ELSEVIER

Biochimica et Biophysica Acta 1461 (1999) 207–215

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.elsevier.com/locate/bba

Review

Mechanism of the ArsA ATPase

Barry P. Rosen [a,*], Hiranmoy Bhattacharjee [a], Tongqing Zhou [a], Adrian R. Walmsley [b]

[a] *Department of Biochemistry and Molecular Biology, Wayne State University, School of Medicine, Detroit, MI 48201, USA*
[b] *Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ UK*

Received 31 August 1999; accepted 1 September 1999

Abstract

The ArsAB ATPase confers metalloid resistance in *Escherichia coli* by pumping toxic anions out of the cells. This transport ATPase shares structural and perhaps mechanism features with ABC transporters. The ArsAB pump is composed of a membrane subunit that has two groups of six transmembrane segments, and the catalytic subunit, the ArsA ATPase. As is the case with many ABC transporters, ArsA has an internal repeat, each with an ATP binding domain, and is allosterically activated by substrates of the pump. The mechanism of allosteric activation of the ArsA ATPase has been elucidated at the molecular level. Binding of the activator produces a conformational change that forms a tight interface of the nucleotide binding domains. In the rate-limiting step in the overall reaction, the enzyme undergoes a slow conformational change. The allosteric activator accelerates catalysis by increasing the velocity of this rate-limiting step. We postulate that similar conformational changes may be rate-limiting in the mechanism of ABC transporters.

Keywords: Resistance ATPase; Efflux pump; Arsenite; Antimonite; Tryptophan fluorescence

Contents

* Corresponding author. Fax: +1-313-577-2765; E-mail: brosen@med.wayne.edu

0005-2736/99/$ – see front matter
PII: S0005-2736(99)00159-5

1. Introduction

All organisms, prokaryotic, eukaryotic or archea, have evolved resistances to drugs and toxic metals. It is likely that life evolved in waters rich in dissolved metals and metalloids, such as arsenic. Thus resistance to toxic metal ions probably evolved early, vastly predating the evolution of drug resistance. In *Escherichia coli*, high level resistance to the salts of the trivalent metalloids arsenite and antimonite is conferred by the *ars* operon of plasmid R773 [1]. The *arsA* and *arsB* genes encode the subunits of the ArsAB As(III)/Sb(III)-translocating ATPase, which extrudes the metalloid oxyanions (Fig. 1). ArsB is the 45-kDa membrane sector of the pump, and has 12 membrane spanning segments. ArsA, the catalytic sector of the pump, is normally bound to ArsB, but, when expressed in the absence of ArsB, ArsA can be purified as a soluble ATPase. The 63-kDa ArsA ATPase has two homologous halves, A1 and A2, connected by a 25-residue linker peptide. Each half has a consensus nucleotide binding domain (NBD), both of which are required for activity. The enzyme is allosterically activated by binding of As(III) or Sb(III). The mechanism by which drugs activate the efflux pumps that confer resistance to those drugs is a major unanswered question in the field of resistance. In this chapter, the mechanism of allosteric activation of ArsA is described. The relationship of the allosteric and nucleotide binding domains has been elucidated, and a reaction scheme has been proposed.

2. The nucleotide binding domains

The first indication that the R773 *ars* operon encoded a primary ATP-coupled pump was from an in vivo analysis of the energetics of arsenical efflux [2]. In intact cells of *E. coli*, a primary pump is dependent on intracellular chemical energy, while a secondary porter requires electrochemical energy. This study was performed in an *unc* strain that is defective in the H^+-translocating ATPase (F_oF_1) and is thus unable to equilibrate chemical and electrochemical energy. In the presence of both glucose, which generates ATP through glycolysis, and cyanide, which inhibits respiratory-driven formation of an electrochemical potential, efflux was rapid. In the presence of respiratory substrates that form an electrochemical potential but not ATP, efflux was slow. These results are consistent with the operon encoding an obligatorily ATP-coupled pump. The operon was subsequently cloned and sequenced [3]. The sequence demonstrated that the *arsA* gene encoded a protein of 583 residues that had an internal repeat of approximately 30 kDa each (Fig. 2). Each half, termed A1 and A2, has a consensus nucleotide binding domain (NBD). ArsA was purified and demonstrated to have arsenite- or antimonite-stimulated ATPase activity [4]. Mutations in either A1 or A2 NBD resulted in loss of resistance, loss of transport and loss of ATPase activity [5,6]. Finally, everted membrane vesicles with the ArsAB complex exhibited ATP-coupled accumulation of $^{73}AsO_2^{-1}$ [7]. Thus ArsAB is an arsenite/antimonite-translocating ATPase.

