Properties of the Arsenate Reductase of Plasmid R773†

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ABSTRACT: Resistance to toxic oxyanions in *Escherichia coli* is conferred by the *ars* operon carried on plasmid R773. The gene products of this operon catalyze extrusion of antimonials and arsenicals from cells of *E. coli*, thus providing resistance to those toxic oxyanions. In addition, resistance to arsenate is conferred by the product of the *arsC* gene. In this report, purified ArsC protein was shown to catalyze reduction of arsenate to arsenite. The enzymatic activity of the ArsC protein required glutaredoxin as a source of reducing equivalents. Other reductants, including glutathione and thioredoxin, were not effective electron donors. A spectrophotometric assay was devised in which arsenate reduction was coupled to NADPH oxidation. The results obtained with the coupled assay corresponded to those found by direct reduction of radioactive arsenate to arsenite. The only substrate of the reaction was arsenate (K_m = 8 mM); other oxyanions including phosphate, sulfate, and antimonate were not reduced. Phosphate and sulfate were weak inhibitors, while the product, arsenite, was a stronger inhibitor (K_i = 0.1 mM). Arsenate reductase activity exhibited a pH optimum of 6.3–6.8. These results indicate that the ArsC protein is a novel reductase, and elucidation of its enzymatic mechanism should be of interest.

Bacterial resistance to arsenicals and antimonials are carried on plasmids, conjugative plasmid R773 in *Escherichia coli* (Hedges & Baumberg, 1973) and plasmid pI258 in *Staphylococcus aureus* (Novick & Roth, 1968). The *ars* operons encode resistance to arsenite, antimonite, arsenate, and tellurite (Chen et al., 1986; Ji & Silver, 1992a; Rosenstein et al., 1992; Turner et al., 1992). Resistance to arsenite (As^{3+}) and antimonite (Sb^{3+}) results from active extrusion of the toxic oxyanions from the cells (Silver et al., 1981; Mobley & Rosen, 1982; Silver & Keach, 1982). Arsenate resistance requires an additional protein, the ArsC protein (Chen et al., 1985). The 131-residue ArsC protein encoded by plasmid pI258 has recently been shown to be an arsenate reductase coupled to thioredoxin (Ji & Silver, 1992b; Ji et al., 1994). The 141-residue ArsC protein encoded by plasmid R773 exhibits less than 20% sequence similarity to the pI258 protein. In vivo reduced glutathione was required for arsenate resistance in *E. coli*, while thioredoxin was not required (Oden et al., 1994). Mutants in the *gor* gene for glutathione reductase or the *gshA* or *gshB* genes for glutathione synthesis were sensitive to arsenate, and resistance was restored. In contrast, the *trx* and *grx* genes for thioredoxin and glutaredoxin, respectively, were not required for arsenate resistance. The R773 ArsC protein has no significant similarity to any known reductase and may be a member of a new family of oxyanion reductases.

In this report, the properties of the R773 ArsC protein are described. The protein was purified in an active form, and a spectrophotometric assay was developed in which the oxidation of NADPH was coupled to reduction of arsenate. The coupled assay required glutathione reductase, glutathione (GSH[1]), glutaredoxin, and the ArsC protein. In parallel assays, reduction of $^{73}AsO_4^{3-}$ to $^{73}AsO_2^-$ was measured, and there was good agreement between the oxidation of NADPH and reduction of arsenate. Arsenate reductase activity exhibited a pH optimum of 6.3–6.8. The K_m for arsenate was 8 mM. Of the oxyanions examined, only arsenate was a substrate. Phosphate and sulfate were not substrates but were weak inhibitors. Arsenite, the product of the reaction, was a potent inhibitor, with a K_i of 0.1 mM.

MATERIALS AND METHODS

Materials. $^{73}AsO_4^{3-}$ was purchased from Los Alamos Laboratories, Los Alamos, NM. Yeast glutathione reductase type IV was obtained from Sigma Chemical Co. 2-Hydroxyethyl disulfide (HED) was purchased from Aldrich Chemical Co. All other chemicals were obtained from commercial sources.

