Arsenate Reductase of *Staphylococcus aureus* Plasmid pI258†

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ABSTRACT: Arsenate reductase encoded by *Staphylococcus aureus* arsenic-resistance plasmid pI258 was overproduced in *Escherichia coli* and purified. The purified enzyme reduced radioactive arsenate to arsenite when coupled to thioredoxin, thioredoxin reductase, and NADPH. NADPH oxidation coupled to arsenate reduction also required thioredoxin and thioredoxin reductase. Glutaredoxin and reduced glutathione did not stimulate arsenate reduction. NADPH oxidation showed Michaelis–Menten kinetics with a K_m of 1 μM AsO_4^{3-} and an apparent V_{max} of 200 nmol/min per mg of protein. At high substrate concentration (above 1 mM AsO_4^{3-}), a secondary rise in the reaction rate was observed, with a K_m of 2 mM and an apparent V_{max} of 450 nmol/min per mg of protein. This secondary rise also occurred upon addition of phosphate or nitrate (which were not substrates for the enzyme). Arsenite (the product of the enzyme), tellurite, and antimonite [Sb(III)] were inhibitors. Selenate (but not selenite or sulfate) was a substrate for reductase-dependent NADPH oxidation, with an apparent K_m of 13 mM SeO_4^{2-}. Arsenate reductase was purified as a monomer of 14.5 kDa, consistent with the DNA sequence. Electrospray mass spectrometry showed two molecular masses of 14 810.5 and 14 436.0 Da, suggesting that 70% of the purified protein lacked the N-terminal three amino acids; HPLC coupled to electrospray mass spectroscopy of protease digest products confirmed this conclusion and verified the entire amino acid sequence.

Bacterial arsenic resistance is determined by plasmids in a wide range of microorganisms. Resistance to arsenate, arsenite, and antimony(III) is governed by an operon of five genes in *Escherichia coli* or three genes in *Staphylococcus aureus* (Rosen et al., 1992; Silver et al., 1993). The *arsC* gene from both bacteria encodes an arsenate reductase (Ji & Silver, 1992b), which reduces intracellular less toxic As(V) (arsenate) to more toxic As(III) (arsenite). The arsenite is rapidly exported out from the cells by an ATP-dependent (in *E. coli*) or membrane-potential-dependent (in *S. aureus*) transport system (Rosen et al., 1992; Silver et al., 1993; Bröer et al., 1993).

The arsenate reductase of *S. aureus* plasmid pI258 is a small, soluble, heat-stable protein (Ji & Silver, 1992b). Studies with cell-free extracts of *S. aureus* cells containing the *arsC* gene showed that arsenate reduction required dithiothreitol (DTT[1]) or NADPH, but was not stimulated by reduced glutathione. Purified arsenate reductase by itself lacks enzyme activity (Ji & Silver, 1992b). *In vitro* coupling of arsenate reductase requires thioredoxin [the small intracellular protein that functions as a general disulfide reducing agent; see Fuchs (1989) and Holmgren (1989) for reviews] plus either DTT (Ji & Silver, 1992b) or thioredoxin reductase and NADPH (see below).

This report includes the overproduction and purification of plasmid pI258 arsenate reductase, plus some basic biochemical and physical properties of this enzyme. Enzyme kinetics and the effects of various oxyanions were studied to establish substrate range and specificity. Measurements with electrospray mass spectroscopy (Jardine, 1990; Parveen et al., 1993) confirm the primary amino acid sequence previously deduced (Ji & Silver, 1992a) from the corresponding DNA sequence.

MATERIALS AND METHODS

Cloning, Overexpression, and Purification of Arsenate Reductase. The *arsC* gene of plasmid pI258 was cloned by polymerase chain reaction (PCR) using plasmid pGJ103 (Ji & Silver, 1992a) as template and 33-mer (5′-ATCG-CTAATGGATCCAAGGAGGCTTTAATTATG-3′, containing a *Bam*HI site) and 27-mer (5′-GCATGCAAG-CTTTGATTATCTCAATTT-3′, containing a *Hin*dIII site) oligomer nucleotide primers, which were complementary to the 5′ and 3′ ends of *arsC*, respectively. The PCR product sequence was confirmed, and the product was cut with *Bam*HI and *Hin*dIII and cloned into the pBR322 derivative vector pHN1 (Liu et al., 1990), in which the *arsC* gene was under the control of the *tac* promoter and inducible by IPTG. The resulting plasmid, pArsC, was electroporated into *E. coli* strain JM109 (Sambrook et al., 1989). Strain *E. coli* JM109(pArsC) was grown in 1 L of LB broth and induced in the log phase with 0.5 mM IPTG for 3 h at 37 °C. The cells were harvested by centrifugation at 4 °C, washed twice with cold 20 mM Tris-HCl (pH 7.5), and suspended in 10 mL of cold buffer

