Deciphering the Role of Histidine 252 in Mycobacterial Adenosine 5′-Phosphosulfate (APS) Reductase Catalysis*

Jiyoun A. Hong† and Kate S. Carroll§

From the †Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109 and the §Department of Chemistry, The Scripps Research Institute, Jupiter, Florida 33458

Mycobacterium tuberculosis adenosine 5′-phosphosulfate reductase (APR) catalyzes the first committed step in sulfate reduction for the biosynthesis of cysteine and is essential for survival in the latent phase of tuberculosis infection. The reaction catalyzed by APR involves the nucleophilic attack by conserved Cys-249 on adenosine 5′-phosphosulfate, resulting in a covalent S-sulfocysteine intermediate that is reduced in subsequent steps by thioredoxin to yield the sulfite product. Cys-249 resides on a mobile active site lid at the C terminus, within a K(R/T)ECG(L/I)H motif. Owing to its strict conservation among sulfonucleotide reductases and its proximity to the active site cysteine, it has been suggested that His-252 plays a key role in APR catalysis, specifically as a general base to deprotonate Cys-249. Using site-directed mutagenesis, we have changed His-252 to an alanine residue and analyzed the effect of this mutation on APR catalysis, specifically as a general base to deprotonate Cys-249. Using site-directed mutagenesis, we have changed His-252 to an alanine residue and analyzed the effect of this mutation on APR catalysis, specifically as a general base to deprotonate Cys-249. Interestingly, our data demonstrate that His-252 enhances substrate affinity via interaction with the α-phosphate and the endocyclic ribose oxygen. These findings were further supported by isothermal titration calorimetry to provide a thermodynamic profile of ligand-protein interactions. From an applied standpoint, our study suggests that small-molecules targeting residues in the dynamic C-terminal segment, particularly His-252, may lead to inhibitors with improved binding affinity.

Tuberculosis remains a serious threat to public health, and new drugs are needed to simply and shorten treatment as well as fight multidrug-resistant tuberculosis. Toward this end, the inhibition of cysteine biosynthesis and, by extension, associated downstream metabolites represents fertile ground for the development of novel antibiotics (1, 2). In mycobacteria, the enzyme adenosine 5′-phosphosulfate reductase (APR) catalyzes the committed step in the biosynthesis of cysteine (Scheme 1) and is a validated target to develop new anti-tuberculosis agents, particularly for the treatment of latent infection (3, 4). APR lacks a human homolog but is highly conserved across a wide range of bacterial species (5), raising the possibility that APR may also represent an attractive target for the discovery or rational design of broad spectrum antibiotics. APR is also present in plants and is recognized as a potential target for herbicide development (6–8).

The importance of APR for microbial and plant survival has motivated investigations of its catalytic mechanism (9–12). These studies provide support for the two-step mechanism shown in Scheme 2, which involves the nucleophilic attack by conserved Cys-249 on adenosine 5′-phosphosulfate (APS) leading to the formation of a covalent enzyme S-sulfocysteine intermediate, \(E\text{-Cys-S}^\{\text{SO}_3\}^\text{−}\) bound to AMP. The sulfite product is then released via thiol-disulfide exchange with free thioredoxin in bacterial APR or via the action of the C-terminal thioredoxin-like protein domain in plant APR. In addition, APR contains an \([4Fe-4S]\) cluster that is essential for catalytic activity (6, 13–15). However, it is not involved in redox chemistry, and its role remains an active area of investigation (16, 17).

In 2006, the crystal structure of Pseudomonas aeruginosa APR (PaAPR) was solved in complex with APS, providing direct insight into substrate recognition (18). PaAPR and Mycobacterium tuberculosis APR (MtAPR) are homologous proteins sharing 27% identity and 41% similarity (supplemental Fig. S1), particularly in residues that line the active site (84% identity and 92% similarity). PaAPR and MtAPR have comparable reaction kinetics and ligand binding affinity (10, 19). Likewise, the PaAPR structure has been successfully employed in virtual ligand screening to identify low micromolar chemical inhibitors of MtAPR (20).