Purification of the ArsC Protein. The ArsC protein was purified by a modification of the procedure of Rosen et al. (1991). Three-liter cultures of *E. coli* strain JM109 bearing plasmid pArsC, which has the *arsC* gene cloned into plasmid PUC18, were grown with shaking at 37 °C in 2 × YT medium (Miller, 1972) with 0.1 mg/mL ampicillin. In plasmid pArsC, expression of the *arsC* gene is under control of the *lac* promoter. At an absorbance at 600 nm of 0.7, 50 μM isopropyl β-D-thiogalactopyranoside was added as an inducer, and the cells were grown for an additional 2 h at 37 °C. Cells were harvested by centrifugation at 4 °C, washed with a phosphate-buffered saline solution consisting of 11 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 0.14 M NaCl, and 2.65 mM KCl, pH 7.5, and stored at −70 °C overnight. Thawed cells were suspended in five volumes per gram of wet cells in a buffer consisting of 10 mM 3-[*N*-morpholino]propanesulfonate adjusted to pH 7.5 with Tris base, 2 mM Na_2EDTA, and 2 mM DTT (buffer A). The cells were lysed by a single passage through a French pressure cell at 20 000 psi, and the serine protease inhibitor of diisopropylfluorophosphate was added immediately at 5 μg per gram of wet cells. Unbroken cells and membranes were removed by centrifugation at 200000*g* for 1 h at 4 °C. The

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[1] Abbreviations: GSH, reduced glutathione; GSSG, glutathione disulfide; DTT, dithiothreitol; NEM, *N*-ethylmaleimide; Na_2EDTA, disodium ethylenediaminetetraacetate; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis, HED, 2-hydroxyethyl disulfide.

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supernatant solution was applied at a rate of 1 mL/min onto a 2.5-cm-diameter column packed with 170 mL of Q Sepharose anion exchanger (Sigma Chemical Co.) equilibrated with buffer A. The column was eluted with 170 mL of buffer A and then with a linear gradient of 0.15–0.25 M NaCl in 570 mL of buffer A at a flow rate of 1 mL/min. Fractions of 8 mL were collected and analyzed by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) on 15% acrylamide gels according to the method of Laemmli (1970). The peak fractions were concentrated to about 1 mL using a Centriprep-10 centrifugal concentrator (Amicon Corp.). The concentrated protein was applied to a 50-cm column filled with 100 mL of Superose 12 (Pharmacia) equilibrated with buffer A containing 0.2 mM NaCl. Fractions of 1 mL were collected at a flow rate of 0.5 mL/min. The location of the ArsC protein was determined by SDS–PAGE, and the peak fractions were stored at 4 °C.

Purification of Glutaredoxin. Glutaredoxin (GRX) was prepared as a modification of the method of Björnberg and Holmgren (1991). A 2-L culture of *E. coli* strain N4830 carrying the glutaredoxin expression plasmid pAHOB1, which carries the *grx* gene under control of the λP_L promoter (Björnberg & Holmgren, 1991), was grown with shaking at 30 °C in 2 × YT medium with 0.1 μg/mL ampicillin. At an absorbance at 640 nm of 0.7, the temperature was raised to 40 °C to inactivate the temperature-sensitive *cI*857 repressor, and the cells were harvested by centrifugation after 5 h. The cells were washed once with PBS and stored at −70 °C overnight. Thawed cells were suspended in five volumes per gram of wet cells in buffer A lacking DTT and containing 1 mM phenylmethanesulfonyl fluoride and lysed by a single passage through a French pressure cell at 20 000 psi. Unbroken cells and membranes were removed by centrifugation at 160000*g* for 1 h at 4 °C. The supernatant solution was applied at a rate of 1 mL/min onto the same Q Sepharose column described above equilibrated with buffer A without DTT. The column was eluted with 340 mL of the same buffer and then with 340 mL of 0.2 M NaCl in the same buffer at a flow rate of 1 mL/min. Fractions of 8 mL were collected and analyzed by SDS–PAGE on 15% acrylamide gels. Glutaredoxin was stored as a lyophilized powder at 4 °C.

