The Anion-stimulated ATPase ArsA Shows Unisite and Multisite Catalytic Activity*

(Received for publication, February 19, 1999, and in revised form, June 8, 1999)

Parjit Kaur‡

From the Department of Biology, Georgia State University, Atlanta, Georgia 30303

ArsA, an anion-stimulated ATPase, consists of two nucleotide binding domains, A1 in the N terminus and A2 in the C terminus of the protein, connected by a linker. The A1 domain contains a high affinity ATP binding site, whereas the A2 domain has low affinity and it requires the allosteric ligand antimonite for binding ATP. ArsA is known to form a UV-activated adduct with [α-32P]ATP in the linker region. This study shows that on addition of antimonite, much more adduct is formed. Characterization of the nature of the adduct suggests that it is between ArsA and ADP, instead of ATP, indicating that the adduct formation reflects hydrolysis of ATP. The present study also demonstrates that the A1 domain is capable of carrying out unisite catalysis in the absence of antimonite. On addition of antimonite, multisite catalysis involving both A1 and A2 sites occurs, resulting in a 40-fold increase in ATPase activity. Studies with mutant proteins suggest that the A2 site may be second in the sequence of events, so that its role in catalysis is dependent on a functional A1 site. It is also proposed that ArsA goes through an ATP-bound and an ADP-bound conformation, and the linker region, where ADP binds under both unisite and multisite catalytic conditions, may play an important role in the energy transduction process.

ArsA and ArsB proteins together form a transport complex for efflux of arsenite or antimonite in the inner membrane of Escherichia coli. ArsA is a peripheral membrane protein that forms the catalytic component of this transport complex (1, 2). It is of interest to determine how the energy of ATP hydrolysis by ArsA is transduced to the integral membrane protein ArsB that forms the carrier for the anions through the membrane. To obtain insights into how ArsA and ArsB proteins interact to form this primary pump, it is important to understand the mechanism of function of ArsA.

ArsA is an anion-stimulated ATPase that consists of two consensus nucleotide binding sites, one in the N-terminal (A1) and other in the C-terminal (A2) half of the protein (1). The A1 and A2 domains of ArsA show significant internal sequence homology. An alignment of their primary amino acid sequence suggests that these two domains are connected by a linker region of about 40 amino acid residues (residues 283–323), the significance of which is not clear (1). Mutations in either nucleotide binding fold have been shown to result in a loss of the anion-stimulated ATPase activity (3, 4), implying that both sites are required for function of the protein. It is not understood how the A1 and the A2 sites participate in catalysis or whether both sites are catalytic in nature. ATP binding studies carried out between ArsA and [α-32P]ATP by UV cross-linking showed that only the A1 domain is involved in forming a UV-activated adduct in the presence of ATP (4, 5). The site of the adduct was identified to lie in the linker region connecting the A1 and the A2 domains (6). Mutations in the A1 nucleotide binding fold resulted in an inability of the protein to form the adduct, whereas mutations in the A2 domain had no effect (3, 4), indicating that the A1 domain is crucial for formation of the ATP adduct with ArsA. The reason for lack of a similar adduct with ATP in the A2 domain has not been clear. That the A2 half of the protein actually binds nucleotide was recently shown by use of an ATP analogue, 5′-p-fluorosulfonylbenzoyladenosine, which was shown to bind preferentially to the A2 site (7). In that study, it was suggested that the A1 and A2 sites of ArsA have different conformations and different affinities for ATP. It was proposed that the A1 site is a high affinity ATP site that binds ATP in the absence of the ligand, whereas the A2 site has a very low affinity, and the binding of the ligand, arsenite or antimonite, acts as a switch that allows ATP binding to the A2 site. However, it is not clear whether two sites in ArsA have independent catalytic activity or what is the mechanism for catalytic co-operativity in this protein. The present study shows that the A1 domain of ArsA is capable of carrying out unisite catalysis in the absence of antimonite at a low rate. When the A2 site is also occupied with ATP, in the presence of antimonite, the catalytic activity increases. The data shown here also suggest that the A2 site is not an independent site and that it comes into action only after the A1 site is occupied, indicating antimonite-induced interaction between the A1 and A2 sites and thus providing first biochemical evidence of co-operativity between the the two sites in ArsA.

