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Molecular Characterization of an Anion Pump

THE ArsB PROTEIN IS THE MEMBRANE ANCHOR FOR THE ArsA PROTEIN*

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R-factor mediated bacterial resistance to arsenical salts occurs by active extrusion of the toxic oxyanions from cells of gram negative bacteria. The *ars* operon of the conjugative plasmid R773 encodes an anion pump. The pump has two polypeptide components. The catalytic subunit, the ArsA protein, is an oxyanion-stimulated ATPase. The membrane component, the ArsB protein, has been localized in the inner membrane of *Escherichia coli*. The ArsA and ArsB proteins have been postulated to form a membrane complex which functions as an anion-translocating ATPase. In this study evidence is presented showing that expression of the *arsB* gene is required to anchor the ArsA protein to the inner membrane. Binding studies with purified ArsA to membranes with and without the *arsB* gene product confirm this requirement. Membranes of *uncA* mutants containing both the ArsA and ArsB proteins exhibit arsenite(antimonite)-stimulated ATPase activity. These results support the model in which the ArsA protein is the catalytic energy transducing component of the anion pump, whereas the integral membrane ArsB protein serves as both the anion channel and membrane binding site for the ArsA protein.

We have recently described the existence of a novel anion pump, the first identified member of a new family of ion-translocating ATPases (Rosen *et al.*, 1988; Rosen *et al.*, 1989). The conjugative plasmid R773 confers resistance in *Escherichia coli* to arsenical salts by active extrusion of the toxic oxyanions from the cell. *In vivo* extrusion is energized by direct coupling to chemical energy, most likely ATP, suggesting the action of an anion-translocating ATPase (Silver *et al.*, 1981; Mobley and Rosen, 1982; Silver and Keach, 1982; Rosen and Borbolla, 1984). *In vitro* analysis has demonstrated that gene products of the arsenical pump form an oxyanion-stimulated ATPase.

From genetics and nucleotide sequence analysis the arsenical resistance operon has been shown to consist of three structural genes, of which two, *arsA* and *arsB*, are required for resistance and transport of AsO_2^- (Mobley *et al.*, 1983; Chen *et al.*, 1985, 1986). The ArsB protein has been identified as an integral membrane protein localized in the inner membrane (Chen *et al.*, 1986; San Francisco *et al.*, 1989) and has been postulated to be the anion channel component of the pump. When expressed from a high efficiency promoter, the

ArsA protein is produced to about 10% of total cell protein and can be easily purified from the cytosol of *E. coli*. The purified protein exhibits arsenite(antimonite)-stimulated ATPase activity. However the *arsB* gene is not highly expressed even though it is part of the same transcript as *arsA*.

We have postulated that the ArsA and ArsB polypeptides form a membrane complex which functions as an oxyanion-translocating ATPase (Chen *et al.*, 1986). Our model predicts that the ArsA protein functions as the catalytic energy transducing component, while the integral membrane ArsB protein acts as an anion channel. This model implies a direct specific interaction between the ArsA and ArsB proteins. In this report we demonstrate that expression of the *arsB* gene is required for membrane association of the ArsA protein. A preliminary report of this work has appeared (Rosen *et al.*, 1989).

MATERIALS AND METHODS

Strains and Plasmids—*E. coli* strains and plasmids used in this study are described in Table I. Cells were grown in Luria broth medium containing 0.2% glucose. Where required ampicillin or kanamycin (40 μg/ml) or arsenite (1 mM) were added to the growth medium. Cultures were incubated with aeration at 37 °C, unless otherwise stated.