Assay of Reductase Activity. Arsenate reductase activity was assayed by a modification of a glutathione disulfide oxidoreductase-dependent assay for glutaredoxin (Holmgren, 1979a; Björnberg & Holmgren, 1991). For glutaredoxin activity, the assay was performed at 37 °C in 1 mL of 0.1 M Tris-Cl, 2 mM Na_2EDTA, pH 8.0, containing 0.1 mg/mL BSA, 0.25 mM NADPH, 1 mM GSH, 50 nM yeast glutathione reductase, and 0.7 mM HED. For arsenate reductase activity, the assay buffer was 50 mM 3-[*N*-morpholino]propanesulfonate and 50 mM 2-[*N*-morpholino]-ethanesulfonate, pH 6.5, containing 0.1 mg/mL BSA, 0.25 mM NADPH, and 50 nM yeast glutathione reductase. Glutaredoxin, GSH, ArsC protein, and arsenate were added as indicated below. Reductase activity was monitored by the decrease in NADPH absorption at 340 nm and is expressed as micromoles of NADPH oxidized per minute using a molar extinction coefficient of 6200 for NADPH.

Assay of $NaH_2{}^{73}AsO_4$ Reduction. Reduction was assayed at 37 °C in 0.1 mL of a reaction mixture buffered with 50 mM 3-[*N*-morpholino]propanesulfonate and 50 mM 2-[*N*-morpholino]ethanesulfonate, pH 6.5, containing 0.1 mg/mL bovine serum albumin, 1 mM reduced glutathione, and 2 mM $Na_2H^{73}AsO_4$ (3000–5000 cpm/μL). NADPH (0.25 mM) and 50 nM yeast glutathione reductase were added where indicated. The reaction was initiated at 37 °C by addition of purified ArsC protein, arsenate, or glutaredoxin, as described below. Portions (2 μL) were removed at intervals and spotted onto a silica gel thin-layer chromatography plate (EM Separations 5553 or 5554). The samples were overlaid with 2–3 μL of 10 mM DTT and allowed to dry. In this chromatographic system, the R_f values for arsenate and arsenite were 0.08 and 0.2, respectively. To enhance the separation of substrate and product, DTT was added to convert arsenite to a cyclic arsenite–DTT compound (Zahler & Cleland, 1968), which chromatographs with an R_f of 0.75. The plates were chromatographed in 95% ethanol:1 N NH_4-OH (9:1) (Rosen & Borbolla, 1984) and dried, and the radioactivity in each spot was quantified with an Ambis β-scanner (Ambis Systems, San Diego, CA).

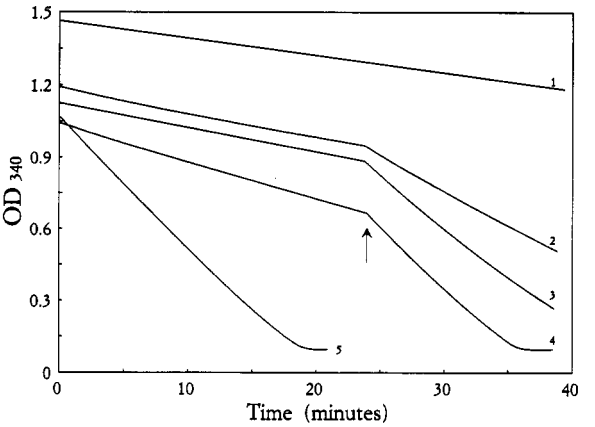

FIGURE 1: Requirements for arsenate reductase activity using a coupled assay. Arsenate reductase activity was determined from the decrease in the absorption of 0.25 mM NADPH at 340 nm, as described under Materials and Methods. In each assay, a combination of all of the following was present at the start of the assay, with the final constituent added at the time indicated by the arrow: 10 μg of glutaredoxin, 1 mM GSH, 3–6 μg of glutathione reductase, 0.1 mg of purified ArsC protein, and 2 mM sodium arsenate. Addition at arrow: curve 2, GSH; curve 3, ArsC protein; curve 4, glutaredoxin. In curve 5, all constituents were added at the start of the assay. Curve 1 shows the nonenzymatic decomposition of NADPH in buffer.