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[1] Abbreviations: IPTG, isopropyl β-thiogalactoside; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; HPLC, high-pressure liquid chromatography; CD, circular dichroism; Trx, thioredoxin; TR, thioredoxin reductase; DTT, dithiothreitol; GSH, reduced glutathione.

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A [20 mM Tris-HCl (pH 7.5) plus 2 mM β-mercaptoethanol]. The cells were disrupted by passing them twice through a French pressure cell at 16000–18000 psi and sonicated briefly to disrupt the remaining cells. The cell lysate was centrifuged at 100000*g* for 1 h at 4 °C, and the pellet fraction was washed with 10 mL of buffer A plus 0.5 M NaCl. After centrifugation at 100000*g* at 4 °C for 1 h, the pellet was suspended in 5 mL of buffer A. The pellet (which contained the arsenate reductase) was dissolved by adding urea to a final concentration of 8 M.

The crude arsenate reductase preparation was dialyzed against 2 L of buffer A plus 0.2 mM PMSF overnight at 4 °C and centrifuged at 100000*g* at 4 °C for 1 h. The supernatant fluid was concentrated by ultrafiltration (Amicon PM10 filter), and 0.2 M NaCl was added. The arsenate reductase was purified by chromatography on a Sephadex G75 gel filtration column (2.5 × 100 cm), eluting with buffer A plus 0.2 M NaCl. The arsenate reductase peak fractions were concentrated by ultrafiltration to about 10 mg/mL protein and stored at −70 °C.

Protein samples at each purification step were monitored by electrophoresis on a 0.1% SDS–18% polyacrylamide gel and stained with Coomassie Blue R-250. Protein concentration was determined by using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA) with bovine serum albumin as the standard or occasionally by absorption at 280 nm.

Arsenate Reductase Enzyme Assays. Radioactive $^{73}AsO_4^{3-}$ reduction to arsenite was assayed as described (Ji & Silver, 1992b) by TLC separation of arsenate from arsenite and quantitation with an AMBIS β-scanner. Purified coupling proteins were added as indicated for each experiment. Trx and TR (Gleason et al., 1990) and glutaredoxin (Holmgren, 1979) were purified as described. A spectrophotometric assay measuring NADPH oxidation (by the decrease in absorption, OD_{340nm}) coupled to arsenate reduction was developed in a buffer consisting of 20 mM Tris-HCl (pH 7.5). Assays generally were run at 250 μM NADPH, and coupling enzymes, Trx and TR, were added as indicated. OD_{340nm} was recorded at 37 °C in 1-mL cuvettes in a Beckman DU-7 recording spectrophotometer (Beckman Instruments Inc., Fullerton, CA).

Protein Denaturation and Alkylation. Arsenate reductase (in 0.1 mL) was denatured and reduced by the addition of 6 M guanidine hydrochloride plus 2 mg of DTT and incubated at room temperature for 30 min under argon. The cysteines of reduced and denatured proteins were alkylated by adding 2 μL of 4-vinylpyridine (also under argon) and incubating at room temperature for 2 h. The protein was purified immediately by HPLC.

Peptide Mapping. Arsenate reductase was digested with endoproteinase Glu-C [in 25 mM acetate buffer (pH 4) at a protein to enzyme ratio of 100:1 and incubation at 25 °C for 40 h]. The reaction was stopped by the addition of 1% trifluoroacetic acid, and the reaction mixture was stored at −20 °C until analysis by HPLC.

