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Role of conserved histidine residues in metalloactivation of the ArsA ATPase

Hiranmoy Bhattacharjee & Barry P. Rosen*
*Department of Biochemistry and Molecular Biology, Wayne State University School of Medicine, Detroit, MI 48201, USA; *Author for correspondence (E-mail: brosen@med.wayne.edu)*

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Abstract

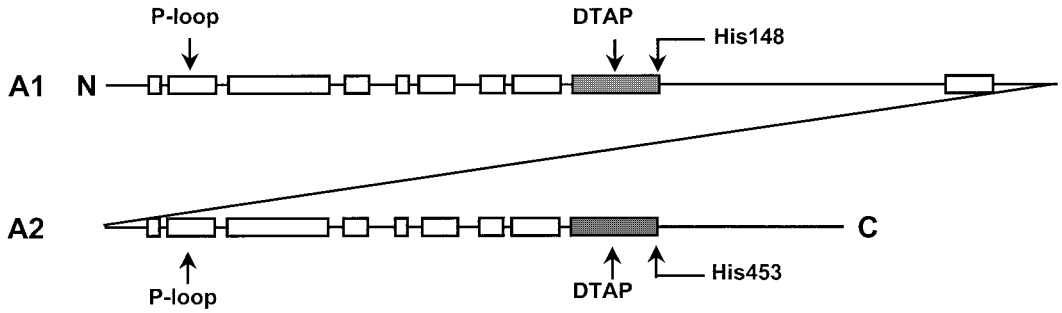
The ArsA ATPase is the catalytic subunit of a pump that is responsible for resistance to arsenicals and antimonials in *Escherichia coli*. Arsenite or antimonite allosterically activates the ArsA ATPase activity. ArsA homologues from eubacteria, archaea and eukarya have a signature sequence (DTAPTGHT) that includes a conserved histidine. The ArsA ATPase has two such conserved motifs, one in the NH_2-terminal (A1) half and the other in the COOH-terminal (A2) half of the protein. These sequences have been proposed to be signal transduction domains that transmit the information of metal occupancy at the allosteric to the catalytic site to activate ATP hydrolysis. The role of the conserved residues His148 and His453, which reside in the A1 and A2 signal transduction domains respectively, was investigated by mutagenesis to create H148A, H453A or H148A/H453A ArsAs. Each altered protein exhibited a decrease in the V_{max} of metalloid-activated ATP hydrolysis, in the order wild type ArsA>H148A>H453A>H148A/H453A. These results suggest that the histidine residues play a role in transmission of the signal between the catalytic and allosteric sites.

Introduction

The conjugative R-factor R773 confers arsenical or antimonial resistance in *Escherichia coli* by coding for an ion-translocating ATPase. This pump actively extrudes arsenite or antimonite out of the cell, thereby reducing their intracellular concentration to subtoxic levels (Bhattacharjee *et al.* 1999). The pump is composed of two proteins, ArsA and ArsB. ArsA is the catalytic subunit of the pump and hydrolyzes ATP in the presence of arsenite or antimonite. The chemical energy so released is coupled to extrusion of arsenite or antimonite out of the cell through ArsB, an integral membrane protein. ArsB serves both as a membrane anchor for ArsA and also as an ion-conducting channel. When expressed at high levels, ArsA is found predominantly as a soluble protein in the cytosol. Soluble ArsA has been purified and shown to be an As(III)- or Sb(III)-stimulated ATPase (Rosen *et al.* 1988).

The 583-residue ArsA polypeptide is composed of homologous N-terminal (A1) and C-terminal (A2) halves (Figure 1), suggesting that the *arsA* arose by gene duplication and fusion of a primordial ancestor. Each half has a nucleotide binding site (NBS). The A1 and A2 halves are held together by a short linker peptide (Li & Rosen 2000). The molecular mechanism of ArsA activation has been well studied (Rosen *et al.* 1999). In the absence of allosteric activator, ArsA has basal level of ATPase activity. The allosteric activators, As(III) or Sb(III), coordinate with Cys113 and Cys172 in the A1 half and Cys422 in the A2 half, producing a conformational change that brings the A1 and A2 NBDs into contact with each other, hence accelerating catalysis (Bhattacharjee *et al.* 1995; Bhattacharjee & Rosen 1996).