Why do many transport ATPases, including ABC transporters, require two or more nucleotide binding domains? From genetic results it appears that the two sites in ArsA interact [8]. The codon for Gly-15 in A1 NBD was mutated to a cysteine codon (Fig. 2). Cells expressing the $arsA_{G15C}$ mutation resulted in substantial reductions in arsenite resistance, transport and ATPase activity. Mutants were selected that suppressed the arsenite-sensitive phenotype of the G15C mutation. One mutation that restored arsenite resistance was a substitution of valine for Ala-344 which is located adjacent to A2 NBD binding sequence, indicating spatial proximity of Gly-15 and Ala-344. These data support a model in which interaction of the two NBDs promotes catalysis (Fig. 3). Alteration of Ala-344 to bulkier residues also suppressed substitutions in the A1 NBD [9]. Less bulky residues, such as glycine or serine, did not efficiently suppress primary A1 NBD mutations. Thus, the larger the residue, the greater the suppression. The altered ArsAs were purified, and a relationship was observed between activity and residue volume. Each altered enzyme exhibited both an increase in the K_m for ATP and in the concentration of anti-

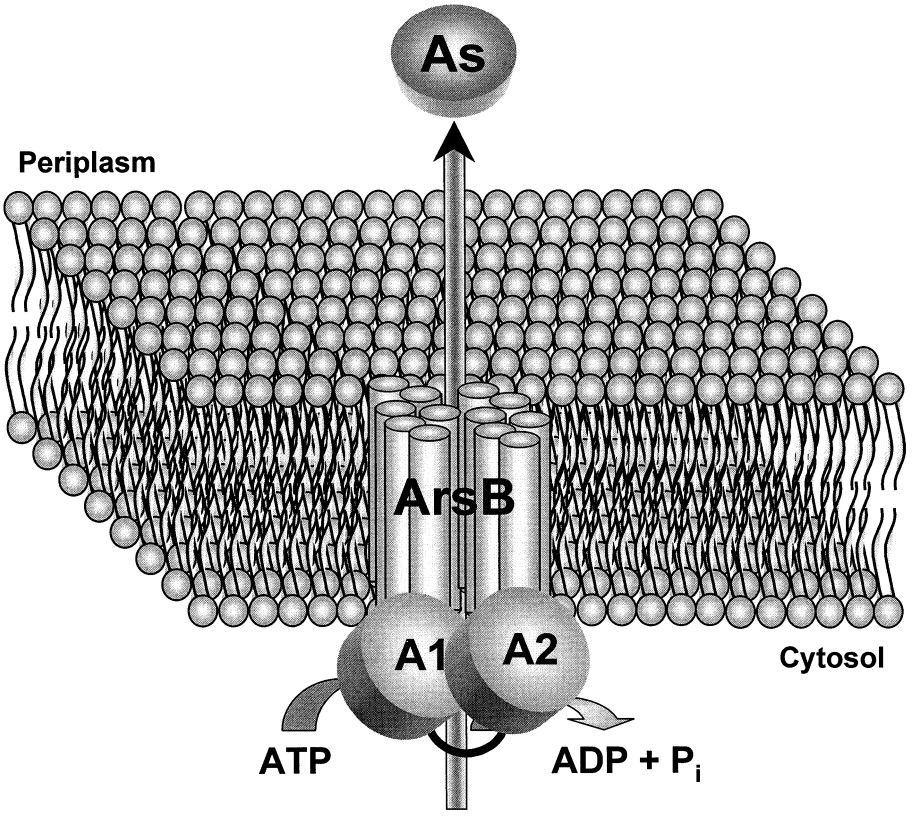
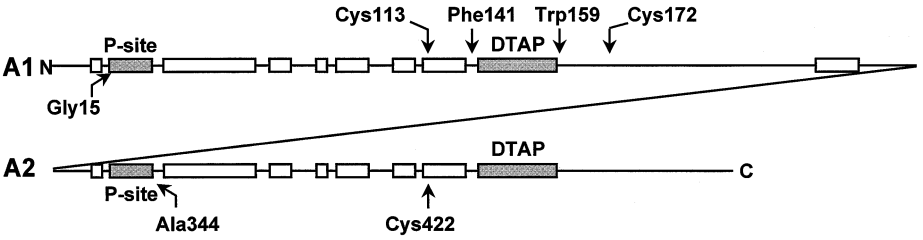


Fig. 1. The ArsAB pump. The complex of ArsA and ArsB forms an anion-translocating ATPase that catalyzes extrusion of arsenite or antimonite. ArsA has two homologous halves, A1 (N-terminal) and A2 (C-terminal). ArsA is the catalytic subunit, exhibiting As(III)/Sb(III)-stimulated ATPase activity. ArsB is an inner membrane protein in *E. coli* and serves both as the membrane anchor for ArsA and as the anion conducting subunit of this ATP-coupled pump.

monite required for half maximal allosteric activation. The results are consistent with a physical interaction of the two NBDs and indicate that the geometry at the interface between A1 and A2 NBDs impose steric constraints on allowable residues in that interface.