HPLC Chromatography. HPLC characterization of arsenate reductase, its alkylation products, and the peptides generated by endoproteinase digestion was performed on an Applied Biosystems Inc. (San Jose, CA) model 130A microbore HPLC using an Aquapore RP300 7-μm-bead Brownlee C-8 column (2.1 × 100 mm) (Applied Biosystems Inc.). The samples were injected in 0.1% trifluoroacetic acid plus 3.5% acetonitrile and eluted with a 3.5%–70% acetonitrile linear gradient over a 90-min period at 35 °C.

Electrospray mass spectrometry was carried out in a Perkin-Elmer Sciex APIII triple quadrupole mass spectrometer (PE Sciex Instruments, Norwalk, CT) (Jardine, 1990; Parveen et al., 1993).

Materials. The materials used were described in previous reports (Ji & Silver, 1992a,b) or as indicated. Endoproteinase Glu-C (sequencing grade; also referred to as *S. aureus* V8 protease) was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). *E. coli* Trx and TR were purified as described (Gleason et al., 1990). *Spirulina* thioredoxin was obtained from Sigma Chemical Co. (St. Louis, MO). ^{73}As (arsenate) was purchased from Los Alamos National Laboratory (Los Alamos, NM).

RESULTS

Purification of Arsenate Reductase. Purified arsenate reductase was obtained from the *arsC* gene of plasmid pI258, cloned into *E. coli* plasmid pArsC as described in Materials and Methods. When IPTG-induced cells were disrupted, the arsenate reductase was found in the pellet fraction (as it forms inclusion bodies under these conditions of overproduction) (data not shown). Arsenate reductase was solubilized with 8 M urea and dialyzed against buffer, during which time a precipitate formed that does not include arsenate reductase (data not shown). The precipitate was removed by centrifugation, and the supernatant fluid was concentrated in an Amicon ultrafiltration cell with a PM10 filter and chromatographed on a Sephadex G75 column. After gel filtration, the protein appeared to be more than 95% pure by SDS–PAGE analysis (data not shown).

Superose 12 chromatography separated the purified arsenate reductase into two peaks, with approximately two-thirds of the protein at a position consistent with a dimeric 29-kDa protein and the remainder at a position consistent with a 14.5-kDa monomeric protein (data not shown). When 0.5 mM dithiothreitol was added prior to Superose 12 chromatography, the arsenate reductase chromatographed as a single peak at a position corresponding to 14.5 kDa (data not shown).

The purified arsenate reductase used in a previous report (Ji & Silver, 1992b) was obtained in lower yields from a different construct. Comparison of the proteins purified by both methods showed equivalent specific activities for both (data not shown). The yield from the earlier procedure was less than 0.1 mg of protein per 1 L of culture, compared with the current 5 mg/L.

Enzyme Activity of Arsenate Reductase. Purified arsenate reductase carried out the reduction of radioactive $^{73}AsO_4^{3-}$ to $^{73}AsO_2^-$ in the presence of thioredoxin and thioredoxin reductase plus NADPH (data not shown). All three protein components, arsenate reductase, thioredoxin, and thioredoxin reductase, were required for arsenate reduction; however, the source of the thioredoxin was not crucial, and thioredoxin from *E. coli* (343 μmol/g of protein in 10 min) and that from the eukaryotic algae *Spirulina* (302 μmol/g of protein in 10 min) were essentially equivalent. With plasmid pI258 arsenate reductase, thioredoxin was required and glutaredoxin (data not shown) or reduced glutathione (Ji & Silver, 1992b) did not function.

A non-radioactive *in vitro* enzyme assay measuring the oxidation of NADPH coupled to arsenate reduction was developed (Figure 1). The addition of thioredoxin, with or without thioredoxin reductase, had no effect on NADPH oxidation unless arsenate reductase was also present (Figure 1). Similarly, the addition of arsenate reductase without thioredoxin reductase and/or thioredoxin did not result in