ArsA homologues have been identified in representatives from each of the three kingdoms (Mukhopadhyay *et al.* 1998). All known bacterial ArsAs have the repeated A1-A2 duplicated structure. Archaea have either an A1-A2 structure or just a single

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136  FDHIIFDTAPTGHTIRLLQLPGAWS  R773 ArsA A1
441  KRFVVMDTAPTGHTLLLLDATGAYH  R773 ArsA A2
136  FDHIIFDTAPTGHTIRLLQLPGAWS  R46 ArsA A1
441  KRFVVMDTAPTGHTLLLLDATGAYH  R46 ArsA A2
136  FDHIIFDTAPTGHTIRLLQLPGAWS  Acidiphilium multivorum A1
441  KRFVVMDTAPTGHTLLLLDATGAYH  Acidiphilium multivorum A2
143  YDVVVFDTAPTGHTIRLMELPSDWN  Halobacterium salinarum A1
475  YDIVVFDTAPTGHTLRLLELPSDWK  Halobacterium salinarum A2
139  YDIVIFDTAPTGHTLRLLSFPEIMD  Methanobacterium thermoautotrophicum
152  FDVVIFDTAPTGHTLRFLGMPEVMD  Methanococcus jannaschii
160  FDTVIFDTAPTGHTLRFLQLPNTLS  Saccharomyces cerevisiae
152  FDVVVFDTAPTGHTLRLLQFPTLLE  Caenorhabditis elegans
128  YDYIVVDTAPTGHTLGLLKTVRNLG  Aquifex aeolicus
160  FSVVVFDTAPTGHTLRLLNFPTIVE  Homo sapiens
160  FSVVVFDTAPTGHTLRLLNFPTIVE  Mus musculus
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Figure 1. Consensus sequences in ArsA homologues. *Top*, Diagram of the linear sequence of the 583-amino acid ArsA ATPase. The two homologous A1 and A2 halves are connected by a 25-residue linker (not drawn to scale). Regions of the highest similarity are boxed. Both A1 and A2 halves have ATP binding sites; the phosphate binding loops (*P-loops*) of each are identified. The shaded box indicates the conserved DTAPTGHT sequence, with the location of His148 and His453 indicated. *Bottom*, The conserved histidine is shaded inside the boxed sequence. Shown are the relevant sequences from R773 ArsA (P08690) and from ArsA homologues R46 ArsA (P52145), *Acidiphilium multivorum* (O50593), *Halobacterium salinarum* (O52027), *Methanobacterium thermoautotrophicum* (O27555), *Methanococcus jannaschii* (Q58542), *Saccharomyces cerevisiae* (NP_010183), *Caenorhabditis elegans* (P30632), *Aquifex aeolicus* (O66674), *Homo sapiens* (O43681), and *Mus musculus* (O54984). Accession numbers are given in parentheses. Homologues having A1 and A2 domains are indicated.

'A' arrangement. Halobacteria have an A1-A2 organization while methanogenic archaea have single 'A' structure. All eukaryotes known to date are single 'A' domain proteins. A common feature of the ArsA homologues is the presence of a Walker A motif – the glycine-rich P loop (GKGGVGKT) that interacts with the phosphate moiety of ATP or GTP (Walker *et al.* 1982). The *Escherichia coli* R773 ArsA has two such Walker A motifs (Figure 1), located in the A1 and A2 halves of the protein, both being required for catalytic activity and resistance (Karkaria *et al.* 1990; Kaur & Rosen 1992). In what is the likely Walker B motif, ArsA homologues have a highly conserved Asp-Pro sequence; the conserved Asp45 in the A1 half of the R773 ArsA has been shown to be required for Mg^{2+} binding (Zhou & Rosen 1999).