MATERIALS AND METHODS

Purification of the ArsA Protein—Wild type or mutant His-tagged ArsA proteins were purified from E. coli cells containing the plasmid pET16bArSA as described earlier (7).

Photolabeling of the ArsA Protein with [32P]ATP or [3H]ADP—Purified wild type and mutant ArsA proteins were photolabeled in the presence of [32P]ATP (3000 Ci/mmole) or [3H]ADP (25 Ci/mmole) by a modification of the procedure described earlier (5, 8). Each reaction contained 10 μCi of labeled ATP or ADP. The samples were precipitated in the presence or absence of antimonite or magnesium for 10 min at 37 °C as indicated in the figure legends. Photolabeling reactions were carried out on ice for 30 min with a short wavelength (254 nm) handheld torch. The photolabeled samples were analyzed by SDS-polyacrylamide gel electrophoresis on a 10% polyacrylamide gel, followed by autoradiography. Where indicated, the reactions were quenched by the addition of excess ADP or ATP to the sample before exposure to UV light.

Digestion with V-8 Staphylococcal Protease—The labeled proteins from above were precipitated with 4 volumes of 10% trichloroacetic acid for 30 min on ice. The precipitated protein was washed three times with ether, air dried, and dissolved in 0.1 ml of 4 μl urea, pH 8.0. V-8 protease was added at a 1:10 ratio of protein to protease, and the samples were incubated at 37 °C for 16 h. The samples were analyzed by a 12%
polycrylamide gel using the Tricine-SDS electrophoresis system (9).**

**ATPase Activity of ArsA—**ATPase activity of ArsA was determined by measurement of inorganic phosphate released from [γ-32P]ATP. Activity was measured in a 40-μl reaction volume containing 50 mM MOPS-KOH, pH 7.5, 1 mM dithiothreitol and the indicated amounts of the ArsA protein and [γ-32P]ATP (3000 Ci/mmole). Where indicated, the samples were preincubated with 0.5 mM antimonite at 37 °C for 10 min. The reaction was started by addition of 5 mM magnesium chloride and the incubation was continued at 37 °C. At the indicated time points, 5-μl samples were withdrawn, and the reactions were stopped by adding 2 μl of 10% SDS solution. The reactants and the products were separated by spotting 2 or 3 μl of the reaction on a polyethyleneimine TLC plate. Chromatography was carried out with a mobile phase consisting of 1 M formic acid and 0.7 M LiCl (10). The radioactivity in the resolved spots was quantitated with a Fuji phosphoimager, and the amount of 32P, released was calculated in nmol/mg of the ArsA protein. To determine kinetic parameters of hydrolysis for unisite or multisite catalysis, ATPase activity at different concentrations of ATP was determined in the absence or presence of antimonite. The specific activity of [γ-32P]ATP was kept constant at all the concentrations of ATP tested. The data were analyzed by the enzyme analysis software Enzfitter using the Michaelis-Menten kinetics equation.

**RESULTS**

**UV Cross-linking of ArsA with [γ-32P]ATP**

ArsA has previously been shown to form an adduct with [α-32P]ATP on activation with UV light (6, 11). In the present study, effect of antimonite on the cross-linking reaction was investigated. These experiments led to a reevaluation of the nature of the adduct and have provided significant new insights into the mechanism of function of the enzyme.