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

RESULTS

Arsenate Reductase Activity Requires the ArsC Protein. By modifying an assay for glutaredoxin activity (Holmgren, 1979a; Björnberg & Holmgren, 1991), a coupled spectrophotometric assay was devised for measurement of ArsC activity. In this assay, the primary source of reducing equivalents is NADPH, which reduces glutathione disulfide (GSSG) to GSH in a reaction catalyzed by glutathione reductase. Reduced glutathione then serves as the reductant for subsequent reactions (Figure 1). At pH 6.5 in the absence of any additions, NADPH absorbance slowly decreased (curve 1). At pH 7.5, the rate of nonenzymatic decomposition was considerably slower (data not shown). Oxidation increased to 26 nmol/min/mL when the combination of GSH, glutaredoxin, ArsC protein, and arsenate was added (curve 5). No activity was observed in the absence of any of the components (curves 2–4). Addition of the missing ingredient increased activity, suggesting that order of addition was not critical. Note that addition of the artificial electron acceptor HED increased the rate of NADPH oxidation in the presence

of glutathione reductase, GSH, and glutaredoxin (data not shown). In the assay shown in Figure 1, the rate of NADPH oxidation in the presence of excess HED was 100 nmol/min/mL, 4-fold higher than that of the ArsC-catalyzed reaction. The activity of the coupling system was measured by addition of HED at the end of every assay; the reactions of the coupling system were never rate limiting in any of the experiments described in this report. GSH was used at 1 mM in most assays; at higher concentrations, GSH appeared to react with NADPH in a glutathione reductase-catalyzed reaction, although the nature of the terminal electron acceptor is not clear.

Optimum Conditions for Arsenate Reductase Activity. The optimum pH of the reaction was 6.3–6.8 (data not shown). Reductase activity increased with temperature from 10 °C to 40 °C with an energy of activation of 8 kcal/mol (data not shown).

Reduction of Radioactive Arsenate. To demonstrate that the oxidation of NADPH corresponded to the production of arsenite, the production of radioactive arsenite was measured under the same conditions as the spectrophotometric assay (Figure 2). No arsenite was produced in the absence of the ArsC protein, even after 30 min (Figure 2A and 2C, lower curve). Arsenite production commenced immediately upon addition of ArsC protein. In the presence of the ArsC protein but absence of glutaredoxin, there was a slow rate of arsenite production (Figure 2B and 2C, upper curve) that was stimulated by addition of glutaredoxin. In the absence of NADPH and glutathione reductase, the reaction attained a low steady-state level after 10 min, while reduction continued for as long as 1 h in the presence of the NADPH and glutathione reductase (Figure 3), indicating the necessity for a GSH-regenerating system.

Substrate Specificity. Antimonate, phosphate, and nitrate were examined as substrates of the ArsC protein at 2 and 20 mM. There was no increase in NADPH oxidation with any of those oxyanions (data not shown). Except for high concentrations of phosphate, none of the oxyanions inhibited arsenate reduction when arsenate was added after the other oxyanions. Although the *arsC* gene provides tellurite resistance (Turner et al., 1992), interaction of tellurite with the ArsC protein could not be determined with this assay due to rapid reduction of tellurite by glutathione reductase (data not shown).

Kinetic Parameters of Arsenate Reductase. The rate of arsenate reduction as a function of arsenate concentration was determined (Figure 4). The K_m for arsenate was determined to be 8 mM. The V_{max} varied from preparation to preparation but was usually in the range of 0.8–1.5 μmol/min/mg of purified ArsC protein.

The rate of reduction as a function of the concentration of the glutaredoxin at a saturating concentration of arsenate was determined (data not shown). Half-maximal arsenate reductase activity was observed with 17 nM glutaredoxin. In these assays, the concentration of the ArsC protein was 0.2 μM. Reduced thioredoxin likewise binds with high affinity to ribonucleotide reductase (K_d = 0.13 μM) (Holmgren, 1979b).

Inhibitors of Arsenate Reductase. A variety of oxyanions were examined as inhibitors of the ArsC arsenate reductase, including arsenite, phosphate, antimonate, and nitrate. Only arsenite and phosphate were competitive inhibitors. Arsenite, the product of the reaction, was a potent inhibitor, with a K_i of 0.1 mM (Figure 5). Phosphate and sulfate, on the other hand, were poorer inhibitors, with K_i values of approximately