ArsA homologues can be identified by a signature sequence, DTAPTGHT (Zhou & Rosen 1997). This sequence is highly conserved in ArsA homologues from every kingdom, indicating that this common motif has a conserved function (Figure 1). The R773 ArsA has two such sequences, located in the A1 and A2 halves of the protein (Figure 1). During ATP hydrolysis the carboxy-terminal end of the A1 sequence becomes exposed to a less polar environment, whereas the amino-terminal end becomes exposed to a more hydrophilic environment as the product, ADP, is formed, indicating that this sequence exhibits considerable conformational mobility during the catalytic cycle. From these results the DTAPTGHT sequences have been proposed to be signal transduction domains that are involved in cross talk between the NBSs and the allosteric domain (Zhou & Rosen 1997). Recently

the 2.3 Å crystal structure of ArsA has been reported (Zhou *et al.* 2000). The two DTAPTGHT sequences clearly spans the spaces between the MgADP-filled NBSs and the metalated allosteric site.

The present study investigates the role of conserved ArsA residues His148 and His453 in the A1 and A2 DTAPTGHT sequences, respectively. His148 and His453 were either individually or simultaneously changed to alanine by site directed mutagenesis of their respective codons in the *arsA* gene. Characterization of these altered ArsAs indicates that the two histidines are not involved in either substrate binding or catalysis, since the altered enzymes had affinities for ATP and Sb(III) that were essentially wild type. On the other hand, the alanine-substituted enzymes had reduced ability to couple antimonite binding to allosteric activation of catalysis. These results support the hypothesis that the DTAPTGHT sequences are part of the signal transduction domains of ArsA, and that His148 and His453 are involved in transmission of information on metalloid occupancy in the allosteric domain to stimulation of ATP hydrolysis by the catalytic domain.

Materials and methods

Materials

All restriction enzymes, nucleic acid modifying enzymes and oligonucleotides were obtained from Life Technologies, Inc. Ni-NTA Superflow resin was purchased from QIAGEN. All other chemicals were purchased from commercial sources.

Medium and growth conditions

E. coli strains and plasmids used in this study are described in Table 1. Cells were grown at 37 °C in LB medium (Sambrook *et al.* 1989). Ampicillin (125 μg/ml) and tetracycline (10 μg/ml) were added as required.

Oligonucleotide-directed mutagenesis

Mutations in the sequence of the *arsA* gene were introduced by site-directed mutagenesis using the Altered Sites^{TM} *in vitro* Mutagenesis System (Promega). Plasmid pABH6 containing the *arsA* and *arsB* genes was used as the template to produce H148A, H453A, or H18A/H453A substitutions. The mutagenic oligonucleotides used and the respective changes introduced (underlined) were as follows:

H148A, 5′-CAGAAGGCGAATGGTGGCACCCGT CGGCGCGGT-3′
H453A, 5′-CAGCAGCAACAGCGTGGCTCCGGT CGGAGCCGT-3′

DNA manipulation and sequence analysis

Plasmid DNA was purified using QIAprep Spin Miniprep Kit (QIAGEN). DNA restriction endonuclease analysis, ligation and transformation were performed as described (Sambrook *et al.* 1989). All mutations were confirmed by sequencing the entire *arsA* gene using an ALFexpress system (Amersham Pharmacia Biotech) and Cy5-labeled primers. Plasmid DNA for sequencing was isolated with a QIAGEN Plasmid Mini Kit.

Purification and assay of ArsA ATPase

Altered ArsA proteins were purified from cultures of *E. coli* strain JM109 harboring the indicated plasmids. Cells were grown at 37 °C in LB medium to the mid-exponential phase of growth, at which point 0.1 mM isopropyl β-D-thiogalactopyranoside was added to induce ArsA expression. The cells were grown for another 3 h before being harvested by centrifugation. The soluble ArsA proteins were purified as described (Zhou & Rosen 1997), except that glycine was replaced with imidazole in all of the buffers. The concentration of ArsA in purified preparations was determined from the absorption at 280 nm using a molar extinction coefficient of 33 480 (Rosen *et al.* 1988). ATPase activity was assayed using an NADH-coupled assay method (Hsu & Rosen 1989; Vogel & Steinhart 1976). Unless otherwise noted, assays were done with 5 mM ATP and 2.5 mM $MgCl_2$.