**Role of Antimonite—**To determine the effect of antimonite on UV cross-linking of ArsA with [α-32P]ATP, one set of samples was preincubated in the presence of antimonite at 37 °C before exposure to UV light. Data shown in Fig. 1A indicate that the sample that had been preincubated with antimonite contained much higher levels of the adduct (lane 3) compared with the sample that either contained no antimonite (lane 2) or the sample that contained antimonite but was not preincubated at 37 °C (lane 1). Because antimonite is known to bring about stimulation of hydrolysis of ATP by ArsA (2), increased labeling in the presence of antimonite may be resulting from increased hydrolysis of ATP by ArsA rather than due to enhanced binding of ATP. Addition of excess cold ADP or ATP to the reaction just before exposure to UV light was found to quench the adduct. At 0.5 and 1.0 mM concentrations of the cold nucleotide, ADP was found to be more effective at quenching the reaction compared with ATP just before cross-linking. Data in Fig. 1A indicate that much less adduct is formed in the presence of antimonite (final concentration, 20 μM) as indicated A, effect of antimonite on adduct formation with [α-32P]ATP. All samples contained magnesium. Lanes 1 and 3, with antimonite. Lanes 2 and 4, no antimonite. Lanes 5 and 6, no preincubation at 37 °C. Lanes 3 and 4, samples were preincubated at 37 °C for 10 min before cross-linking. B, effect of excess cold ADP or ATP on adduct formation with [α-32P]ATP. Preincubations were carried out as indicated. The indicated concentrations of cold ADP or ATP were then added, and samples were exposed to UV light as described below. Lanes 1 and 2, no addition. Lanes 3 and 4, 0.5 mM. Lanes 5 and 6, 1.0 mM. Lanes 7 and 8, 5.0 mM. Lanes 3 and 5, 7, ADP. Lanes 4, 6, and 8, ATP C, comparison of UV-activated adduct formation between ArsA and [α-32P]ATP or [γ-32P]ATP. The reactions contained either [α-32P]ATP or [γ-32P]ATP. Lanes 1, 2, 3, 5, and 7, no antimonite. Lanes 2, 4, 6, and 8, with antimonite. Lanes 3, 4, 7, and 8, preincubated at 37 °C for 10 min. Lanes 1, 2, 5, and 6, not preincubated. Following the preincubation in A, B, or C, the samples were transferred to ice and subjected to UV cross-linking for 30 min. The samples were analyzed by 10% polyacrylamide-SDS gel electrophoresis, followed by autoradiography. About 20 μg of protein was loaded in each lane.

ArsA and ADP and that increased hydrolysis of ATP by ArsA in the presence of antimonite results in increased adduct with ADP.

**Labeling of the A1 or A2 Mutants with [α-32P]ATP—**Mutants in the A2 domain of ArsA, which contain a normal A1 domain, have previously been shown to form an adduct in the presence of ATP on UV cross-linking (4), whereas mutants in the A1 domain do not form the adduct (3). Data in Fig. 3A indicate that, unlike the case with the wild type protein (lanes 1 and 2), preincubation with antimonite has no significant effect on the adduct formed with the A2 mutants GA337 or GR337 (lanes 3–6). A1 mutant GS20 showed no adduct in the presence or absence of antimonite (lanes 7 and 8).
Unisite and Multisite Catalytic Activity in ArsA

Fig. 2. UV-activated adduct between ArsA and [3H]ADP. Light-activated adduct formation between ArsA (10 μM) and [3H]ADP (10 μM) was carried out as described under "Materials and Methods." The reaction mix contained antimonite or magnesium as indicated. Lane 1, with antimonite. Lane 2, no magnesium. Lane 3, with antimonite. Lane 4, with antimonite and magnesium. Lane 5, with 5 mM cold ADP.

Fig. 3. Characterization of the UV-activated adduct between A1 or A2 mutant proteins or A1 peptide N35 and [α-32P]ATP. N35 peptide was expressed in E. coli cells, and the protein was recovered from the inclusion bodies as described earlier (17). All samples were preincubated at 37 °C for 10 min. The experimental conditions for analysis of the light-activated adduct were same as described in the legend to Fig. 1. A, A1 (G20S) or A2 (G337A or G337R) mutants. Lanes 1 and 2, wild type. Lanes 3 and 4, GA337. Lanes 5 and 6, GR337. Lanes 7 and 8, GS20 B, N35. Lanes 1–4, N35. Lanes 5–8, wild type

Labeling of the A1 Peptide with [α-32P]ATP—Previous studies have indicated that the N-terminal peptide N35 (residues 1–323), consisting of the A1 domain plus the linker, forms an independent domain capable of forming an adduct in the presence of ATP on exposure to UV light (5). Results in Fig. 3B indicate that, as in the case of A2 mutants, antimonite has no effect on the amount of the adduct formed with N35 (lanes 1 and 2), indicating that the A2 domain is required for the stimulatory effect of antimonite.