Mg^{2+} is essential for ATPase activity of ArsA [10]. An effect of Mg^{2+} on the conformation of ArsA was observed only in the presence of ATP, suggesting that Mg^{2+} binds to ArsA as a complex with ATP [11]. Coordination of Mg^{2+} in ATP- and GTP-binding proteins frequently involves aspartate residues. ArsA has several aspartate residues that are highly conserved in homologs and in other nucleotide binding enzymes such as the nitrogenase iron protein (NifH) [12], RecA [13], and GTP-binding proteins, including Ras p21 [14]. In Ras p21 the conserved Asp residues are Mg^{2+} ligands [14]. The magnesium ion was shown to bring together diverse components of the GTP-binding core, facilitating the information flow between domains. ArsA residue 45 is a conserved aspartic acid. To examine the role of Asp-45, mutants were constructed in which Asp-45 was changed to Glu, Asn, or Ala [10]. Cells expressing those mutated *arsA* genes have reduced arsenite resistance. Purified D45A and D45N ArsAs were inactive, while the purified D45E ArsA exhibited approximately 5% of the wild-type activity, with about 5-fold decrease in affinity for Mg^{2+}. These results indicate that an aspartate residue at position 45 promotes catalytic activity. Moreover, Mg^{2+} produces conformational changes in ArsA that can be detected from the effect of the cation on the surface accessibility of the protein to trypsin or on the intrinsic fluorescence of Trp-159. These results strongly suggest that Asp-45 is a Mg^{2+} ligand.



136	FDHII**F**DTAPTGHTIRLL**W**LPGA	ArsA1
441	KRFVVMDTAPTGHTLLLLDATGAY	ArsA2
125	YDALVLDTAPTGETLRLLSLPDTL	*C. vibrioforme*
160	FDTVIFDTAPTGHTLRFLQLPNTL	*S. cerevisiae*
152	FDVVIFDTAPTGHTLRFLGMPEVM	*M. jannaschii*
231	FTRIVFDTAPTGHTLRLLSLPDFL	*A. thaliana*
152	FDVVVFDTAPTGHTLRLLQFPTLL	*C. elegans*
160	FSVVVFDTAPTGHTLRLLNFPTIV	*M. musculus*
144	FSVVVFDTAPTGHTLRLLNFPTIV	*H. sapiens*

Fig. 2. ArsA primary sequence model. Top: the 583-amino acid residue ArsA ATPase consists of two homologous halves, N-terminal A1 and C-terminal A2, connected by a 25-residue linker sequence. The aligned boxes in the two halves indicate the regions of greatest sequence similarity. Shown are the P-loop of the A1 and A2 NBDs (shaded). Indicated are the locations of Gly-15 in the A1 NBD and Ala-344 in the A2 NBD and the three cysteine residues in the allosteric binding site. Also shaded are the two DTAP domains, with the A1 sequence bounded by Phe-141 and Trp-159. Bottom: conserved residues in the DTAP consensus sequences in nucleotide binding proteins are shaded. Phe-141 and Trp-159 are underlined. Shown are sequences from the A1 and A2 halves of ArsA, and homologs from *Chlorobium vibrioforme* (U09867), *Saccharomyces cerevisiae* (S67642), *Methanococcus jannaschii* (E64442), *Arabidopsis thaliana* (AB005246), *Caenorhabditis elegans* (P30632), *Mus musculus* (AAB94772) and *Homo sapiens* (NP 004308). Accession numbers are given in parentheses.