Limited trypsin digestion of ArsA

Limited trypsin digestion was performed at room temperature and terminated at the indicated times by the addition of a 2-fold excess of soybean trypsin inhibitor to the reaction mixture (Hsu & Rosen 1989). Samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using 12% polyacrylamide gel and Coomassie Blue staining.

Table 1. Strains and plasmids.

Strain/Plasmid	Genotype/description	Reference
E. coli strains		
JM109	*endA*1, *rec*A1, *gyrA*96, *thi*, *hsdR*17 (r_k^-, m_k^+), *rel*A1, *supE*44, λ^-, Δ(*lac-proAB*), [F′, *traD*36, *proA*$^+$*B*$^+$, *lacI*^{q}ZΔM15]	Sambrook *et al.* 1989
ES1301	*lacZ*53, *mutS*201::Tn5, *thyA*36, *rha*-5, *metB*1, *deoC*, IN(*rrnD-rrnE*)	Promega
Plasmids		
pALTER^{TM}-1	Cloning and mutagenesis vector, Tc^r	Promega
pALTER-AB	3.2-kilobase pair *Hin*dIII-*Kpn*I fragment containing *ars*A and *ars*B genes cloned into the multiple cloning site of pALTER-1 vector, *arsAB*, Tc^r	Bhattacharjee *et al.* 1995
pABH6	pALTER-AB with six histidine codons added to 3′-end of *arsA*, Tc^r	Zhou & Rosen Unpublished work
pH148AH6	Site directed mutagenesis of codon 148 to Ala codon in *arsA* gene of pABH6, Tc^r, Amp^r	This study
pH453AH6	Site directed mutagenesis of codon 453 to Ala codon in *arsA* gene of pABH6, Tc^r, Amp^r	This study
pH148A/H453AH6	Site directed mutagenesis of both codons 148 and 453 to Ala codons in *arsA* gene of pABH6, Tc^r, Amp^r	This study

Results

Analysis of altered ArsA proteins

His148 and His453 were changed, either individually or simultaneously, to alanine residues, producing ArsA derivatives H148A/H453A and H148A/H453A. The steady-state level of production of the altered ArsA proteins was found to be the same as the wild type ArsA protein, as judged by Western Blot analysis using an antiserum against wild type ArsA protein (data not shown). Wild type and altered ArsA proteins were purified by Ni^{2+} affinity chromatography to $>95\%$ homogeneity. There was no difference between the wild type and altered proteins in mobility on SDS-PAGE, nor was abnormal degradation of the altered proteins observed.

The purified proteins were analyzed for their ability to hydrolyze ATP in the presence of antimonite or arsenite. The K_m for ATP was between 20 and 70 μM for the wild type and three substituted ArsAs (Table 2). The concentration of either arsenite or antimonite required for half maximal activation was observed to be similar in magnitude between the wild type and altered ArsA proteins (Table 2). Similarly, no difference in the affinity for Mg^{2+} was noted between wild type and altered ArsA proteins (Table 2). These results indicate that the nucleotide and metalloid binding domains in the alanine-substituted enzymes were relatively unchanged. The basal rate of ATPase activity was similar between the wild type and altered ArsA proteins (Table 3). However, the activated rates in presence of arsenite or antimonite differed considerably. Wild type ArsA showed a 10-fold stimulation in ATPase activity in presence of Sb(III). H148A and H453A ArsA exhibited a 5-6-fold Sb(III)-stimulated ATPase activity. The double histidine substituted H148A/H453A ArsA exhibited only a 3-fold stimulation of ATPase activity. The results with arsenite as activator were consistent with that of antimonite. Compared to wild type ArsA that exhibited a 5-fold As(III)-stimulated ATPase activity, H148A ArsA exhibited only a 2-fold stimulation in activated rate. The H453A ArsA and H148A/H453A ArsAs were practically unresponsive to As(III).