The structure of PaAPR shows that APS binds in a deep active site cavity with the phosphosulfate extending toward the protein surface (see Fig. 1A). Conserved and semiconserved residues participate in four main chain hydrogen bonds with adenine and the ribose \(\text{O}_2\text{−}\) hydroxyl. Interaction between the phosphosulfate and APR occurs via strictly conserved residues Lys-145, Arg-237, and Arg-240. The phosphosulfate is also positioned opposite the \([4Fe-4S]\) cluster. The C-terminal 18

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§1 The abbreviations used are: APR, adenosine 5′-phosphosulfate reductase; APS, adenosine 5′-phosphosulfate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate reductase; PaAPR, Pseudomonas aeruginosa APR; MtAPR, Mycobacterium tuberculosis APR; ScPAPR, Saccharomyces cerevisiae PAPR; ITC, isothermal titration calorimetry; L, ligand.

§2 The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S7 and an additional reference.

† To whom correspondence should be addressed: The Scripps Research Institute, 130 Scripps Way #2B2, Jupiter, FL 33458. Tel.: 561-228-2460; Fax: 561-228-2919; E-mail: kcarroll@scripps.edu.

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residues, carrying the catalytically essential Cys-249, were disordered in the structure of PaAPR. The lack of electron density information, coupled with limited proteolysis studies, led to the proposal that Cys-249 resides on a flexible “lid peptide” that closes over the active site pocket upon ligand binding (18).

This conformational change hypothesis was later confirmed when Fisher and co-workers (21) reported the crystal structure of the related enzyme, 3′-phosphoadenosine-5′-phosphosulfate (PAPS) reductase from Saccharomyces cerevisiae (ScPAPR) in complex with adenosine 3′,5′-diphosphate (PAP). Although APS and PAPS differ by a 3′-phosphate and PAPR lacks the [4Fe-4S] cluster, structural and functional studies show that the two-step mechanism for sulfite production in Scheme 2 is conserved among this family of enzymes, known collectively as sulfonucleotide reductases (10, 18, 22, 23). Sulfonucleotide reductases share ~25% overall amino acid identity, including two highly conserved domains, the sulfonucleotide-binding pocket, and C-terminal segment containing the K(R/T)EGG(L/I)H catalytic motif (supplemental Fig. S1; see also Ref. 18 for an alignment of 38 APR and 34 PAPR amino acid sequences). Likewise, sulfonucleotide reductases have virtually identical three-dimensional structures (superposition Cα backbone atoms from PaAPR and ScPAPR yields an root mean square deviation of 0.98 Å; see Fig. 1B). The crystal structure of ScPAPR is especially significant as it shows the flexible C-terminal segment folded over the active site pocket. In this conformation, a strictly conserved histidine residue His-252 within the K(R/T)EGG(L/I)H motif is proximal to the active site ligand (~3 Å) and Cys-249 (~4.5 Å) (see Fig. 1B). These three-dimensional relationships are recapitulated well in the homology model of MtAPR, generated on the basis of sequence alignment and the ScPAPR template structure (root mean square deviation of 0.1 Å for Cα backbone atoms; Fig. 1C).

Experimental Procedures

Materials—All chemicals, unless stated otherwise, were purchased from the Sigma and were of the highest purity available. The C-terminal peptide (AKTECGLHASW) was synthesized by solid-phase peptide synthesis using Fmoc-based chemistry and HPLC-purified to >98%. The molecular mass of the peptide was confirmed by mass spectrometry (1202.4 Da). Aristeromycin was synthesized from dimethyl-3-cyclopentene-1,1-dicarboxylate as described previously (24). 5′-Phosphoaristeromycin was prepared by chemical phosphorylation of aristeromycin using established methods (25). The physical and spectral data for 5′-phosphoaristeromycin were consistent with values reported in the literature for this nucleotide (25).

Mutagenesis and Protein Expression—The construction of the expression vector encoding wild-type MtAPR cloned into the vector pET24b (Novagen) has been described previously (10). The H252A mutant plasmid was prepared using QuikChange site-directed mutagenesis (Stratagene). Wild-type and mutant MtAPR were overexpressed and purified to homogeneity according to published procedures using nickel affinity and gel filtration column chromatography (17).

General APS Reductase Assay—APR assays were performed as described previously (17, 19). All assays were conducted at 30 °C. Unless otherwise indicated, the reaction conditions included 100 mM Bis-Tris propane (pH 6.5), supplemented with 5 mM DTT, and 10 μM Escherichia coli thioredoxin. Production of 35SO4 2− from 35S-labeled APS was monitored using charcoal-based separation and scintillation counting as reported previously (19). For each time point, the fraction product was calculated according to Equation 1,

\[ F = P / (P + S) \]

(Eq. 1)

where \( F \) is the fraction converted to product, \( P \) is product, and \( S \) is intact substrate. Reactions progress curves were analyzed using Kaleidograph (Synergy Software) as described below.