3. The allosteric site

ArsA ATPase activity has been shown to be specifically activated by antimonite (Sb(III)) or arsenite (As(III)). In the absence of metalloid ion, ArsA has a low level of ATPase activity. Sb(III) stimulates ATP hydrolysis 10- to 20-fold, while a 3- to 5-fold stimulation is observed with As(III). Other oxyanions tested were neither stimulatory nor inhibitory [15]. It was possible that activation of ArsA by arsenite or antimonite might be through binding of these compounds as oxyanions to anion binding sites. However, as metalloids, they might react as soft metals through coordinate interactions with cysteine thi-

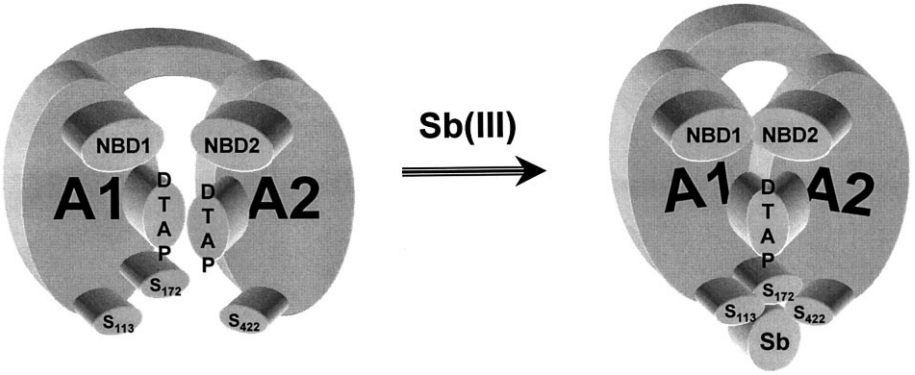


Fig. 3. Allosteric model of ArsA catalysis. Binding of effector Sb(III) (or As(III)) to Cys-113, Cys-172, and Cys-422 (with the thiolates represented as *S*) brings together the A1 and A2 NBDs of ArsA. This produces a large increase in the rate of the conformational change that occurs in the DTAP domains during the catalytic cycle, promoting catalysis.

olates. In the reaction of arsenite with thiolates, such as dithiothreitol or glutathione, arsenic acts as a soft metal, forming direct As–S bonds [16,17]. The ArsR repressor binds either Sb(III) or As(III) as a soft metal through interaction with three cysteinyl residues, and metalloid binding results in derepression [18].

Therefore, if activation of ArsA by antimonite or arsenite involves direct metal–sulfur bonds, then alteration of participating cysteine residues would be expected to have a dramatic effect on activation. ArsA catalysis was found to be extremely sensitive to sulfhydryl reagent, methyl methanethiosulfonate (MMTS) [19]. MMTS reacts specifically with thiol groups on the surface of proteins, forming a disulfide between the cysteinyl residue and the small $-SCH_3$ group. A two-fold molar excess of MMTS over ArsA rapidly and completely inactivated ArsA ATPase activity. The MMTS-modified ArsA could be reactivated to about 70% of its original activity upon addition of dithiothreitol. These data indicated that one or more cysteines is involved in activation.

As(III) binds to spatially proximate thiol groups [20]. This implies that for arsenite or antimonite to activate ArsA, the protein would be expected to have two or more cysteine residues in close proximity in the tertiary structure, if not in the primary sequence. In ArsA there are four cysteines, Cys-26, Cys-113, Cys-172, and Cys-422, all located in different regions of the primary sequence (Fig. 2). For arsenite or antimonite to act as an allosteric modulator, two or more of the cysteines must be close enough in the folded protein to coordinate with the metalloid.

To investigate the role of cysteine residues in the allosteric activation of ArsA, each of the four cysteines were altered to serine residues by site-directed mutagenesis of the *arsA* gene [19]. Cells expressing the $arsA_{C26S}$ mutant exhibited no change in resistance to arsenite or antimonite, and the purified C26S ArsA had wild-type ATPase activity. In contrast, cells expressing the $arsA_{C113S}$, $arsA_{C172S}$ and $arsA_{C422S}$ mutants were sensitive to arsenite or antimonite. Each of the three altered proteins exhibited reduced arsenite or antimonite stimulated ATPase activity. Substitution of each of the cysteines with serine residues did not appear to grossly perturb the tertiary structure of ArsA. The kinetic parameters of the C26S ArsA were essentially the same as that of wild-type protein. Thus, Cys-26 does not appear to be involved in either activation or catalysis. Each cysteine-to-serine substituted protein was produced in normal amounts and exhibited the same tryptic digestion patterns as the wild-type, indicating that the altered proteins had relatively unaltered conformations. When the kinetic parameters of the purified C113S, C172S and C422S ArsAs were investigated, the K_m for ATP for each of the mutant was found to be within an order of magnitude of the wild-type enzyme. However, the concentration of Sb(III) or As(III) required for activation was substantially increased, most likely reflecting a decrease in affinity for metalloid. Alteration of Cys-422 appeared to have more of an effect than alteration of Cys-113 or Cys-172. The C113S and C172S proteins exhibited a 20-fold increase in the concentration of antimonite required for half maximal activation, while the C422S required 200-fold more. Similar results were observed when arsenite was used as the activator. These results suggested that all three cysteines, Cys-113, Cys-172, and Cys-422 are involved in activation.