Table 2. ArsA ATPase activity: dependence on the concentrations of ATP, Mg (II) and oxyanions.

ArsA	ATP[a,b] K_m (μM)	[Sb(III)][c] $[C]_{50\%\ activation}$ (μM)	[As(III)][c] $[C]_{50\%\ activation}$ (mM)	[Mg (II)][d] $[C]_{50\%\ activation}$ (mM)
Wild type	70	4	0.8	0.7
H148A	35	4	1.8	0.6
H453A	30	2	1.1	0.5
H148A/H453A	20	2	n.d[e]	0.4

[a]ATPase activity assayed with 0.1 mM Sb(III) in the form of potassium antimonyl tartrate.
[b]ATPase activity corrected for basal activity in the absence of Sb(III).
[c]Assays performed with 5 mM ATP.
[d]Assays performed with 5 mM ATP and 0.1 mM Sb(III).
[e]not determined.

Table 3. V_{max}[a,b] of oxyanion-stimulated ATPase activity of ArsA proteins.

ArsA	−As(III) or −Sb(III)	+As(III)	As(III)-stimulated	+Sb(III)	Sb(III)-stimulated
Wild type	90	350	260	1025	935
H148A	65	140	75	400	335
H453A	60	85	25	285	225
H148A/H453A	55	65	10	180	125

[a]Assays performed with 5 mM ATP.
[b]Units of ATPase activity in nanomoles of ATP hydrolyzed/min/mg of protein.

Limited trypsin digestion of the altered ArsAs

Limited trypsin digestion has been used as a sensitive tool to assess the surface accessibility and ligand-induced conformational changes of the 63-kDa ArsA protein (Hsu & Rosen 1989). In the absence of substrates, trypsin initially cleaves ArsA at Arg290 (Ramaswamy & Kaur 1998), which is located in the short linker peptide that connects the A1 and A2 halves of the protein (Li & Rosen 2000). This is followed by rapid digestion of the A2 half, leaving the A1 half intact. When either wild type or H148A/H453A ArsA was treated with trypsin in the presence of the activator Sb(III) or As(III) the protein was rapidly digested to the 30-kDa species that represents the A1 half of the protein (Figures 2A and 2C). A similar accessibility to trypsin indicates that the wild type and double alanine-substituted enzymes have overall similar conformations. With both proteins addition of ATP decreases the rate of production of the 30-kDa species, with production of a 50-kDa species not observed in the absence of ATP (Figures 2A and 2C). Addition of ATP and Sb(III) together conferred substantial protection from trypsin with both proteins. The full-length 63-kDa ArsA remained present for up to 30 min of trypsin treatment in the presence of both ligands for both wild type and altered proteins (Figures 2A and 2C). Again, the similarity in trypsin accessibility in the presence ligands points to similar conformational changes produced by binding of ligands to either wild type or altered ArsA. Thus, no differences in the conformation of the wild type and alanine-substituted proteins could be detected by trypsin accessibility under these noncatalytic conditions.

In contrast, when Mg^{2+} was added to produce catalysis, the altered enzyme exhibited a clear difference from the wild type (Figures 2B and 2D). Under catalytic conditions [ATP, Sb(III) or As(III) and Mg^{2+}], the wild type enzyme was rapidly digested to a 50-kDa species that was quite stable to further trypsin attack. ATP and Sb(III) or As(III) protected the 63-kDa wild type ArsA species to a significant extent of time; under catalytic conditions produced by addition of Mg^{2+}, wild type ArsA was rapidly cleaved to a 50-kDa species that was then resistant to trypsin (Figure 2B). In contrast, under catalytic conditions, the H148A/H453A ArsA exhibited a different response to trypsin, with greater stability of the full-length protein and no build-up of the 50-kDa species (Figure 2D).