Cell Fractionation—Isolation of everted membrane vesicles and separation into inner and outer membranes was performed as described previously (San Francisco *et al.*, 1989). For most membrane preparations cultures (0.5 liter) of exponentially growing cells at 37 °C (OD_{590} = 0.8–1.0) were harvested at 4 °C at 10,000 × g. Cells were suspended in 10 ml of a solution of 10 mM Tris-HCl, pH 7.8, containing 0.75 M sucrose. Lysozyme was added to final concentration of 0.1 mg/ml, and the suspension was incubated on ice for 2 min. The cells were diluted slowly and gently by adding 2 volumes of a solution of 10 mM Tris-HCl, pH 8.0, containing 5 mM EDTA (buffer A) over a period of 10 min. The resulting spheroplasts were lysed by a single passage through a French pressure cell at 20,000 p.s.i. Immediately prior to lysis, phenylmethylsulfonyl fluoride was added to the suspension to a final concentration of 1.0 mM. The lysate was centrifuged for 10 min at 10,000 × g, and the resulting supernatant solution was centrifuged at 100,000 × g for 90 min. The pelleted membranes were suspended in a solution of buffer A containing 0.5 M KCl and 20% glycerol (v/v). The suspension was centrifuged at 100,000 × g for 90 min. The pelleted everted membrane vesicles were suspended in 1 ml of buffer A. The membrane vesicles could be stored at −20 °C after the addition of glycerol to a final concentration of 50% (v/v). For separation into inner and outer membranes, everted membrane vesicles were layered onto a continuous sucrose gradient and centrifuged for 12–14 h in a swinging bucket rotor (Sorvall TH641) at 208,500 × g. Continuous sucrose gradients were prepared by four cycles of rapid freezing and thawing of 10 ml of a 45% sucrose (w/w) solution in buffer A. The top two-thirds of the upper band and the lowest band were removed and washed in a solution of buffer A containing 20% (v/v) glycerol. The pellets were suspended in buffer A with 50% (v/v) glycerol for storage at −20 °C. These fractions represent inner and outer membrane fractions, respectively (Osborne *et al.*, 1972). Strains containing the T7 expression system were grown overnight at 30 °C in Luria broth medium with ampicillin and kanamycin. The culture was diluted 5-fold into fresh medium and incubated for 3 h at 37 °C

TABLE I

Strains, plasmids, and phage

Strain/Plasmid/Phage	Genotype/Description[a]	Source or reference
HB101	F⁻, *hsds20*, *recA13*, *ara14*, *proA2*, *lacY1*, *galK2*, *rpsL20* (*sm*ʳ), *xyl-5*, *mtl-1*, *supE44*	Maniatis *et al.*, 1982
K38	HfcC (λ)	Russel and Model, 1984
AN120	F⁻, *uncA401*, *arg*, *rpsL*	Butlin *et al.*, 1971
pBR322	Apʳ, Tetʳ	Maniatis *et al.*, 1982
pUM3	pBR322 with the ars operon (*arsA*, *arsB*, and *arsC* genes) cloned into the *Hind*III site of pBR322 (Apʳ)	Mobley *et al.*, 1983
pT7-5	Multiple cloning site downstream of T7 phage RNA polymerase promoter	Tabor and Richardson, 1985
pGP1.2	T7 phage RNA polymerase gene under control of λpₗ promotor; λc*I*ₜₛ	Tabor and Richardson, 1985
pT7-5-16	*Pst*I-*Hind*III fragment of pUM3 containing the *arsB* and *arsC* genes inserted into *Pst*I-*Hind*III-digested pT7-5	San Francisco *et al.*, 1989
pT7-5-69	*Eco*RI-*Hind*III fragment of M13mCMC69 inserted into *Eco*RI-*Hind*III-digested pT7-5 (entire *arsA* gene and 642 nucleotides of the *arsB* gene).	San Francisco *et al.*, 1989
pT7-5-91	*Eco*RI-*Hind*III fragment of M13mCMC91 inserted into *Eco*RI-*Hind*III-digested pT7-5	San Francisco *et al.*, 1989
pT7-5-1538	*Eco*RI-*Hind*III fragment of M13mCMC49 containing the *arsA*, *arsB*, *arsC* genes inserted into *Eco*RI-*Hind*III-digested pT7-5	San Francisco *et al.*, 1989
M13mCMC49	mWB373 with 4.3-kb *Hind*III fragment of pUM3 (the *arsA*, *arsB*, and *arsC* genes)	Chen *et al.*, 1986
M13mCMC69	*Bal*31 deletion clone of M13mCMC49 containing 2.6 kb of the ars operon (the entire *arsA* gene and 642 nucleotides of the *arsB* gene)	Chen *et al.*, 1986
M13mCMC91	*Bal*31 deletion clone of M13mCMC49 containing 1.9 kb of the ars operon (entire *arsA* gene)	San Francisco *et al.*, 1989

[a] kb, kilobase(s).

to allow for overexpression of genes under control of the T7 transcription system and for efficient formation of spheroplasts for cell fractionation.

