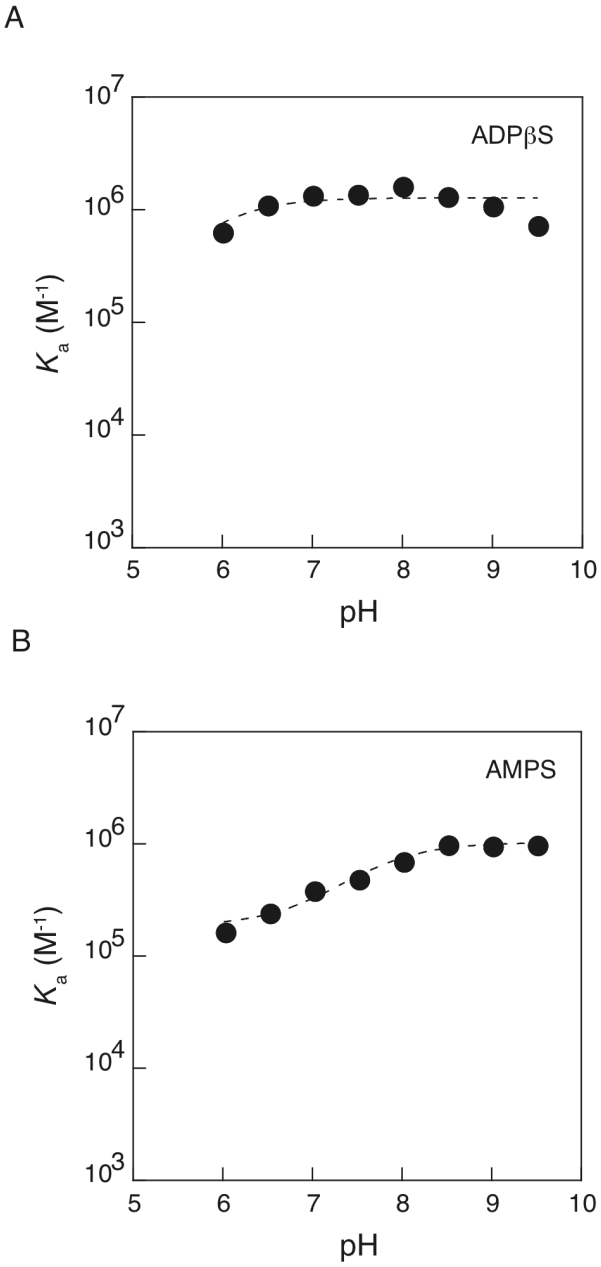
A
ADPβS
10^7
10^6
10^5
10^4
10^3
K_a (M^{-1})
5
6
7
8
9
10
pH
B
AMPS
10^7
10^6
10^5
10^4
10^3
K_a (M^{-1})
5
6
7
8
9
10
pH

Figure S3

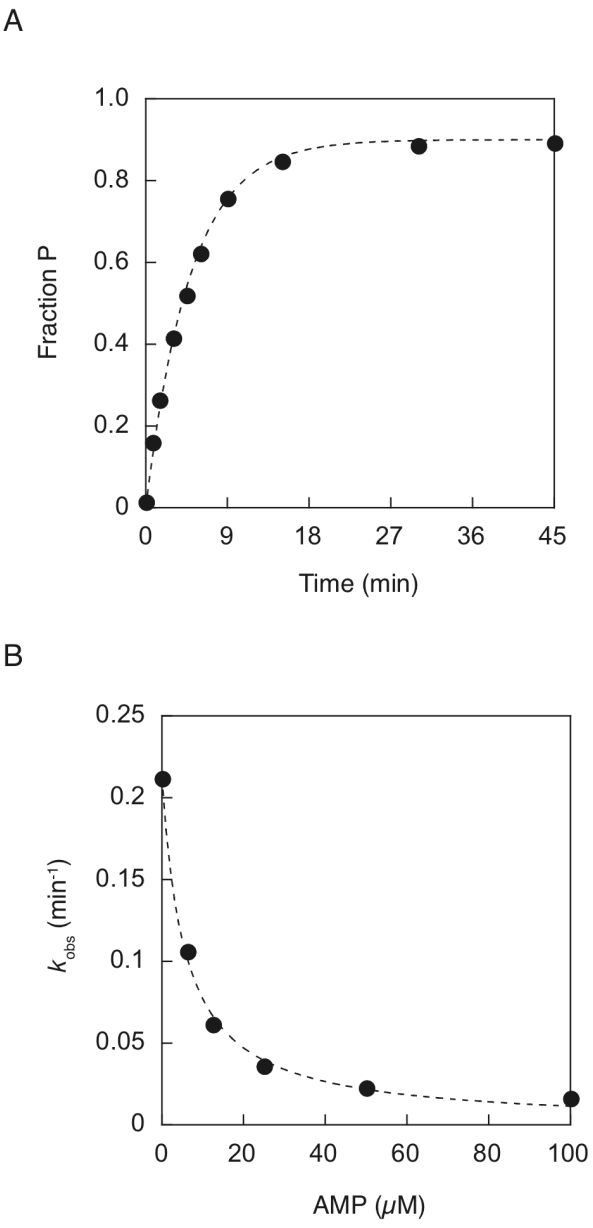
A
1.0
0.8
0.6
0.4
0.2
0
Fraction P
0
9
18
27
36
45
Time (min)
B
0.25
0.2
0.15
0.1
0.05
0
k_{obs} (min^{-1})
0
20
40
60
80
100
AMP (μM)

Figure S4