The UV cross-linking experiments described above suggest that adduct in the absence of antimonite results primarily from the function of A1 site, whereas increased adduct on addition of antimonite is the result of involvement of both A1 and A2. Because the adduct is between ArsA and ADP, these data suggest that ArsA carries out unisite catalysis from the A1 site in the absence of antimonite and multisite catalysis from both A1 and A2 in the presence of antimonite.

A1 and A2 in the presence of antimonite. This is further supported by the observation that mutants in the A2 site are still able to form the adduct due to catalysis by A1 and the adduct in these mutants is not affected by antimonite, whereas mutants in the A1 site do not form the adduct due to absence of both unisite and multisite catalysis.

Site of the UV-induced ATP Adduct in the Presence or Absence of Antimonite

The site where the adduct forms in the absence of antimonite has previously been identified to lie in a 40-amino acid stretch in the linker region (residues 283–320) connecting the A1 and A2 domains (6) (see also Fig. 4, lane 4). Because enhanced adduct formation was seen under conditions where antimonite was present, it was of interest to determine whether this was due to the formation of the adduct at an additional site, perhaps resulting from catalysis in the A2 domain. In the present study, the same amounts of the the ArsA protein labeled with [α-32P]ATP in the absence or presence of antimonite were subjected to complete V-8 protease digestion as described under "Materials and Methods." The samples were analyzed by 12% polyacrylamide Tricine-SDS gel electrophoresis. Lanes 1 and 3, labeled in the presence of antimonite. Lanes 2 and 4, labeled in the absence of antimonite. Lanes 1 and 2, undigested controls. Lanes 3 and 4, digested with V-8 protease. 25 μg of protein was loaded in each lane.

To further analyze unisite and multisite catalysis by ArsA, the amounts of phosphate released upon hydrolysis of ATP from A1 or from A1 and A2 together were determined. [γ-32P]ATP was employed in these experiments, and the amount of 32P released was determined by TLC as described under "Materials and Methods." The autoradiogram of a TLC obtained from a typical experiment is shown in Fig. 5. In this case, the autoradiogram was of the TLC obtained from a typical experiment is shown in Fig. 5. In this

Unisite and Multisite Hydrolysis of [γ-32P]ATP by ArsA

Fig. 4. Localization of the UV-activated adduct between ArsA and [α-32P]ATP. The ArsA protein UV cross-linked with [α-32P]ATP in the presence or absence of antimonite was subjected to complete V-8 digestion as described under "Materials and Methods." The samples were analyzed by 12% polyacrylamide Tricine-SDS gel electrophoresis. Lanes 1 and 3, labeled in the presence of antimonite. Lanes 2 and 4, labeled in the absence of antimonite. Lanes 1 and 2, undigested controls. Lanes 3 and 4, digested with V-8 protease. 25 μg of protein was loaded in each lane.

A1 and A2 in the presence of antimonite. This is further supported by the observation that mutants in the A2 site are still able to form the adduct due to catalysis by A1 and the adduct in these mutants is not affected by antimonite, whereas mutants in the A1 site do not form the adduct due to absence of both unisite and multisite catalysis.

Site of the UV-induced ATP Adduct in the Presence or Absence of Antimonite

The site where the adduct forms in the absence of antimonite has previously been identified to lie in a 40-amino acid stretch in the linker region (residues 283–320) connecting the A1 and A2 domains (6) (see also Fig. 4, lane 4). Because enhanced adduct formation was seen under conditions where antimonite was present, it was of interest to determine whether this was due to the formation of the adduct at an additional site, perhaps resulting from catalysis in the A2 domain. In the present study, the same amounts of the the ArsA protein labeled with [α-32P]ATP in the absence or presence of antimonite were subjected to complete V-8 protease digestion as described under "Materials and Methods." The samples were analyzed by 12% polyacrylamide Tricine-SDS gel electrophoresis. Lanes 1 and 3, labeled in the presence of antimonite. Lanes 2 and 4, labeled in the absence of antimonite. Lanes 1 and 2, undigested controls. Lanes 3 and 4, digested with V-8 protease. 25 μg of protein was loaded in each lane.