Immunoblot Procedure—Samples were prepared by boiling in sodium dodecyl sulfate (SDS)[1] sample buffer for 5 min. SDS-polyacrylamide gel electrophoresis was carried out as described by Laemmli (1970). Proteins were electrophoretically transferred from polyacrylamide gels to nitrocellulose for 12–16 h at 50 mA. Unoccupied sites on the nitrocellulose filter were blocked using 5% non-fat dried milk in phosphate buffered saline (21 mM KH_2PO_4, 11 mM $Na_2HPO_4\cdot7H_2O$, 0.138 M NaCl, 2.5 mM KCl, pH 7.4) (buffer B). The filter was incubated with rabbit anti-ArsA serum (1:2000) for 1 h and washed three times for 10 min each time with 15 ml of buffer B containing 5% dried milk, all at 37 °C. To detect the antigen antibody complex the filter was incubated with goat anti-rabbit IgG (1:5000) to which horseradish peroxidase was conjugated. The filter was incubated with the conjugated second antibody for 1 h at 37 °C and washed three times with 15 ml of buffer B. Hydrogen peroxide (0.03%) and 4-chloro-1-naphthol (3 mg/ml) were added in buffer B to produce a color reaction. The intensity of individual lanes on immunoblots were examined by reflectance densitometry using a Hoefer densitometer.

Binding Assay—Purified ArsA protein (28 µg) was added to everted membrane vesicles (1 mg of membrane protein) in a buffer consisting of 25 mM Tris-HCl, pH 7.4, in a total volume of 0.4 ml. The suspension was incubated on ice for 20 min and then diluted with 8 ml of buffer A containing 0.2 M KCl and 20% (v/v) glycerol. The membranes were sedimented by centrifugation at 363,000 × *g* for 40 min at 4 °C. The membrane pellet was suspended in the same buffer and sedimented. The pelleted membrane vesicles were suspended in SDS sample buffer and boiled 5 min. The presence of bound ArsA was detected by immunoblot analysis.

ArsA Antibodies—Polyclonal antibodies were raised in New Zealand rabbits immunized with purified ArsA protein.

Urea Treatment of Membranes—Membranes were incubated in buffer A containing 6 M urea on ice for 30 min. This suspension was centrifuged at 100,000 × *g* for 90 min. The pelleted membranes were

suspended in a solution of buffer A containing 20% glycerol (v/v). The membranes were sedimented by centrifugation at 363,000 × *g* for 40 min at 4 °C. The membrane pellet was suspended in the same buffer and sedimented. The pelleted urea treated everted membrane vesicles were suspended in 1 ml of buffer A. The membrane vesicles were stored at −20 °C after the addition of glycerol to a final concentration of 50% (v/v).

ATPase Assay—ATPase activity was determined by measuring the amount of ADP produced using a coupled assay (Vogel and Steinhart, 1976). The assay mixture consisted of 1 ml of a 0.1 M Mops buffer adjusted to pH 7.5 with KOH, containing 2 units each of pyruvate kinase and lactic dehydrogenase, 1.25 mM phosphoenolpyruvate, 0.25 mM NADH and 5 mM ATP. NaCN (20 mM) was added to inhibit NADH oxidase activity. Oxyanion stimulation was examined by addition of 0.1 mM potassium antimonyl tartrate hemihydrate. Membranes were preincubated in assay buffer at 37 °C for 10 min before the initiation of the reaction by the addition of $MgCl_2$ to 2.5 mM. ATPase activity was estimated from the rate of decrease in A_{340} absorption expressed as nanomoles/min/mg protein.

Enzyme Assays—Glucose-6-phosphate dehydrogenase activity was estimated from the rate of increase in A_{340} absorption in an assay mixture containing 55 mM Tris-HCl, pH 7.8, 3.3 mM $MgCl_2$, 0.2 mM NADP⁺, and 3.3 mM glucose 6-phosphate in a total volume of 1 ml. NADH dehydrogenase activity was estimated from the rate of decrease in A_{340} absorption in an assay mixture containing 0.1 M Tris-HCl, pH 7.4, and 0.15 mM NADH in a total volume of 1 ml. Dehydrogenase activity is expressed as a percentage of total activity of a cell lysate.