Single-Turnover Kinetics—Single-turnover APR assays were performed in the standard reaction buffer as described above. To ensure single-turnover reactions, the concentration enzyme was kept in excess over the concentration of [35S]APS (typically

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6 The only known exception is the enzyme from Bacillus subtilis, which possess an [4Fe-4S] cluster, but can utilize both substrates (13).

7 The distance between His-252 and Cys-249 differs for each monomer of the dimer in the crystal structure of ScPAPR.
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FIGURE 1. Representative structures from the sulfonucleotide reductase enzyme family. A, crystal structure of PaAPR in complex with substrate, APS (Protein Data Bank code 2GOY). Hydrogen bond interactions are depicted as yellow dashes. B, structure superposition of PaAPR bound to APS (gold, Protein Data Bank code 2GOY) and ScPAPR bound to PAP (orange, Protein Data Bank code 2OQ2) yields a root mean square deviation of 0.98 Å over Ca backbone atoms. The structure of ScPAPR shows that the C-terminal segment (orange) containing the conserved K(R/T)ECG(L/I)H motif folds over the active site. Hydrogen bond interactions are depicted as yellow dashes. C, no empirical three-dimensional structure information is currently available for MtAPR; however, the amino acid sequence of sulfonucleotide reductases is highly conserved, particularly among residues that define the active site (supplemental Fig. S1). In view of this, a homology model was built using the structure of ScPAPR (Protein Data Bank code 2OQ2) as a template and the Swiss-Model server. The model predicts several interactions between APS and His-252, analogous to those observed in the crystal structure of ScPAPR bound to PAP. Hydrogen bond interactions are depicted as yellow dashes. The magenta dashes indicate the predicted distance between the two nearest atoms of His-252 and Cys-249 (3 Å).

Reactions were followed to completion (≥5 half-lives) except for very slow reactions. The reaction progress curve was plotted as the fraction of product versus time and was fit by a single exponential using Kaleidagraph (Equation 2), where F is the fraction product, A is the fraction of substrate converted to product at completion, k_{obs} is the observed rate constant, and t is the time.

\[
F = A[1 - \exp(-k_{obs}t)] \tag{Eq. 2}
\]

\[
k_{obs} = k_{max}[E]/(K_{1/2} + [E]) \tag{Eq. 3}
\]

Under single-turnover conditions, the concentration dependence of the enzyme is hyperbolic (Equation 3). The maximal observed rate constant (k_{max}) corresponds to the rate of reaction at a saturating enzyme concentration, and the K_{1/2} value indicates the concentration at which half of the substrate is bound. For K_{1/2} determinations, the APR concentration was varied over a wide range, and reactions were carried out in the absence of thioredoxin, as described previously (19). Although we refer to the K_{1/2} value for maximal activity as K_{m'}, the value could differ from the K_{m} value for multiple turnover because the latter can be affected by product release. At least two or more enzyme concentrations were averaged, and K_{1/2} values were fit to a model for a single rate-controlling ionization as described by Equation 5.

\[
(k_{cat}/K_{m})_{obs} = (k_{cat}/K_{m})_{max}/(1 + [H^+]/K_a) \tag{Eq. 4}
\]

\[
(k_{cat}/K_{m}) = (k_{cat}/K_{m})_{max}/(1 + [H^+]/K_a) \tag{Eq. 5}
\]

The single-turnover rate constant (k_{max}) was determined at saturating concentration of APR, and this was confirmed by the observation of the same rate constant at two different concentrations of APR. Under these conditions, the observed rate constant is equal to the maximal single-turnover rate constant (k_{obs} = k_{max}) and monitors steps after binding up to and including the chemical step (Equation 3). The inhibition constant (K_i) was measured for various ligands by inhibiting the APR reaction under k_{cat}/K_{m} conditions with varying concentration of inhibitor (l). The data were fit to a simple model for competitive inhibition (Equation 4) and, with subsaturating APR, the K_i is equal to the equilibrium dissociation constant (K_a) of the inhibitor.