Limited trypsin digestion experiments also supported the idea that the activator-binding site of C113S, C172S and C422S was modified. Surface accessibility to trypsin has been used to assess the structure of ArsA [15]. The effect of ATP and oxyanions on the rate of trypsin digestion has been shown to reflect the binding of substrate and activator and the interaction of the two types of binding sites in ArsA. The presence of both antimonite and ATP has been shown to synergistically protect the 63-kDa wild-type ArsA protein [15]. The synergistic effect was observed with C26S but not C113S, C172S or C422S. From those results it was proposed that allosteric activation of ArsA ATPase occurs through coordination of As(III) or Sb(III) by the cysteine thiolates of residues 113, 172, and 422 (Fig. 3).

Although these three cysteines are located distant from each other in the primary sequence, the data imply that they come close together in the quaternary structure to interact with either As(III) or Sb(III). From X-ray crystal data of As(III) or Sb(III) complexed to small molecule dithiols, the lengths of an As–S bond and Sb–S bond are 2.23 and 2.45 Å, respectively, while S–As–S and S–Sb–S angles are 92.7° and 84.8°, respectively [16,21]. Moreover, using

a combination of site-directed mutagenesis and arsenic X-ray absorption spectroscopy, the ArsR repressor was shown to bind As(III) to the thiolates of the cysteine triad of Cys-32, Cys-34, and Cys-37 with As–S bonds of 2.25 Å [22]. Thus, a reasonable model for interaction of ArsA with As(III) or Sb(III) would be coordination of the metalloid to the thiolates of Cys-113, Cys-172, and Cys-422, which requires that the cysteines be in proximity with each other in the folded protein. To examine this possibility, the homobifunctional cross-linker dibromobimane (bBBr) was used to map distance between pair of cysteines in ArsA. bBBr has two equivalent bromomethyl groups that can cross-link a thiol pair located within 3–6 Å of each other [23]. bBBr is nonfluorescent in solution, but becomes fluorescent when both of its alkylating groups have reacted [24]. This property allowed bBBr to be used as a molecular ruler to map distance between cysteine residues

An ArsA in which only one of the three essential cysteine was altered by site-directed mutagenesis still formed fluorescent adducts with dibromobimane. Proteins in which two of the three essential cysteine residues were substituted did not form fluorescent adducts. These results demonstrated that Cys-113, Cys-172, and Cys-422 are within 6 Å from each other in the native enzyme [25], suggesting that a novel As(III)/Sb(III)-thiol structure is involved in allosteric activation of ArsA. ArsA is the only known example of an enzyme that requires As(III)/ Sb(III) for its activity.

4. Interaction of the allosteric and catalytic sites

How does binding of the metalloid result in allosteric activation? As described above, the A1 and A2 NBDs interact to produce a high rate of catalysis. In the absence of allosteric activator, A1 and A2 have independent mobility, held together only by a 25-residue flexible linker peptide. Coordination of As(III) or Sb(III) with Cys-113 and Cys-172 in the A1 and Cys-422 in A2 physically pulls the two halves of the protein together. This in turn brings the two nucleotide-binding sites in close contact with each other and accelerating catalysis. The release of energy from ATP hydrolysis in ArsA is transduced into the ArsB subunit of the pump, thereby driving transport of the metalloid oxyanions.

In addition to the movement of the A1 and A2 halves of the protein, there are more localized conformational changes that are propagated from the allosteric domain to the NBDs. The changes are mediated by the 12-residue consensus sequence DTAPTGHTIRLL (termed the DTAP domain) that is found in each half of ArsA and ArsA homologs from eubacteria, archebacteria, fungi, plants and animals, including humans (Fig. 2) [26]. Two ArsAs were constructed with only a single tryptophan residue, either Trp-141 or Trp-159, at either end of the A1 DTAP domain (Fig. 2). Trp-159 is one of the four native tryptophan residues in ArsA; in the mutant, the other three were changed by mutagenesis to phenylalanine residues. In the Trp-141 protein, all native tryptophans had been changed to phenylalanines, and Phe-141 was altered to tryptophan by mutagenesis. In the absence of ligands, the emission spectrum of the fluorescence of Trp-159 indicated that the C-terminal end of the DTAP domain is in a relatively hydrophilic environment [10,26]. However, upon addition of MgATP there was an increase in intrinsic tryptophan fluorescence and a blue shift of the maximum emission wavelength, indicative of a change in the environment of the domain into a relatively less polar environment. This could be the result of a conformational change in the domain itself. More likely, the domains are located on the surfaces of the A1 and A2 halves that form an interface upon binding of As(III). As the two halves come together, the solvent accessibility of the DTAP domains is altered.