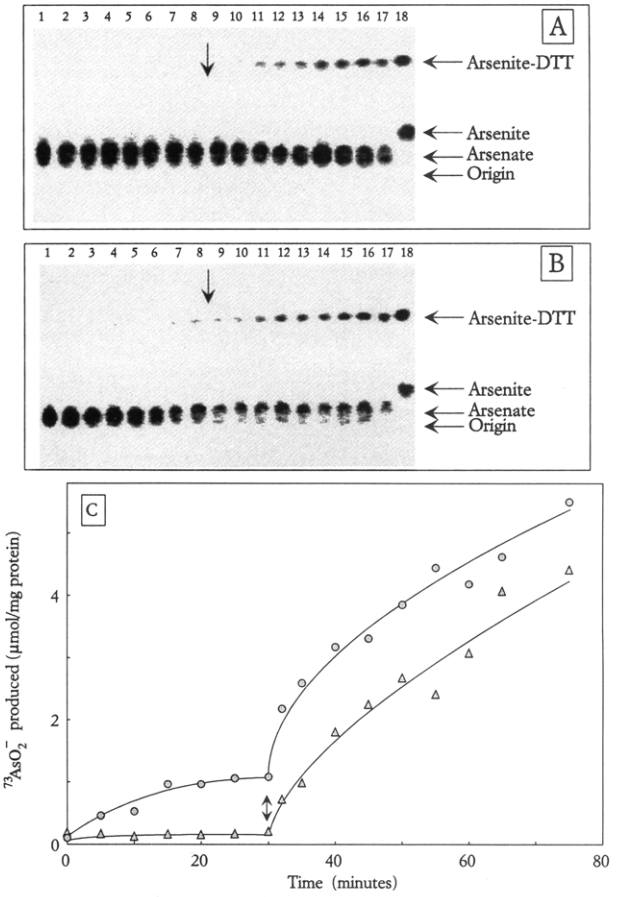

FIGURE 2: ArsC-catalyzed reduction of $NaH_2{}^{73}AsO_4$. Reduction of radioactive arsenate to arsenite was assayed under the conditions described in the legend to Figure 1. Each assay contained in 0.1 mL of 0.5 mM NADPH, 1 mM GSH, 0.3 μg of glutathione reductase, and 2 mM radioactive sodium arsenate. In A, 1 μg of glutaredoxin was present at the start of the assay, and 10 μg of ArsC protein was added at the time indicated by the arrow. In B, 10 μg of ArsC protein was present at the start of the assay, and 1 μg of glutaredoxin was added at the time indicated by the arrow. At 5, 10, 15, 20, 25, 30, 32, 35, 40, 45, 50, 55, 65, and 75 min, corresponding to lanes 1–18 in A and B, samples were removed and analyzed for production of labeled arsenite by thin-layer chromatography, as described under Materials and Methods. A and B show radioanalytic visualizations of thin-layer chromatographs. C shows a densitometric quantification of the data: at the arrows either ArsC protein (●) or glutaredoxin (▲) was added.

30 and 10 mM, respectively (Figure 6). Pretreatment of the ArsC protein with NEM resulted in rapid inactivation, suggesting that at least one cysteine residue is in or near the active site (Figure 7).

DISCUSSION

The *ars* operons of plasmids of both Gram positive and Gram negative bacteria provide resistance to arsenical oxyanions of the 3+ oxidation state (arsenite) and the 5+ oxidation state (arsenate). Resistance to arsenate in either type of bacterium requires the operation of an ArsC protein in addition to an arsenite extrusion system (Chen et al., 1985; Ji & Silver, 1992a). Recently, the ArsC protein of *S. aureus* plasmid pI258 was shown to catalyze arsenate reduction with thioredoxin as the source of reductant (Ji & Silver, 1992b). Neither GSH nor reduced glutaredoxin supports arsenate reduction catalyzed by the pI258 ArsC protein (Ji & Silver, 1992b; G. Ji and T. B. Gladysheva, unpublished).