\[
K_a = K_{d}/(1 + [H^+]/K_a) \tag{Eq. 6}
\]
tration of the enzyme was varied from 0 to 50 μM (wild-type) or 0 to 400 μM (H252A). The enzyme was added to reaction buffer containing 100 mM Bis-Tris propane (pH 6.5) with 5 mM APS at 30 °C and then transferred into ultrafiltration devices (Microcon, 30-kDa cut-off, Millipore), and the free and bound ligand separated by centrifuging the samples at 3000 rpm for 2.5 min. Equal volumes of the filtrate and retentate were removed and quantified using scintillation counting. The ratio of $EL/L_{total}$ was determined as a function of $[E]_{total}$ and the $K_d$ value was obtained by fitting Equation 7 to these data.

$$\frac{EL}{L_{total}} = \frac{(EL/L_{total})_{\text{in}}}{1 + K_d/E_{\text{total}}} + \frac{(EL/L_{total})_{\text{background}}}{1 + K_d/E_{\text{total}}}$$  \hspace{1cm} \text{(Eq. 7)}$$

**Spectrophotometric pH$_a$ Determination of Cys-249—Buffer exchange of APR was performed using a PD-10 column (GE Healthcare) that had been pre-equilibrated with degassed water. Ionization of Cys-249 was monitored by absorption of the thiolate anion at 240 nm (23) using a Cary 50 UV-visible spectrometer (Varian) and a 1-cm path length quartz cuvette. APR was diluted to a final concentration of 20 μM in 10 mM MES buffers of various pH (5.0–8.0), and the absorption of the sample was monitored at 240 and 280 nm after correction for the absorbance of the MES buffer alone. The extinction coefficient at 240 nm ($\varepsilon_{240}$) was calculated using the ratio of absorbance at 240 and 280 nm (Equation 8).

$$\varepsilon_{240} = \varepsilon_{280} \times (A_{240}/A_{280})$$  \hspace{1cm} \text{(Eq. 8)}$$

$A_{240}/A_{280}$ is the ratio of the absorbance of the protein at 240 and 280 nm, $\varepsilon_{280}$ is the known extinction coefficient of APR at 280 nm (36,815 M$^{-1}$ cm$^{-1}$), and $\varepsilon_{240}$ is the extinction coefficient at 240 nm (23). The data were plotted as a function of pH, and the pH$_a$ was determined by fitting a version of the Henderson-Hasselbalch equation to the data (Equation 9).

$$\varepsilon_{240}(pH) = \varepsilon_{240}^{\text{SH}} + (\varepsilon_{240}^{\text{SH}} - \varepsilon_{240}^{\text{SS}})/(1 + 10^{pK_a - pH})$$  \hspace{1cm} \text{(Eq. 9)}$$

**ITC—Wild-type and H252A MtAPR were exchanged into 100 mM Bis-Tris propane buffer (pH 7.5). ITC experiments were performed using an iTC200 calorimeter from MicroCal (Northampton, MA). Experiments were carried out by titrating wild-type MtAPR (50 μM) with APS or AMP (250 μM) and H252A MtAPR (50 μM) with APS or AMP (1 μM) at 30 °C. A total of 20 injections were performed with a spacing of 180 s and a reference power of 5 μcal/s. Control experiments to determine the heat of dilution for each injection were performed by injecting the same volume of APS or AMP into the sample cell containing only buffer. The heat of dilution generated by the compounds was subtracted, and the binding isotherms were plotted and fit (Equation 10) to a single-site binding model using Origin ITC software.

$$q = v \times \Delta H \times [E] \times \left( \frac{K_d[L]^n}{1 + K_d[L]^n} - \frac{K_d[L]^{n-1}}{1 + K_d[L]^{n-1}} \right)$$  \hspace{1cm} \text{(Eq. 10)}$$

where $q$ is the directly measured amount of heat released during each injection, $v$ is the volume of the reaction, and $L_i$ is the ligand concentration at the $i$th injection. The $K_d$ was calculated as the inverse of the $K_a$.

**RESULTS AND DISCUSSION**

To advance our understanding of the molecular recognition and catalytic mechanism of MtAPR, we used site-directed mutagenesis to change His-252 to an alanine residue and characterize single turnover kinetic parameters for wild-type and H252A. Mutation of His-252 to Ala reduced $k_{cat}/K_m$ by 230-fold (Table 1), indicating that this residue contributes to catalytic efficiency by enhancing substrate affinity and/or stabilizing the catalytic transition state. To gain further insight into the role of the conserved active site histidine residue, we compared the saturating single-turnover rate constant ($k_{max}$), the $K_m$, and the substrate dissociation constant ($K_d$) for wild-type and H252A MtAPR (Table 1; see also supplemental Figs. S2–S5 for representative data). The results show that alanine substitution of His-252 decreased the value of $k_{max}$ by only 2-fold, whereas $K_m$ and $K_d$ were both weakened by more than two orders of magnitude. Control experiments showed that there was no difference in iron incorporation or [4Fe-4S] cluster stability between the wild-type and variant MtAPR (supplemental Fig. S6), consistent with the long-range distance (∼ 10 Å) that is predicted between His-252 and the metallocenter.