To further analyze unisite and multisite catalysis by ArsA, the amounts of phosphate released upon hydrolysis of ATP from A1 or from A1 and A2 together were determined. [γ-32P]ATP was employed in these experiments, and the amount of 32P released was determined by TLC as described under "Materials and Methods." The autoradiogram of a TLC obtained from a typical experiment is shown in Fig. 5. In this case, the autoradiogram was of the TLC obtained from a typical experiment is shown in Fig. 5. In this
In the absence of magnesium, no ATP hydrolysis was observed. In this study, it was also found that the A2 site appears to be inaccessible to ATP in the absence of antimonite. Hence, the site of adduct formation, identified earlier is shared between A1 and A2 and that the adduct formation was abolished in mutations in the A1 Walker A sequence but was unaffected by mutations in the A2 domain (3, 4), suggesting that the A1 domain of ArsA is involved in binding ATP in the UV-catalyzed reaction. The reason for the lack of a similar adduct formation in this paper provide an interesting explanation for the absence of the adduct in A2: it might lie in the fact that the site identified earlier is shared between A1 and A2 and that the adduct is the result of stepwise catalytic reactions, involving first A1 and then A2, as described below.

The data shown here suggest that UV cross-linking actually reflects binding of ADP and not binding of ATP as assumed earlier. Hence, the site of adduct formation, identified earlier, is the site where the product of the reaction ADP binds. Second, it is shown that addition of antimonite enhances the adduct formation significantly. If the adduct is between ArsA and ADP, thus reflecting hydrolysis of ATP, then adduct formation in the absence of antimonite reflects unisite catalysis by A1, and adduct formation in the presence of antimonite would reflect multisite catalysis involving A1 and A2. This is supported by the fact that point mutants in the A1 site do not form the adduct, whereas point mutants in the A2 site form the adduct. Interestingly, the addition of antimonite has no significant effect on the adduct formation in A2 mutants, implying the absence of multisite catalysis in these mutants. Hence, A2 mutants seem to carry the first half of the reaction, i.e. unisite catalysis in A1 leading to formation of the ADP adduct, correctly. These results imply that the A1 site is first in the sequence of events, and A2 comes into play later.

**Kinetic Parameters**

Because ATP is hydrolyzed by ArsA at different rates under conditions utilizing the A1 domain alone (in the presence of antimonite) or A1 and A2 domains together (in the presence of antimonite), it was of interest to determine the $K_m$ and $V_{max}$ of the protein under these conditions. Hence, the initial rate of hydrolysis by ArsA at several different concentrations of ATP was determined. Fig. 6 shows the rate of $^{32}$P release at different concentrations of ATP in the absence and presence of antimonite. The kinetic constants obtained upon analysis of the data by the Lineweaver-Burk plot suggest that the $V_{max}$ of the A1 site alone is about $\frac{1}{40}$th the $V_{max}$ obtained for the A1 + A2 sites together. However, $K_m$ of the A1 site alone or A1 + A2 sites together lies within the same range, which is around 150–200 $\mu$M, implying that the A1 site by itself has a high affinity for ATP that is equal to the overall affinity obtained in the presence of antimonite. $K_m$ and $V_{max}$ data in the presence of antimonite have been reported earlier (2), and the values obtained in this study are in the same range as before.

**DISCUSSION**

In most positively regulated allosteric proteins, the binding of an effector serves to increase the affinity of the enzyme for its substrate. Hence, the effect of the ligand can be bypassed by increasing the substrate concentration. However, allosteric regulation of the ATPase activity of ArsA by antimonite appears to occur by a novel mechanism, in which binding of antimonite acts as an “on/off” switch that allows ATP binding to the A2 site (7). The A1 site of ArsA is a high affinity site, and it does not require antimonite for ATP binding; however, the A2 site appears to be inaccessible to ATP in the absence of the ligand (7). This novel mechanism of allosteric regulation in ArsA results from the fact that the two ATP binding sites in ArsA have different conformations, and the A2 site is in an “open” conformation only when the ligand is present. In the present study, it is shown that the A1 site of ArsA is capable of carrying out unisite catalysis in the presence of magnesium alone; however, the addition of antimonite brings about an involvement of the A2 site, thus switching the protein from unisite to multisite catalysis. For full catalytic activity of ArsA, an interaction between A1 and A2 is required which is brought about by antimonite. Whether the A2 site also carries out unisite catalysis is not clear at the moment.