We have shown that arsenate resistance mediated by the *E. coli* plasmid R773 ArsC protein in *E. coli* correlated with

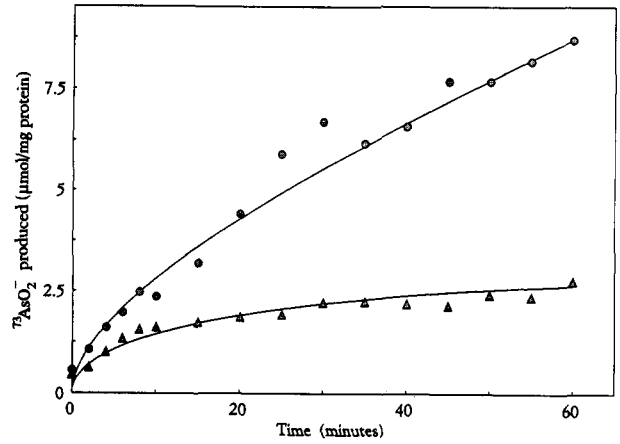

FIGURE 3: Requirement for a GSH-regenerating system in ArsC-catalyzed reduction of $NaH_2{}^{73}AsO_4$. Reduction of radioactive arsenate to arsenite was assayed as described in the legend to Figure 2. (●) Complete coupling system; (▲) no NADPH or glutathione reductase.

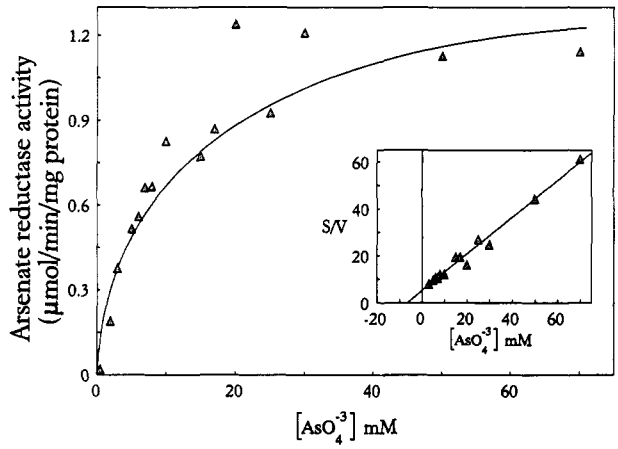

FIGURE 4: Determination of the K_m for arsenate in ArsC-catalyzed reduction of arsenate. Arsenate reductase activity was estimated from the decrease in NADPH absorption, as described in the legend to Figure 1, as sodium arsenate was added at the indicated concentrations. Inset: linearized transformation of the data; kinetic values were determined from a least-squares fit.

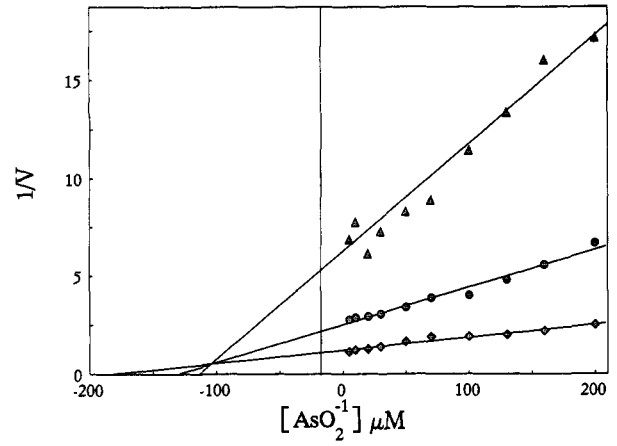

FIGURE 5: Competitive inhibition of ArsC-catalyzed arsenate reductase activity by arsenite. Arsenate reductase activity was estimated from the decrease in NADPH absorption, as described in the legend to Figure 1. The sodium arsenate concentration was 2 (▲), 5 (●), or 20 mM (◆). Sodium arsenite was added at the indicated concentrations.

reduction to arsenite (Oden et al., 1994). In this genetic analysis, glutathione was shown to be required for reduction and resistance. ArsC reductase activity similarly required