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**TABLE 1**

| Enzyme | $k_{cat}/K_m$ | ($k_{cat}/K_m$)$_{\text{rel}}$ | $k_{max}$ | ($k_{max}$)$_{\text{rel}}$ | $K_m$ | ($K_m$)$_{\text{rel}}$ | $K_d$ | ($K_d$)$_{\text{rel}}$ |
|--------|---------------|-----------------------------|----------|-------------------|------|-----------------|------|-----------------|
| Wild-type | 3.0 × 10$^6$ | 230 | 2.8 | 2.0 | 0.2 | >250 | 0.2 | 450 |
| H252A | 1.5 × 10$^4$ | (1) | 1.4 | (1) | >50 | (1) | 90 | (1) |

$^a$ $k_{cat}/K_m$ values were measured as described under "Experimental Procedures."

$^b$ Single-turnover rate constants with saturating wild-type or H252A MtAPR.

$^c$ $K_m$ values for $S$-sulfoxysteine formation were measured in the absence of thioredoxin by varying the concentration of wild-type or H252A MtAPR (see "Experimental Procedures").

$^d$ Dissociation constants were measured using ultrafiltration at 30 °C (100 mM Bis-Tris propane, pH 6.5) as described under "Experimental Procedures."

$^e$ From Ref. 26.

$^f$ In Bis-Tris propane at pH 7.5. Due to technical limitations of the TLC-based assay only a lower limit could be obtained.
limb for reaction of APS with wild-type or H252A has a first-order dependence on the proton concentration, consistent with a single inactivating protonation at acidic pH. For $k_{\text{cat}}/K_m$, these kinetic $pK_a$ values could represent ionization of either free enzyme or substrate. The data described below support the model with ionization of the Cys-249 thiol.

The acidic limb of the pH dependence for the APR-catalyzed reduction of APS is best fit by a $pK_a$ of 6.1 $\pm$ 0.1 and 6.3 $\pm$ 0.1 for wild-type and H252A MtAPR, respectively (Fig. 2). The most likely candidate for this ionization is the enzyme, specifically of catalytic cysteine, because the substrate $pK_a$ falls significantly below this region. To test this proposal, we determined the $pK_a$ of Cys-249 by measuring the change in absorbance of UV light at 240 nm resulting from formation of the thiolate anion, as described previously (23, 27, 28). For these studies, we utilized C59A MtAPR, which has identical kinetic properties to the native enzyme (10, 15) but eliminates a nonconserved cysteine that could confound the analysis.

The $pK_a$ of C59A MtAPR at 240 ($\varepsilon_{240}$) displays a transition with a $pK_a$ of 6.2 $\pm$ 0.1 (Fig. 3A). The change in $\varepsilon_{240}$ is most likely due to ionization of Cys-249, as indicated by the absence of a pH-dependent transition for C59A/C249A MtAPR (Fig. 3A). The pH dependence of the molar extinction coefficient of C59A/H252A MtAPR at 240 ($\varepsilon_{240}$) shows a transition with a $pK_a$ of 6.0 $\pm$ 0.1 (Fig. 3B). For comparison, we evaluated the $pK_a$ of Cys-249 within a synthetic peptide derived from the last 10 C-terminal residues of MtAPR (supplemental Fig. S4). The $pK_a$ of the thiol in the peptide segment was determined as 8.3 $\pm$ 0.1, consistent with the $pK_a$ value of free cysteine solution (29).

Interestingly, our experiments indicate that thiolate formation at Cys-249 correlates with decrease in signal at $\varepsilon_{240}$ as opposed to the increase that is normally observed. Therefore, the ionization constant of Cys-249 was verified by an independent method using the thiol-specific reagent, monobromobimane (24). In this assay, the $pK_a$ value of Cys-249 for C59A MtAPR was determined to be 6.0 $\pm$ 0.1 (supplemental Fig. S4), which is within error of the UV-based method. The similarity of the kinetic $pK_a$ and the $pK_a$ value for Cys-249 deprotonation strongly suggest that the observed inflection in $k_{\text{cat}}/K_m$ corresponds to the ionization of the active site cysteine to form the thiolate anion.