Protein Assay—Protein content was estimated by a micromodification of the procedure of Lowry *et al.* (1951).

RESULTS

Localization of ArsA Protein on the Inner Membrane Requires Expression of the arsB Gene—We have demonstrated previously that the *arsB* gene product is an integral membrane protein located in the inner membrane of *E. coli* (San Francisco *et al.*, 1989). However, *arsB* expression is not dependent on gene dosage, so that both arsenical extrusion and resistance

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[1] The abbreviations used are: SDS, sodium dodecyl sulfate; Mops, 4-morpholinepropanesulfonic acid.

FIG. 1. **Cellular localization of the ArsA protein.** Cells of *E. coli* strain K38(pGP1.2) bearing plasmid pT7-5-1538 (*arsA arsB arsC*) (*curves 1–3*), pT7-5-91 (*arsA*) (*curves 4 and 5*), or pT-7-69 (*arsA and truncated arsB*) (*curves 6 and 7*) were fractionated into cytosol, inner and outer membranes by sucrose gradient centrifugation. Samples were electrophoresed on a 12% SDS-polyacrylamide gel, electrophoretically transferred to nitrocellulose, and incubated with anti-ArsA serum to detect the ArsA polypeptide. The immunoblots were analyzed by reflectance densitometry and are expressed as arbitrary reflectance units as a function of distance from the top of the immunoblot. *Curves 1, 4, and 6*, 8 µg of cytosol; *curves 2, 5, and 7*, 8 µg of inner membrane; *curve 3*, 8 µg of outer membrane. The position of the ArsA protein (*arrow*) was determined from the migration of authentic purified ArsA protein.
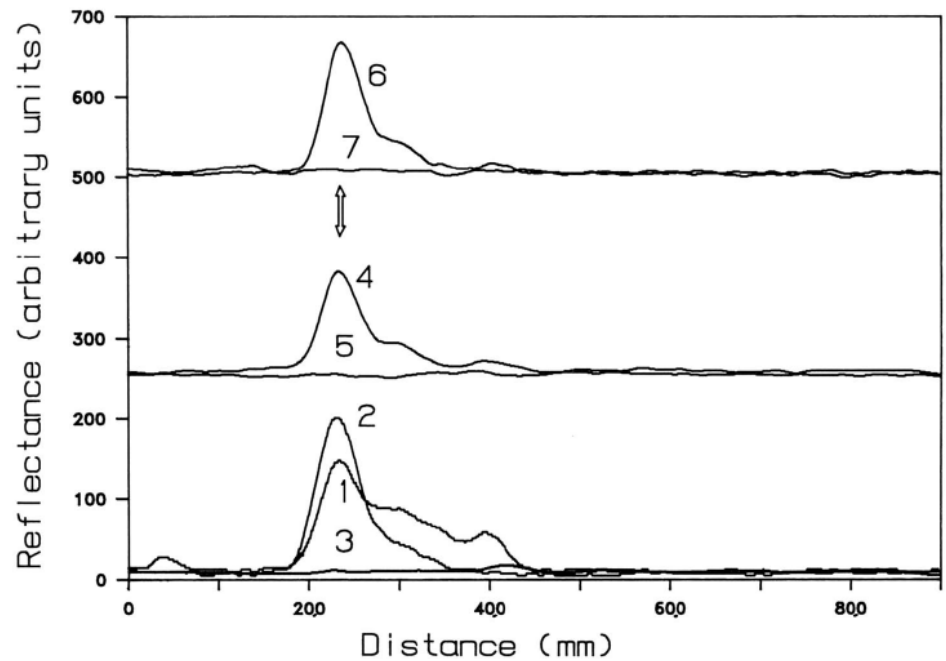
FIG. 2. **Binding of purified ArsA to everted membranes.** Everted membranes prepared from cells of *E. coli* strain K38 (pGP1.2) bearing either plasmid pT7-5 (no insert) (*lanes 2 and 3*) or pT-7-5-16 (*arsB arsC*) (*lanes 4 and 5*) were reconstituted with purified ArsA protein as described under "Materials and Methods." Samples were electrophoresed on a 12% SDS-polyacrylamide gel, electrophoretically transferred to nitrocellulose, and incubated with anti-ArsA serum. *Lanes 1 and 6*: 0.5 µg of purified ArsA; *lanes 2*, 20 µg of control membranes; *lanes 3*, 20 µg of membranes reconstituted with purified ArsA protein; *lanes 4*, 20 µg of control membranes; *lanes 5*, 20 µg of membranes reconstituted with purified ArsA protein.