Unisite catalytic activity of the A1 site is strongly supported by UV cross-linking experiments carried out with [$\gamma^{32}$P]ATP. Previous experiments with ArsA resulted in the identification of a 40- amino acid stretch of protein that forms a linker connecting the A1 and the A2 domains as the site where the UV-activated adduct lies (6). The adduct formation was abolished in mutations in the A1 Walker A sequence but was unaffected by mutations in the A2 domain (3, 4), suggesting that only the A1 domain of ArsA is involved in binding ATP in the UV-catalyzed reaction. The reason for the lack of a similar adduct in the A2 domain has been elusive. Experiments described in this paper provide an interesting explanation for the absence of the adduct in A2: it might lie in the fact that the site identified earlier is shared between A1 and A2 and that the adduct is the result of stepwise catalytic reactions, involving first A1 and then A2, as described below.

The data shown here suggest that UV cross-linking actually reflects binding of ADP and not binding of ATP as assumed earlier. Hence, the site of adduct formation, identified earlier, is the site where the product of the reaction ADP binds. Second, it is shown that addition of antimonite enhances the adduct formation significantly. If the adduct is between ArsA and ADP, thus reflecting hydrolysis of ATP, then adduct formation in the absence of antimonite reflects unisite catalysis by A1, and adduct formation in the presence of antimonite would reflect multisite catalysis involving A1 and A2. This is supported by the fact that point mutants in the A1 site do not form the adduct, whereas point mutants in the A2 site form the adduct. Interestingly, the addition of antimonite has no significant effect on the adduct formation in A2 mutants, implying the absence of multisite catalysis in these mutants. Hence, A2 mutants seem to carry the first half of the reaction, i.e. unisite catalysis in A1 leading to formation of the ADP adduct, correctly. These results imply that the A1 site is first in the sequence of events, and A2 comes into play later.
adduct in GS20 in either the absence or presence of antimonite suggests that the A2 site, by itself, is not catalytic/functional, and even though antimonite brings about the participation of A2 in catalysis, it requires a functional A1 to do that. Hence, it seems reasonable to suggest that antimonite brings about an interaction between an intact A1 and A2 site that may precede ATP binding to A2 resulting in catalytic co-operativity. It is possible that the A2 site may only play a regulatory role so that ATP binding to A2 allows much faster product release from A1 without itself being catalytic in the process. A model showing unisite and multisite catalysis in ArsA is shown in Fig. 7.