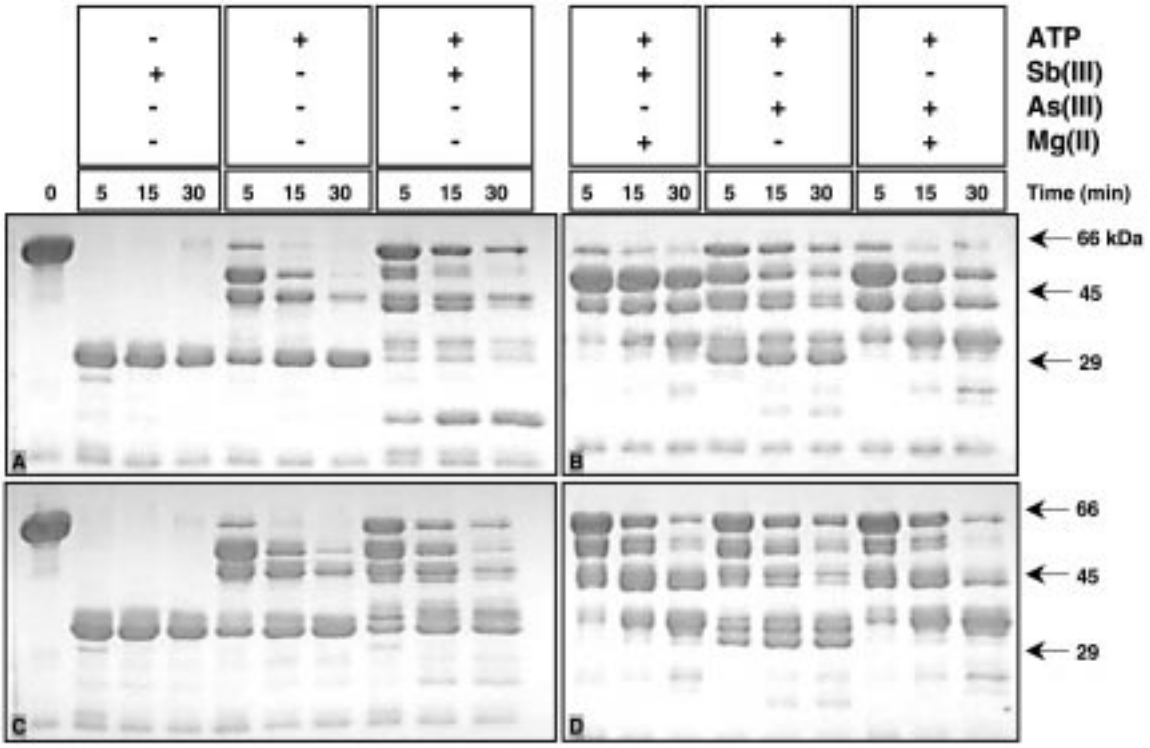

Figure 2. Effect of alanine-to-histidine substitutions on trypsin sensitivity. Trypsin digestion was performed at room temperature with the indicated additions: 5.0 mM ATP, 1.0 mM potassium antimonyl tartrate [Sb(III)], 5.0 mM $MgCl_2$or 10.0 mM sodium arsenite [As(III)]. Wild type ArsA (Panel A and B) or H148A/H453A ArsA (Panel C and D) (1.0 mg/ml, final concentration) were mixed with trypsin (0.01 mg/ml, final concentration). At the indicated times, samples were removed, and the reactions were terminated by the addition of a two fold excess of soybean trypsin inhibitor. The tryptic products were analyzed by SDS-PAGE on 12% polyacrylamide gels and stained with Coomassie Blue. The positions of migration of standards are indicated.

The two single histidine substituted proteins, H148A and H453A, showed the same pattern of trypsin digestion as the doubly substituted H148A/H453A ArsA (data not shown). Thus, the results of the trypsin sensitivity assays demonstrate that the conformation of the altered ArsA is similar to that of wild type when ATP is not being hydrolyzed, but it acquires a different conformation than the wild type during catalysis.