TABLE II

Basal ATPase activity of membranes preparations

Strain (plasmid)[a]	ATPase activity[b]	
	Untreated	Urea-treated[c]
	nmol/min/mg	
AN120(pT7-5-16) grown in 0.2 M KCl	35	46
AN120(pT7-5) grown in 0.2 M KCl	32	35
K38(pT7-5-16)	1706	34
K38(pT7-5)	4948	38

[a] Cultures of *E. coli* strain K38 were grown in Luria broth medium. Cultures of *E. coli* strain AN120 was grown in media supplemented with 0.2 M KCl to repress expression of the *Kdp* genes. Everted membrane vesicles were prepared as described under "Materials and Methods."

[b] ATPase activity was determined by a coupled assay, as described under "Materials and Methods." NaCN (40 mM) was added to the membrane preparations to inhibit NADH oxidase activity. All measurements were corrected for cyanide-insensitive oxidase activity with control samples without ATP. Activity is expressed as nanomoles/min/mg membrane protein.

[c] Everted membranes were urea treated as described under "Materials and Methods."

TABLE III

In vitro binding of purified ArsA protein to membranes

Plasmid[a]	Oxyanion-stimulated ATPase activity[b]	
	Units	%
pT7-5-16 (*arsB arsC*)		
Membranes + ArsA protein	11.8	100
Wash supernatant	5.0	42
Washed membranes	5.5	46
pT7-5 (no insert)		
Membranes + ArsA protein	11.8	100
Wash supernatant	10.6	90
Washed membranes	0	0

[a] Everted membrane vesicles were prepared from *E. coli* strain K38 bearing the indicated plasmid as described under "Materials and Methods." Membranes were incubated with 11.8 units of purified ArsA protein. The membranes were washed three times to remove unbound ArsA protein. The initial mixture, supernatant solution from first wash, and final membrane suspension were assayed for antimonite-stimulated ATPase activity.

[b] Antimonite-stimulated ATPase activity was determined as described under "Materials and Methods" with 0.1 mM potassium antimonyl tartrate. Units of ATPase activity are defined as nanomoles ATP hydrolyzed/min/ml multiplied by the total volume of each fraction. Oxyanion-stimulated activities were corrected for basal activity.

outer membrane fractions. When immunoblots were probed with anti-ArsA serum, the presence of the ArsA protein was observed in the inner membrane fraction of cells bearing plasmid pT-7-1538, which expressed both the ArsA and ArsB proteins (Fig. 1, *curves 1–3*). The presence of ArsA protein in the soluble fraction probably reflects disproportionate synthesis of the ArsA and ArsB proteins. No ArsA protein was found in outer membrane.

Cells bearing plasmid pT7-5-91, which expresses the *arsA* gene but not the *arsB* gene, had ArsA protein only in the cytosolic fraction and not inner membrane (Fig. 1, *curves 4–5*). Similar results were obtained with fractions from cells bearing plasmid pT7-5-69, in which 645 nucleotides were deleted from the 3′ end of the *arsB* gene by *Bal*31 digestion (Fig. 1, *curves 6–7*). The product of this arsB deletion is

are limited by the amount of ArsB protein in the membrane.[2] To demonstrate that binding of the ArsA proteins to inner membrane requires the ArsB protein, cells containing plasmids encoding either the ArsA protein alone or the ArsA and ArsB proteins were fractionated into insoluble, inner and