Interestingly, no fluorescence response was observed with MgADP, with non-hydrolyzable ATP analogs, or with MgATP by inactive ArsAs. Moreover, the time course of the fluorescence change agreed well with the rate of ATP hydrolysis, suggesting that Trp-159 becomes less solvent accessible only during hydrolysis of ATP. Addition of Sb(III) or As(III) rapidly collapsed the ATP-enhanced fluorescence of Trp-159. Since the metalloids increase the rate of catalysis, the data suggest a link between the allosteric transition and the change in environment of the DTAP domain. In contrast, Trp-141, located on the N-terminal side of the A1 DTAP domain, is in a relatively non-polar environment. Addition of

MgADP decreased the fluorescence yield and red shifted the maximum emission wavelength. While MgATP slowly decreased Trp-141 fluorescence, the rate correlated with product formation and was not observed with non-hydrolyzable ATP analogs. These results suggest that the N-terminal end of the DTAP domain becomes exposed to a more polar environment upon product formation. Thus, the C-terminal end of the A1 DTAP domain moves into a less polar environment during catalysis, while the N-terminal end moves into a more hydrophilic environment as product is formed.

5. Reaction mechanism of the ArsA ATPase

While, in the aggregate, the results demonstrate interaction between the two NBDs that is linked to movement of the DTAP domain facilitated by binding of the allosteric effector, what are the molecular and enzymatic steps in activation and catalysis? The kinetic mechanism of the ATPase reaction catalyzed by ArsA has been studied by transient kinetic techniques, and the data has been interpreted in terms of the following multi-step kinetic scheme [27]:

$$\overset{1}{\rightleftharpoons}\ \mathrm{ArsA^2\text{-}MgATP}\ \overset{2}{\rightleftharpoons}\ \mathrm{ArsA^3\text{-}MgATP}\ \overset{3}{\rightleftharpoons}\ \mathrm{ArsA^4\text{-}MgATP}$$

$$\mathrm{ArsA^1 + MgATP}$$

$$\overset{4}{\rightleftharpoons}\ \mathrm{ArsA^5\text{-}MgADP.P_i}\ \overset{5}{\rightleftharpoons}\ \mathrm{ArsA^6\text{-}MgADP + P_i}\ \overset{6}{\rightleftharpoons}\ \mathrm{ArsA^7 + MgADP}\ \overset{7}{\rightleftharpoons}\ \mathrm{ArsA^1}$$

The most notable feature of the mechanism is the fact that the rate-limiting step for the reaction is a conformational change, subsequent to hydrolysis and product release, back to the original conformation (e.g. step 7). The experimental evidence supporting this mechanism is as follows:

1. An analysis of the steady-state kinetics yielded an apparent k_{cat} of 5.4×10^{-3} s^{-1}.
2. The binding of MgATP to ArsA can be monitored directly as a biphasic, increase and decrease, in the intrinsic fluorescence of ArsA. The rate constant for the first step (observed as an increase in fluorescence) increases hyperbolically with the MgATP concentration, while the rate constant for the second step (observed as a decrease in fluorescence) is concentration independent. This behavior is indicative of a three-step binding process, with the rapid equilibrium binding of MgATP (e.g. step 1, with a K_1 of 178 μM) followed by two rate-limiting isomerizations (e.g. steps 2 and 3). The first-order rate constants for these isomerizations of the ArsA–MgATP complex are $k_2 = 54$ s^{-1}, $k_{-2} = 7.3$ s^{-1}, $k_3+k_{-3} = 6.5$ s^{-1}. This cannot be rate-limiting because they are much faster than the steady-state rate.
3. There is a phosphate burst when ArsA is mixed with MgATP. Since there can only be a pre-steady-state product burst (e.g. a build-up of Pi and MgADP on the ArsA) when the hydrolysis steps are faster then subsequent steps (e.g. product release or a conformational change linked to product release) this data indicates that hydrolysis of the MgATP is not rate-limiting. To determine the amount of phosphate produced during the burst phase, a photospectrometric assay was used, which would only detect Pi released to the bathing solution, rather than that complexed with ArsA. Consequently, these data imply that neither hydrolysis nor Pi dissociation are rate-limiting.
4. Because the MgATP–ArsA complex has greater fluorescence than the MgADP–ArsA complex, the dissociation rate constant for MgADP could be measured directly by MgATP displacement. This experiment yielded a value for k_6 of 0.08 s^{-1}, which is too fast for MgADP release to be rate-limiting.
5. Since neither hydrolysis nor product release is rate-limiting, we conclude that a conformational change at the end of the reaction, in which the protein adopts its original conformational form (e.g. $ArsA^1$), is rate-limiting for the ATPase reaction.
6. Stopped-flow fluorescence studies revealed the transient formation of a reaction intermediate (with enhanced fluorescence) that was formed over 100 s (e.g. $k = 0.05$ s^{-1}) and decayed over 1000 s (e.g. $k = 2.3\times10^{-3}$ s^{-1}). The rates at which this intermediate formed and decayed were independent of the MgATP concentration, indicating that its formation preceded the rate-limiting step. A plausible interpretation for this observation is that the intermediate is the ArsA–MgADP·Pi