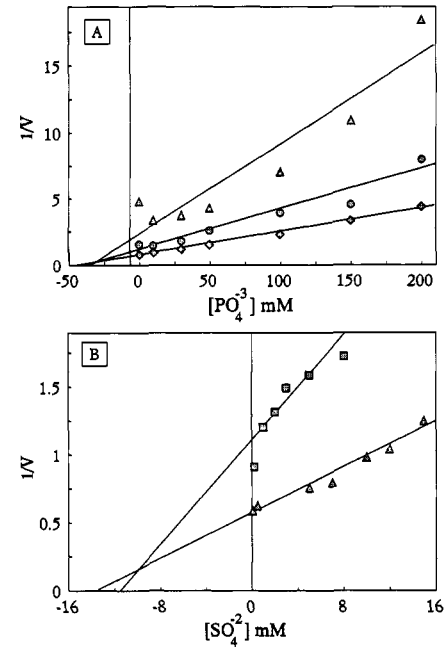

FIGURE 6: Competitive inhibition of ArsC-catalyzed arsenate reductase activity by phosphate and sulfate. Arsenate reductase activity was estimated from the decrease in NADPH absorption, as described in the legend to Figure 1. A: The concentration of sodium arsenate was 2 (▲), 5 (●), or 20 mM (◆). B: The concentration of sodium arsenate was 10 (▲) or 20 mM (■). Sodium phosphate (A) or sodium sulfate (B) was added at the indicated concentrations.

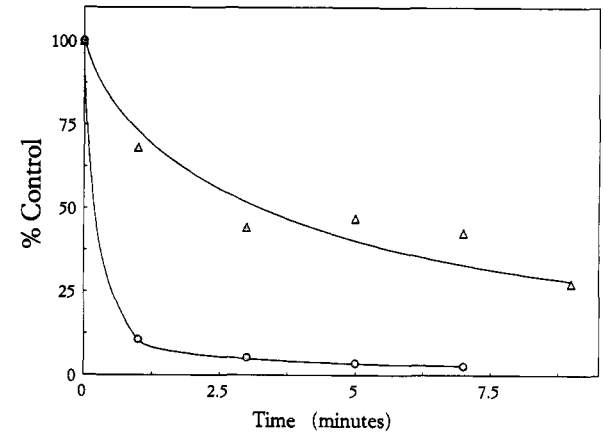

FIGURE 7: Inactivation of ArsC-catalyzed arsenate reductase activity by *N*-ethylmaleimide. Arsenate reductase activity was estimated from the decrease in NADPH absorption, as described in the legend to Figure 1, with 20 mM sodium arsenate. The ArsC protein was preincubated at 8 mg/mL with 10 (▲) or 100 μM (●) NEM for the indicated times, following which the protein was diluted 1000-fold into the assay mixture. Under these conditions, the coupling system was not rate limiting, as measured by HED reduction (data not shown).

GSH in vitro (Figures 1 and 5). Clearly, both the in vivo and the in vitro results demonstrate that the natural source of reducing equivalents is GSH. However, glutaredoxin was required only in vitro (Figures 1 and 8). A strain with an insertion in the gene for glutaredoxin had normal ArsC-mediated arsenate resistance (Oden et al., 1994). The reason for this difference is unknown. Genes for several additional glutaredoxin-like proteins have been identified in *E. coli* (A. Holmgren, personal communication), and it is possible that one or more of the gene products can substitute in vivo for glutaredoxin. Alternatively, the ArsC protein may couple directly to GSH in vivo without the need for a glutaredoxin-like protein.

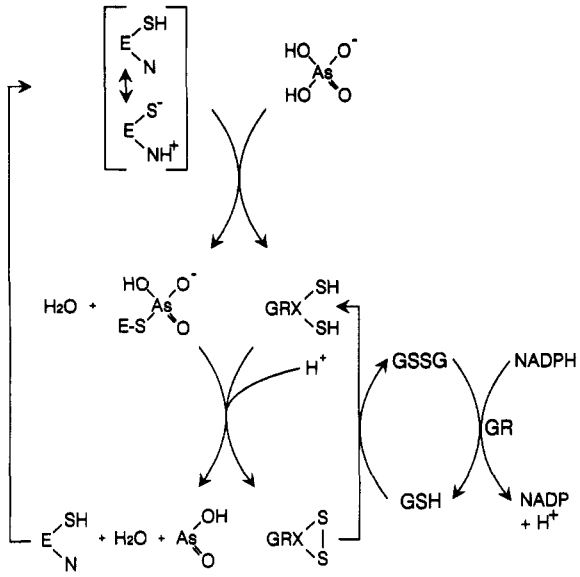

FIGURE 8: Proposed mechanism of the ArsC arsenate reductase. An enzyme–substrate intermediate would form by arsenylating the thiolate anion of Cys12. The cysteine residue is activated by tautomeric stabilization of the thiolate anion in an ion pair with a nearby basic residue in the ArsC protein. In the next step, the complex would interact with glutaredoxin with transfer of electrons to reduce As^{5+} to As^{3+}. The resulting arsenite dissociates from the complex. Reduced glutaredoxin would be regenerated by electron transfer from GSH, which is reduced by glutathione reductase (GR) using NADPH as reductant.