Discussion

A consensus sequence, DTAPTGHT, has been identified in ArsA homologues from prokaryotes, archaea and eukaryotes (Bhattacharjee *et al.* 1999). The high degree of sequence conservation implies that this signature sequence for ArsA homologues, has a conserved function. In ArsA there are two such motifs, one in A1 and the other in A2 half of the protein. These motifs have been proposed to be part of signal transduction domains involved in transmitting the information of metalloid occupancy of the allosteric site to the catalytic site, activating ATP hydrolysis (Zhou & Rosen 1997). Recently the crystal structure of ArsA at 2.3 Å has been reported (Zhou *et al.* 2000). In that structure, with bound Sb(III) and MgADP, the A1 and A2 DTAPTGHT sequences stretch from the two NBDs to the metallated allosteric site (Figure 3). There are three Sb(III) in the allosteric site, and imidazole nitrogen of His148 or His453, respectively, are ligands to two of the metals. The structure is consistent with our hypothesis that the signature sequence is a physical link that transmits information from the allosteric to the catalytic domains and imply that the two histidine are directly involved in signal transduction. For that reason the function of His148 and His453 in the A1 and A2 signature sequences, respectively, were investigated. The codon for His148 and His453 were either individually or simultaneously changed to alanine by site-directed mutagenesis. Replacement of the conserved histidine with alanine residue did not grossly perturb the tertiary structure of ArsA.

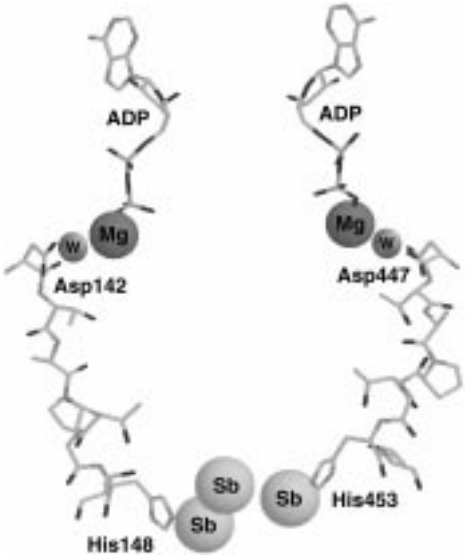

Figure 3. Structure of the DTAP domain. The A1 and A2 DTAPTGHT sequences (residues 142–149 and 447–454, respectively) are shown spanning the space from the metalated allosteric site and the MgADP-filled NBDs. Asp142 and Asp447 are indirectly coordinated to MgADP via water molecules (W). Imidazole nitrogen in His148 and 453 are coordinated to two of the three Sb in the allosteric site. The model was derived from the 2.3 Å crystal structure (Zhou et al., 2000)

Firstly, the altered proteins were produced in normal amounts. Secondly, limited trypsin digestion experiments showed that the rate of production of tryptic peptides in the presence of oxyanions was unaffected by the mutations. Finally, the protection from proteolysis afforded by ATP in the absence or presence of oxyanions was not modified.

The purified proteins H148A, H453A and H148A/H453A ArsA each catalyzed ATP hydrolysis, hence the two-conserved histidine are not required for catalysis. Each altered protein had a K_m for ATP that was within the same order of magnitude as that of the wild type enzyme. The concentration of oxyanions required for half-maximal activation was also essentially unaltered by the mutation. The trypsin digestion experiments clearly showed that the altered proteins bind substrate and metalloid in a similar manner as the wild type ArsA. Therefore, neither of the two histidines is required for binding either nucleotide or metal.

While catalysis is only marginally affected, the major effect of the alanine substitutions for the two histidines appears to be on the transmission of the signal from the allosteric site to the catalytic site. While the basal rate of ATP hydrolysis was not affected by the alanine substitutions, the activated rates were considerably lower than the wild type. Single alanine substitutions showed a 3-5-fold decrease in the Sb(III)-stimulated ATPase activity compared to wild type ArsA. The doubly substituted H148A/H453A ArsA exhibited a 10-fold decrease in the activated rate compared to wild type enzyme. Similar results were observed with arsenite as the activator.