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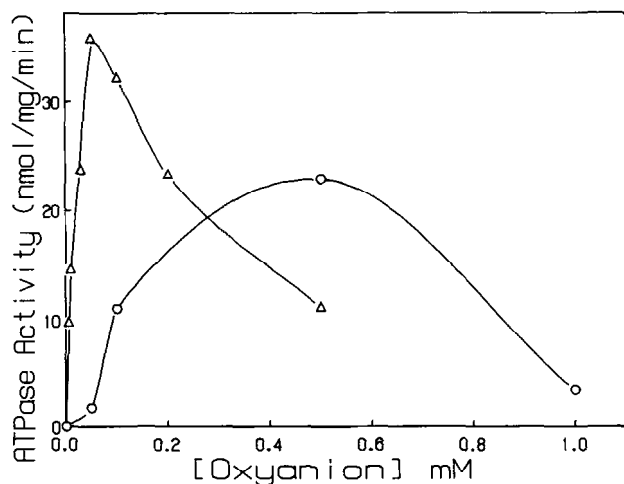[2] L. S. Tisa and B. P. Rosen, unpublished results.

FIG. 3. **Effect of oxyanion on anion-stimulated ATPase activity of membranes from AN120(pUM3).** ATPase activity was measured by a coupled assay system, as described under the "Materials and Methods." Oxyanion-stimulated ATPase activity was corrected for basal levels of ATPase activity and expressed as nanomoles/min/mg protein. △, antimonite-stimulated activity; ○, arsenite-stimulated activity.

inserted into the inner membrane as a truncated ArsB protein.[2] These results demonstrate that the ArsA protein requires an intact ArsB protein in order for association with inner membrane, implying interaction between the two *ars* gene products.

*Binding of Purified ArsA Protein to E. coli Membranes—*The ability of membranes to bind purified ArsA protein was examined by immunoblotting with anti-ArsA serum (Fig. 2). At low ionic strength considerable amounts of ArsA protein was found associated with membranes, but nonspecific binding could be prevented by inclusion of 0.2 M KCl in the binding and washing buffers (data not shown). Under those conditions membranes from cells expressing the *arsB* gene bound exogenously added ArsA protein, whereas membranes from cells without an *arsB* gene did not.

*Membrane-associated ArsA Protein Retains Oxyanion-stimulated ATPase Activity—*ArsA purified from cytosol possesses arsenite(antimonite)-stimulated ATPase activity (Rosen *et al.*, 1988). We reported in a preliminary communication that membrane-bound ArsA protein retains catalytic activity (Rosen *et al.*, 1989). The catalytic activity of the membrane-bound form is at least as great as the soluble form.[2]

However, in the absence of stimulatory oxyanions, high endogenous levels of ATPase activity were observed. Attempts were made to reduce this background (Table II). Cells were grown in the presence of 0.2 M KCl to repress the *kdpB* K+-ATPase (Epstein, 1984), but this had no effect on this background level of ATPase activity. The F_1 from strain AN120 has been reported to retain unisite catalysis at a rate of about 1% of the wild-type enzyme (Kanazawa *et al.*, 1984). Urea treatment of these membranes under conditions which removes F_1 and other extrinsic membrane proteins similarly was without effect. These results suggests that the background ATPase activity is not due to *kdpB*, residual F_1, or other loosely bound ATP hydrolases. Urea treatment of membranes from an *unc+* strain reduced the basal ATPase activity to the same level as the *uncA* strain, allowing use of *unc+* strains for binding studies. Membranes washed with urea were incubated with purified ArsA protein and washed three times in buffer containing 0.2 M KCl. The supernatant from the first wash and the membranes were assayed for ATPase activity (Table III). About 40% of the total number of ATPase units added

was found in the supernatant of the first wash. About half of the added oxyanion-stimulated ATPase activity was found on membranes from cells expressing the *arsB* gene. No membrane-bound antimonite-stimulated ATPase activity was found on membranes lacking ArsB.

*Properties of Membrane-bound Oxyanion-stimulated ATPase Activity—*The effect of oxyanion concentration on membrane-associated ATPase activity was examined (Fig. 3). Antimonite-stimulated ATPase activity exhibited maximum activity at approximately 50 μM antimonite and 0.5 mM arsenite. Higher concentrations of oxyanions were inhibitory. These values are similar to those found for the soluble ArsA protein (Hsu and Rosen, 1989). The catalytic activity of the purified soluble ArsA protein was stable when the enzyme was stored at −80 °C. In contrast, the membrane-bound activity did not retain oxyanion-stimulated ATPase activity for more than a few days (data not shown). However, oxyanion-stimulated ATPase activity was stable for several months with storage at −20 °C when the concentration of glycerol was adjusted to 50% (v/v) glycerol (data not shown).