An insertion into the gene for thioredoxin similarly had no effect on arsenate resistance in *E. coli*, and reduced thioredoxin was unable to donate electrons to arsenate reduction (data not shown). Considering that the R773 and pI258 ArsC proteins are not closely similar in sequence (less than 20% identity and no regions of high similarity), the difference in the source of reducing equivalents may relate more to the reductants available in the host strains than to their common function of arsenate reduction and resistance and their presumed common origin. *S. aureus* has been shown to contain little gluathione (<0.1 mmol/g dry weight) (Fahey & Sundquist, 1991). In contrast, in *E. coli* glutathione is the major intracellular reductant and is present in millimolar amounts. Therefore, while ArsC-mediated reduction of arsenate to arsenite in vivo may occur via redox-active sulfhydryls, the electron donors may be quite different in *E. coli* and *S. aureus*.

In *E. coli*, arsenate is transported by both the Pit and the Pst phosphate transport systems, with affinities of approximately 6 μM for the Pst system and 32 μM for the Pit system (Rosenberg et al., 1977). Although the Pst system is more specific for phosphate, under the low phosphate growth conditions used for determining arsenate resistance, cells would accumulate arsenate though both phosphate transport systems. Since the pool of free phosphate in aerobic *E. coli* is at least 3 mM (Ugurbil et al., 1982), the cells could be expected to accumulate arsenate to similar levels. Thus, even with an affinity for arsenate of 8 mM, the ArsC reductase would function within a physiologically relevant range of intracellular arsenate.

From the substrate specificity of the reduction reaction, the ArsC protein could discriminate between arsenate and other oxyanions, including antimonate, nitrate, phosphate, and sulfate. Although the other oxyanions tested were not reduced, phosphate and sulfate inhibited. Inhibition appeared competitive, indicating that the oxyanion binding site of the ArsC protein may be rather nonspecific.

The pathway of electron transfer and the inhibition by NEM suggests that a redox-active sulfhydryl may participate in ArsC-catalyzed reduction. The 141-residue R773 ArsC protein has only two cysteines, Cys12 and Cys106 (Chen et al., 1986). Preliminary results of mutagenesis of the *arsC* gene to alter those two residue to serines demonstrate that a cysteine residue at position 106 is not required for resistance or reductase activity, while a Cys12-to-Ser alteration resulted in loss of both (J. Liu, T. Gladysheva, and B. P. Rosen, unpublished experiments). While consequences of the Cys12-to-Ser alteration have to be examined in more detail, the fact that the Cys106-to-Ser alteration has no effect on either arsenate resistance in vivo or arsenate reduction with the purified protein demonstrates that this cysteine residue is not involved in catalysis and suggests that a single thiol group may be sufficient for reductase activity. Based on these results, we propose a model for the mechanism of the ArsC protein (Figure 8). The pH optimum of the reaction is about 6.5, considerably lower than that of cysteinyl residues in protein, which are usually in the range of 8.5–9. Enzymes with reactive thiol groups have similar pH optima, and in such proteins there is usually a basic residue near the cysteine that forms a stable ion pair, with the pH optimum reflecting the average of the pK_a values of the acid and base. For example, the thiolate form of Cys149 in glyceraldehyde 3-phosphate dehydrogenase is stabilized by the imidazolium group of His176 (Polgár, 1975). The active site of papain contains Cys25 and His159, which form a complex that is tautomeric with the ion-paired species; thus the thiolate anion is the species that attacks the substrate to generate a tetrahedral intermediate (Fersht, 1985). Following formation of the arsenylated thiol intermediate, reaction with the vicinal cysteine pair of glutaredoxin results in reduction of the arsenic atom to release arsenite. Note that release of the arsenite product from a dithiol enzyme would be highly unfavorable. The reaction is shown as a two-electron transfer, although two one-electron transfers are possible. If this model is correct, *the ArsC protein would be to our knowledge the first member of a new class of single thiol reductases.*

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