complex. The rate of formation of the intermediate would then occur at the rate of hydrolysis of ATP (e.g. $k_4 = 0.05$ s^{-1}), and the intermediate would decay as Pi is released from the ArsA–MgADP·Pi complex (e.g. $k_5 = 2.3 \times 10^{-3}$ s^{-1}). There would then be a relatively rapid release of ADP. An alternative proposal might be that step 4 involves both ATP hydrolysis and ADP release, and that the intermediate is ArsA–Pi. However, the intermediate is sensitive to EDTA, which causes a decrease in the fluorescence back to the baseline, consistent with destabilization of the ArsA–MgADP·Pi complex as the Mg^{2+} is sequestered.

Interestingly, the Pi burst occurred at a rate approximately half that of k_{cat}. A plausible explanation for this behavior is that both the nucleotide-binding sites of ArsA are catalytic. Consistent with this interpretation, approximately 2 moles of phosphate are released per mole of ArsA during the Pi burst.

When ArsA is equilibrated with antimonite prior to mixing with MgATP, the stopped-flow fluorescence profile is characterized by a very rapid increase in fluorescence, which is almost complete within the dead-time of the stopped-flow, followed by a relatively rapid decrease in fluorescence back to the baseline over 100 s. A reasonable interpretation for this behavior is that ArsA does not need to undergo the slow conformational change back to its original conformation in the presence of antimonite and, consequently, there is no build-up of the ArsA–MgADP·Pi intermediate with enhanced fluorescence. The increase in fluorescence is due to rapid formation of the ArsA–MgATP complex, which undergoes rapid hydrolysis and product release, and the fluorescence returns to the basal level during this phase of the reaction. Indeed, when ArsA is mixed with MgATP and the ArsA–MgADP·Pi intermediate, with enhanced fluorescence, is allowed to build up during the first 100 s of the reaction, the addition of antimonite has the effect of rapidly reversing the fluorescence enhancement within a second. This behavior is consistent with the antimonite inducing the rapid displacement of the ADP from the ArsA–MgADP·Pi complex. This rapid dissociation of ADP is not observed when ArsA is pre-equilibrated with antimonite before mixing with MgATP, presumably because ADP dissociation is then rate-limited by ATP hydrolysis. Consistent with this view there is no phosphate burst in the presence of antimonite and k_{cat} increases by about an order of magnitude. A plausible explanation for this behavior is that antimonite stabilizes a conformation of the enzyme from which the products can dissociate rapidly.

6. Conclusions

While it is not clear how closely the ArsAB resistance pump is related to ABC transporters, they share structural and perhaps mechanistic features. The ArsAB pump is composed of two types of subunits, the 45-kDa ArsB membrane sector with two groups of six transmembrane segments, and the catalytic sector, the 63-kDa ArsA ATPase. As is the case with many ABC transporters, ArsA has an internal repeat, clearly the result of an ancestral gene duplication and fusion. Both the A1 and A2 halves have a NBD, and both NBDs are required for resistance. ArsA ATPase activity is allosterically activated by the substrates of the pumps; similarly ABC transporters exhibit increased ATPases activity in the presence of their substrates, and allosteric activation has been proposed [28,29].

The mechanism of allosteric activation of the ArsA ATPase has been elucidated at the molecular level. In brief, the allosteric site is composed of residues from both the A1 and A2 halves, and binding of the activator brings the two halves together, such that the two NBDs have an interface where catalysis is accelerated. The rate-limiting step in the overall reaction is neither substrate binding nor product release. Rather, the enzyme undergoes a conformational change that is slow in the unactivated state. Binding of the allosteric activator increases the rate of movement of the enzyme through this conformation, accelerating catalysis. It is possible that conformational change may be rate-limiting in the mechanism of some ABC transporters.