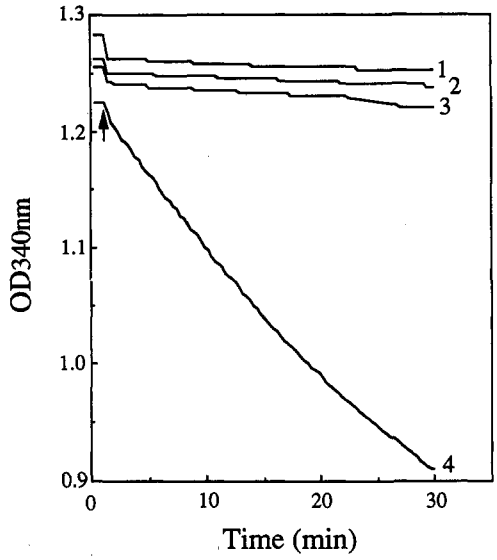

FIGURE 1: Oxidation of NADPH coupled to arsenate reduction. The assay mixture in Tris-HCl buffer (pH 7.5) also contained (1) 250 μM NADPH; (2) NADPH plus 5 μg of thioredoxin reductase; (3) NADPH, thioredoxin reductase, plus 5 μg of thioredoxin; and (4) everything in reaction 3 plus 8 μg of arsenate reductase. The reactions were started (as indicated) by the addition of 50 μM arsenate.

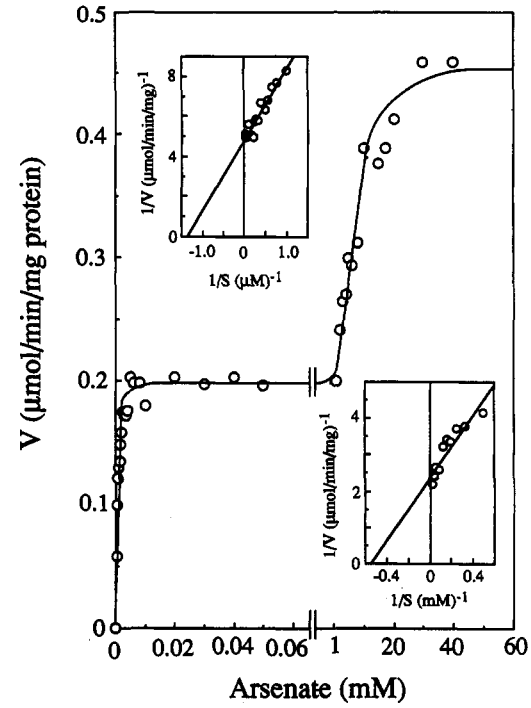

FIGURE 2: Kinetics of NADPH oxidation as a function of arsenate concentration. Reactions were with 250 μM NADPH, thioredoxin, thioredoxin reductase, and arsenate reductase, as in the legend for Figure 1. Arsenate was added from 0.25 μM to 40 mM, and the rate of NADPH oxidation was measured. Note the break in the arsenate concentration scale. Insets: Lineweaver–Burk plots of the same data, from 1 to 50 μM and from 2 to 40 mM.

NADPH oxidation (data not shown). The reduction of radioactive arsenate to arsenite was measured under the conditions of the spectrophotometric NADPH oxidation assay (data not shown). Approximately 2 μmol of NADPH was oxidized per micromole of arsenate added over the range 5–10 μM arsenate, during determination of the K_m. We have not studied the stoichiometry of the reaction further.

Kinetic and Substrate Analysis. The rate of NADPH oxidation coupled to arsenate reduction showed typical Michaelis–Menten kinetics at low substrate concentrations, giving an apparent K_m of approximately 0.8 μM AsO_4^{3-} (Figure 2) and an apparent V_{max} of 0.2 μmol of NADPH oxidized/min per mg of arsenate reductase added (thioredoxin and thioredoxin reductase were always present in excess, and doubling the amounts of these proteins did not affect the NADPH oxidation rate; data not shown). Increases in substrate between 10 μM and 1 mM arsenate did not affect the reaction rate (Figure 2), but above 1 mM arsenate, a second increase in rate with increasing substrate was observed, with an apparent K_m of 2 mM arsenate.

Alternative oxyanions either stimulated or inhibited arsenate-coupled NADPH oxidation. The addition of 10 mM NaH_2AsO_4 (Figure 2), phosphate (Figure 3A), or $NaNO_3$ (data not shown) increased the rate of NADPH oxidation by 2-fold. These stimulations were oxyanion- and not cation-dependent, since 10 mM Na_2SO_4 was without effect (data not shown). Phosphate and nitrate (data not shown) were not substrates for the reaction themselves, and the addition of NADPH, thioredoxin, and thioredoxin reductase plus arsenate reductase plus phosphate (but without arsenate) did not cause NADPH oxidation (data not shown). In the presence of 10 mM arsenate, the addition of 20 mM phosphate did not further stimulate NADPH oxidation (data not shown).