Unisite and multisite catalytic activity is well documented for the F_0F_1 ATPase (12). F_1 ATPase contains three catalytic sites on three separate β subunits. The three sites in F1 are equivalent; however, they are asymmetric in their affinity for ATP, resulting in three $K_m$ values (13). Which site is the high affinity site at any particular time is determined by the rotational position of the γ subunit with respect to the αβ_3 head. Hence, each site in turn becomes a high affinity site (14). At substoichiometric concentrations of ATP, the high affinity site in F_1 is filled with ATP and is able to carry out unisite catalysis. At higher concentrations, ATP binds to the second and the third sites, and it causes a 10^6-fold higher rate of product release from the first high affinity site (13). ArsA is different in that the asymmetry between the two nucleotide binding sites in ArsA is present in the structure of the protein. The A1 site is a high affinity site and is able to carry out unisite catalysis just like each of the three sites in F_1, whereas binding of ATP to A2 seems to be controlled by a switch, which is the binding of the ligand antimonite. Filling of the A2 site with ATP results in catalytic co-operativity between A1 and A2; however, it is not clear whether, like each of the three catalytic sites in F_1, the A2 site is able to carry out catalysis.
Even though, under multisite catalytic conditions, the amount of the UV-activated adduct formed with ADP is significantly increased, it was found that the adduct under both unisite and multisite conditions lies in the same location, i.e., in the linker region. As discussed above, UV cross-linking between ArsA and $[\alpha-^{32}\text{P}]\text{ATP}$ identifies a site where the product of the reaction, ADP, binds. The experiments described here suggest that there is only one such site in ArsA, thus explaining why it has not been possible to identify an adduct site specific for the A2 domain. Binding of ADP to the linker under either condition (unisite using A1 or multisite using A1 and A2) suggests that the linker region might lie at the interface of the A1 and the A2 domains, and the ADP binding site in the linker region is a site common to both A1 and A2. In addition, such a location for binding of ADP might have significant implications in the energy transduction process. The fact that there is an ADP-bound conformation resulting from hydrolysis suggests that the ArsA protein switches between the ATP-bound and the ADP-bound states. It seems likely that one of these two conformations may be active in terms of the ability of the protein to interact with the ArsB protein. Hence, the linker region might be actively involved in the energy transduction process. Switching between the ADP- and ATP-bound forms of a protein has been shown for other ATPases, and it has been suggested that this switching is involved in the transduction of energy (15, 16). In the RecA protein, it has been suggested that, in addition to the Walker A and Walker B motifs that form the nucleotide binding site, the protein contains a motif C that undergoes conformational changes upon hydrolysis of ATP causing it to interact with its substrate DNA (16). In ArsA, the linker region might be equivalent to the region C of RecA, and it might be directly involved in interacting with ArsB. This is an interesting extension of the studies described herein, which needs to be investigated further. Hence, understanding the biochemical basis of catalysis in proteins such as ArsA and the conformational changes that result from catalysis would eventually lead to an understanding of the mechanism of energy transduction between the catalytic component and the membrane component of the pump.

Acknowledgment—I thank P. C. Tai for critical comments and discussions during preparation of the manuscript.

REFERENCES
1. Kaur, P. (1998) in *Advances in Molecular and Cell Biology* (Andersen, J. P., ed), Vol. 23B, pp. 453–488, Jai Press, Inc., Greenwich, CT
2. Hsu, C.-M., and Rosen, B. P. (1989) *J. Biol. Chem.* 264, 17349–17354
3. Karkaria, C. E., Chen, C. M., and Rosen, B. P. (1990) *J. Biol. Chem.* 265, 7832–7836
4. Kaur, P., and Rosen, B. P. (1992) *J. Biol. Chem.* 267, 19272–19277
5. Kaur, P., and Rosen, B. P. (1994) *J. Bacteriol.* 175, 351–357
6. Kaur, P., and Rosen, B. P. (1994) *Biochemistry* 33, 6456–6461
7. Ramaswamy, S., and Kaur, P. (1998) *J. Biol. Chem.* 273, 9243–9248
8. Yue, V. T., and Schimmel, P. R. (1997) *Biochemistry* 16, 4678–4684
9. Schagger, H., and Jagow, G. V. (1987) *Analytical Biochem.* 166, 368–379
10. Hiromura, M., Yano, M., Mori, H., Inoue, M., and Kido, H. (1998) *J. Biol. Chem.* 273, 5435–5438
11. Rosen, B. P., Weigel, U., Karkaria, C., and Gangola, P. (1988) *J. Biol. Chem.* 263, 3067–3070
12. Boyer, P. D. (1989) *FASEB J.* 3, 2164–2178
13. Cross, R. L., Grubmeyer, C., and Penefsky, H. S. (1982) *J. Biol. Chem.* 257, 12101–12105
14. Abrahams, J. P., Leslie, A. G. W., Lutter, R., and Walker, J. E. (1994) *Nature* 370, 621–628
15. Milburn, M., Tong, L., DeVos, A. M., Brunger, A., Yamazumi, Z., Nishimura, S., and Kim, S.-H. (1990) *Science* 247, 939–945
16. Story, R., and Steitz, A. T. (1992) *Nature* 355, 374–376
17. Kaur, P., and Rosen, B. P. (1994) *J. Biol. Chem.* 269, 9698–9704