DISCUSSION

We have previously demonstrated the existence of an anion pump encoded by the arsenical resistance operon of plasmid R773 (Rosen *et al.*, 1988; Rosen *et al.*, 1989). This pump reduces the intracellular concentration of the toxic oxyanions arsenite and antimonite, thus providing resistance (Mobley and Rosen, 1982; Rosen and Borbolla, 1984). The operon encodes three structural genes, of which the *arsA* and *arsB* are necessary for resistance and transport of arsenite (Chen *et al.*, 1985; Rosen and Borbolla, 1984). We have proposed a model in which the catalytic ArsA protein (Rosen *et al.*, 1988) and the inner membrane ArsB protein (San Francisco *et al.*, 1989) form a membrane-bound ATPase which catalyzes oxyanion transport. This model requires direct interaction between the two proteins. However, nearly all of the ArsA protein produced by cells bearing high copy number recombinant plasmids is found in the cytosol (Rosen *et al.*, 1988), and attempts to demonstrate a membrane-bound form were not conclusive. In part the difficulty in demonstrating direct interaction of the two proteins stems from the poor expression of the arsB gene (San Francisco *et al.*, 1989), such that about 100-fold more ArsA protein is produced than ArsB. The reason for differential expression is not entirely clear. Analysis of mRNA structure suggests a potential translational block to synthesis of the ArsB protein. Northern analysis of the *ars* message demonstrates rapid degradation of the mRNA in the *arsB* region.[3] Moreover, the ArsB protein cannot be visualized with protein stains on SDS gels, and the protein has not proven sufficiently antigenic to allow for production of antibodies. In spite of these difficulties, the ArsB protein has been identified by the creation of in-frame gene fusions of the *arsB* gene with *lacZ* and localized in the inner membrane (San Francisco *et al.*, 1989). In addition use of the T7 expression system has allowed visualization of the ArsB protein as a [35S] methionine-labeled membrane protein.

For those reasons a less direct approach was used to demonstrate interaction of the two proteins. The presence of ArsA protein on membranes from cells expressing the *arsB* gene could be clearly demonstrated by immunoblotting of membranes with antiserum prepared against the ArsA protein (Fig. 1). Cells producing ArsA protein and either a truncated ArsB protein or no ArsB protein at all contained the ArsA protein only in the cytosol. We have shown previously that

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[3] J. B. Owolabi and B. P. Rosen, unpublished results.

the ArsB protein is an integral membrane protein and that the ArsB protein in inserted into the inner membrane even in strains lacking the *arsA* gene (San Francisco *et al.*, 1989). Membranes from such cells were capable of binding purified ArsA protein added exogenously (Fig. 2), suggesting that the ArsB protein is inserted into the membrane in a functional form in the absence of the ArsA protein.

Membrane-bound ArsA protein was catalytically active as an oxyanion-stimulated ATPase, whether isolated from cells as a complex (Rosen *et al.*, 1989) or bound to isolated membranes *in vitro* (Table III). Properties of the membrane-bound form were similar to those of soluble. When corrected for the amount of ArsA protein on the membrane, the specific activity of the membrane-bound form was the same or greater than the soluble form, and the concentrations of arsenite and antimonite sufficient for half-maximal ATPase activity were the same order of magnitude.

These results demonstrate that expression of the *arsB* gene is essential for association of the ArsA protein with inner membrane, supporting the hypothesis that the integral membrane ArsB protein serves as the membrane anchor for the ArsA protein. The ability to reconstitute a functional ArsA· ArsB complex allows for future analysis of subunit stoichiometry and determination of the contact points between the two subunits of the anion pump. Finally, we would predict that the complex should catalyze ATP-driven oxyanion transport into everted membrane vesicles. The ability to bind purified ArsA protein to ArsB-containing membranes is a prerequisite for those studies.

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