Acknowledgements

The work in B.P.R.'s laboratory was supported by United States Public Health Service Grants

GM55425 and GM52216. The work in A.R.W.'s laboratory is supported by the Biotechnology and Biological Sciences Research Council, the Medical Research Council, the Wellcome Trust and the Royal Society. Collaboration between the laboratories was supported by the Burroughs Wellcome Fund and the NATO International Scientific Exchange Programs.

References

[1] B.P. Rosen, Trends Microbiol. 7 (1999) 207–212.
[2] H.L. Mobley, B.P. Rosen, Proc. Natl. Acad. Sci. USA 79 (1982) 6119–6122.
[3] C.M. Chen, T.K. Misra, S. Silver, B.P. Rosen, J. Biol. Chem. 261 (1986) 15030–15038.
[4] B.P. Rosen, U. Weigel, C. Karkaria, P. Gangola, J. Biol. Chem. 263 (1988) 3067–3070.
[5] P. Kaur, B.P. Rosen, J. Biol. Chem. 267 (1992) 19272–19277.
[6] C.E. Karkaria, C.M. Chen, B.P. Rosen, J. Biol. Chem. 265 (1990) 7832–7836.
[7] S. Dey, D. Dou, B.P. Rosen, J. Biol. Chem. 269 (1994) 25442–25446.
[8] J. Li, S. Liu, B.P. Rosen, J. Biol. Chem. 271 (1996) 25247–25252.
[9] J. Li, B.P. Rosen, J. Biol. Chem. 273 (1998) 6796–6800.
[10] T. Zhou, B.P. Rosen, J. Biol. Chem. 274 (1999) 13854–13858.
[11] T. Zhou, S. Liu, B.P. Rosen, Biochemistry 34 (1995) 13622–13626.
[12] R.D. Joerger, P.E. Bishop, J. Bacteriol. 170 (1988) 1475–1487.
[13] R.M. Story, T.A. Steitz, Nature 355 (1992) 374–376.
[14] E.F. Pai, U. Krengel, G.A. Petsko, R.S. Goody, W. Kabsch, A. Wittinghofer, EMBO J. 9 (1990) 2351–2359.
[15] C.M. Hsu, B.P. Rosen, J. Biol. Chem. 264 (1989) 17349–17354.
[16] W.B.T. Cruse, M.N.G. James, Acta Crystallogr. Sec. B 28 (1972) 1325–1331.
[17] M. Delnomdedieu, M.M. Basti, J.D. Otvos, D.J. Thomas, Chem. Biol. Interact. 90 (1994) 139–155.
[18] W. Shi, J. Wu, B.P. Rosen, J. Biol. Chem. 269 (1994) 19826–19829.
[19] H. Bhattacharjee, J. Li, M.Y. Ksenzenko, B.P. Rosen, J. Biol. Chem. 270 (1995) 11245–11250.
[20] R.D. Hoffman, M.D. Lane, J. Biol. Chem. 267 (1992) 14005–14011.
[21] D.B. Sowerby, in: S. Patai (Ed.), The Chemistry of Organic Arsenic, Antimony and Bismuth Compounds, Wiley, New York, 1994, pp. 25–88.
[22] W. Shi, J. Dong, R.A. Scott, M.Y. Ksenzenko, B.P. Rosen, J. Biol. Chem. 271 (1996) 9291–9297.
[23] D. Mornet, K. Ue, M.F. Morales, Proc. Natl. Acad. Sci. USA 82 (1985) 1658–1662.
[24] N.S. Kosower, E.M. Kosower, G.L. Newton, H.M. Ranney, Proc. Natl. Acad. Sci. USA 76 (1979) 3382–3386.
[25] H. Bhattacharjee, B.P. Rosen, J. Biol. Chem. 271 (1996) 24465–24470.
[26] T. Zhou, B.P. Rosen, J. Biol. Chem. 272 (1997) 19731–19737.
[27] A.R. Walmsley, T. Zhou, M.I. Borges-Walmsley, B.P. Rosen, J. Biol. Chem. 274 (1999) 16153–16161.
[28] A.E. Senior, M.K. al-Shawi, I.L. Urbatsch, FEBS Lett. 377 (1995) 285–289.
[29] S.V. Ambudkar, S. Dey, C.A. Hrycyna, M. Ramachandra, I. Pastan, M.M. Gottesman, Annu. Rev. Pharmacol. Toxicol. 39 (1999) 361–398.