The results of limited trypsin digestion support the idea that the histidines of the DTAP domain are involved in signal transduction. Under noncatalytic conditions there were no differences between wild type and alanine-substituted enzymes. Only under catalytic conditions did the altered ArsAs exhibit increased sensitivity to trypsin, indicating that the histidine residues have an effect on the conformation of the enzyme only during the catalytic cycle. Thus, substitution of the conserved histidine residues disrupts transmission of the information of metalloid occupancy of the allosteric site to the catalytic site.

Acknowledgement

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References

Bhattacharjee H, Ghosh M, Mukhopadhyay R, Rosen BP. 1999 Arsenic transporters from *E. coli* to humans in Broome-Smith JK, Baumberg S, Sterling CJ,Ward FB (Eds), *Transport of molecules across microbial membranes*, Society for General Micriobiology, pp. 58–79.

Bhattacharjee H, Li J, Ksenzenko MY, Rosen BP. 1995 Role of cysteinyl residues in metalloactivation of the oxyanion-translocating ArsA ATPase. *J Biol Chem* **270**, 11245–11250.

Bhattacharjee H, Rosen BP. 1996 Spatial proximity of Cys113, Cys172, and Cys422 in the metalloactivation domain of the ArsA ATPase. *J Biol Chem* **271**, 24465–24470.

Hsu CM, Rosen BP. 1989 Characterization of the catalytic subunit of an anion pump. *J Biol Chem* **264**, 17349–17354.

Karkaria CE, Chen CM, Rosen BP. 1990 Mutagenesis of a nucleotide-binding site of an anion-translocating ATPase. *J Biol Chem* **265**, 7832–7836.

Kaur P, Rosen BP. 1992 Mutagenesis of the C-terminal nucleotide-binding site of an anion- translocating ATPase. *J Biol Chem* **267**, 19272–19277.

Li J, Rosen BP. 2000 The linker peptide of the ArsA ATPase. *Mol Microbiol* **35**, 361–367.

Mukhopadhyay R, Li J, Bhattacharjee H, Rosen BP. 1998 Metalloid resistance mechanisms in Rosen BP,Mobashery S (Eds), *Rosolving the antibiotic paradox*, Plenum Publishers, pp. 159–181.

Ramaswamy S, Kaur P. 1998 Nucleotide binding to the C-terminal nucleotide binding domain of ArsA: Studies with an ATP analogue, 5′-p-fluorosulfonylbenzoyladenosine. *J Biol Chem* **273**, 9243–9248.

Rosen BP, Bhattacharjee H, Zhou T, Walmsley AR. 1999 Mechanism of the ArsA ATPase. *Biochim Biophys Acta* **1461**, 207–215.

Rosen BP, Weigel U, Karkaria C, Gangola P. 1988 Molecular characterization of an anion pump. The *arsA* gene product is an arsenite(antimonate)-stimulated ATPase. *J Biol Chem* **263**, 3067–3070.

Sambrook J, Fritsch EF, Maniatis T. 1989 *Molecular cloning, a laboratory manual*. Cold Spring Harbor Laboratory.

Vogel G, Steinhart R. 1976 ATPase of *Escherichia coli*: purification, dissociation, and reconstitution of the active complex from the isolated subunits. *Biochemistry* **15**, 208–216.

Walker JE, Saraste M, Runswick MJ, Gay NJ. 1982 Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. *EMBO Journal* **1**, 945–951.

Zhou T, Radaev S, Rosen BP, Gatti DL. 2000 Structure of the ArsA ATPase: the catalytic subunit of a heavy metal resistance pump. *Embo J*, **19**, 4838–4845.

Zhou T, Rosen BP. 1997 Tryptophan fluorescence reports nucleotide-induced conformational changes in a domain of the ArsA ATPase. *J Biol Chem* **272**, 19731-19737.

Zhou TQ, Rosen BP. 1999 Asp45 is a Mg^{2+} ligand in the ArsA ATPase. *J Biol Chem* **274**, 13854–13858.