To further investigate the molecular recognition properties for wild-type and H252A MtAPR, we compared the pH dependence for binding of the nucleotide product, AMP. As shown in Fig. 4, the logarithm of the association constant ($K_a = 1/K_d$) shows a first-order dependence on the proton concentration. The acidic limb for wild-type MtAPR binding to AMP has a $pK_a$ of 8.1 $\pm$ 0.1, as reported previously (19). The $pK_a$ values observed in product affinity could reflect ionizations in either or both the ligand and the enzyme, analogous to the pH dependence for $k_{\text{cat}}/K_m$ discussed above. A likely explanation...
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for the weaker binding of AMP below pH 8 is that the dianion binds more tightly than the monoanion. However, the apparent pKₐ of AMP differs from the pKₐ of AMP in solution (~6.8) by more than one unit. The discrepancy between the experimental data and this model is most likely due to concurrent ionization of the enzyme that affects ligand binding, leading to shift in the apparent pKₐ of AMP. One model that could account for this upward deviation is that an enzymatic group with a pKₐ of ~6 contributes slightly (~5-fold) to AMP binding when protonated. Given its proximity to the α-phosphate, the most likely residue to exert such an effect on ligand binding is His-252. Consistent with this proposal, the acidic limb for H252A MtAPR binding to AMP displays a pKₐ of 6.4 ± 0.1 (Fig. 4). An additional observation from these data is that binding of the nucleotide product to H252A is weaker at physiological pH and above, as compared with wild-type MtAPR. For example, at pH 7.5 wild-type and H252A MtAPR bind to AMP with respective pKₐ values of 5.4 ± 0.2 µM and 50.5 ± 3 µM.

The crystal structure of scPAPR (21) and the model of MtAPR shown in Fig. 1 indicate that the side chain of His-252 is positioned within hydrogen bonding distance of the α-phosphate and the endocyclic ribose oxygen of the active site ligand. Previous studies have demonstrated the relative importance of the α-phosphate group for AMP binding to MtAPR (~3 kcal/mol) (19); however, the contribution of O-4 in the ribose sugar has not been investigated. To examine the importance of the hydrogen bond contact between His-252 and the endocyclic ribose oxygen, we synthesized 5′-phosphoaristeromycin, which replaces O-4 in AMP with a methylene unit. Binding studies indicate that at pH 7.5, this analog binds to MtAPR with a Kₐ value of 25 µM ± 2.5 µM (Fig. 5) or 5-fold more weakly than AMP. These data indicate that the interaction of His-252 with the ribose O-4 makes a modest contribution to ligand recognition (~1 kcal/mol).

To substantiate the role of His-252 in substrate binding, we performed additional biophysical experiments. In initial experiments, we attempted to monitor spectral perturba-

**FIGURE 4.** pH dependence for AMP binding. The association equilibrium constant (Kₛ = 1/Kᵣₛ) is plotted as a function of pH. Values of Kₛ were determined by inhibition of APS reduction with [S] < Kₛ such that Kₛ is expected to be Kᵣₛ. The average of three independent determinations is shown, and the error bars indicate S.D. Nonlinear least-squares fit of the data to a model for simple competitive inhibition (Equation 4) gave a dissociation constant (Kᵣₛ) of 25 µM.

**FIGURE 5.** Binding of 5′-phosphoaristeromycin to MtAPR. The average of three independent determinations is shown, and the error bars indicate the S.D. Nonlinear least-squares fit of the data to a model for simple competitive inhibition (Equation 4) gave a dissociation constant (Kᵣₛ) of 25 µM.
(4). This key discovery has led to the proposal that small molecule inhibitors of APR might be a source for new drugs to treat latent tuberculosis infection (1, 3, 20). The increasing number of antibiotic-resistant strains suggests that the availability of such compounds could play an important role in treating the disease and minimizing the impact on human health. Defining active site residues that are essential for molecular recognition in MtAPR sets the stage for the development of such drugs. Toward this end, results from the present study suggest that targeting dynamic elements within the active site, particularly Cys-249 and His-252, may increase the potency of APR inhibitors.

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