With 50 μM arsenate as substrate, arsenite, antimonite, or tellurite inhibited NADPH oxidation with half-inhibitory concentrations of 500 μM AsO_2^-, 10 μM SbO_2^-, or 0.5 μM TeO_3^{2-} (Figure 3B). The level of arsenate reductase protein in these assays was 0.5 μM, so it appears that tellurite inhibited reductase activity when added in about a 2:1 ratio with the protein. Sulfate was without effect even at 10 mM (data not shown), and the substrate, arsenate, was not inhibitory at concentrations up to 40 mM (Figure 2).

Selenate Is a Poor Substrate for Arsenate Reductase. The selenate oxyanion [SeO_4^{2-}; Se(VI)] was a substrate for NADPH oxidation coupled to arsenate reductase (Figure 4). Standard Michaelis–Menten kinetics was followed with a K_m = 13 mM SeO_4^{2-} (compared with the 0.8 μM K_m for arsenate; Figure 2; 16000:1 ratio). The selenate response is not likely to be due to arsenate contamination of selenate, since the apparent V_{max} was different (compare Figure 4 with Figure 2). Thioredoxin reductase alone did not catalyze NADPH oxidation in the presence of 10 mM selenate, but the addition of thioredoxin produced a background rate of NADPH oxidation (data not shown) and the addition of arsenate reductase increased the rate of selenate-dependent NADPH oxidation (Figure 4). With selenite [SeO_3^{2-}; Se(IV)] instead of selenate, the addition of thioredoxin to thioredoxin reductase increased the rate NADPH oxidation, but no further increase occurred upon addition of arsenate reductase (data not shown). Intact cells of *S. aureus* grew in up to 20 mM selenate, without inhibition and without visible production of red metallic Se^0, whether or not the *ars* operon-containing plasmid was present (data not shown).

Confirmation of the protein primary sequence was obtained with endoproteinase digestion, HPLC analysis, and electrospray mass spectrometry. Reverse-phase HPLC showed the presence of two major protein peaks, which eluted from the C-8 column at 40% and 41% acetonitrile (data not shown). The second peak contained 70% of the protein in two independent protein preparations. Mass spectral analysis of the purified ArsC protein yielded values of 14 436.0 Da for the more abundant species and 14 810.5 Da for the less abundant species (data not shown). The predicted molecular

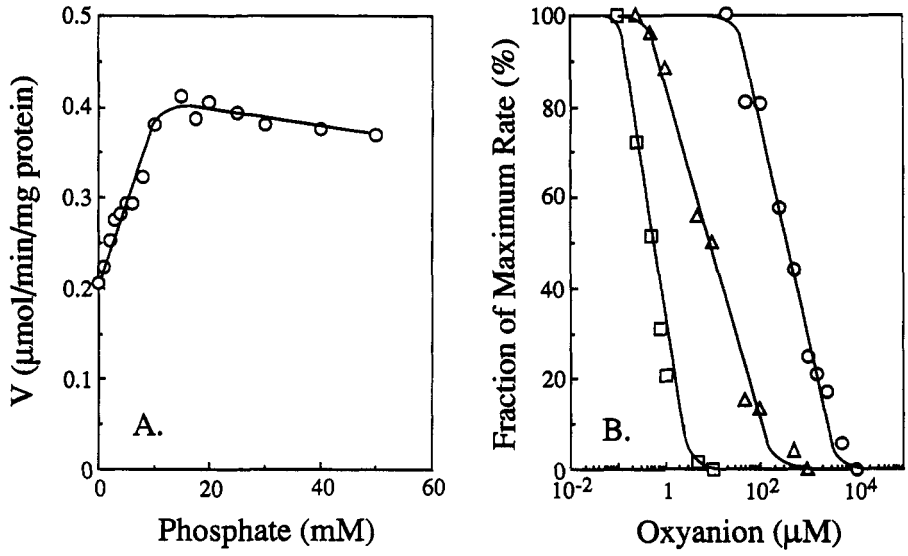

FIGURE 3: (A) Phosphate stimulation of NADPH oxidation coupled to arsenate reduction. Effect of varying phosphate concentration on the rate of reduction of 50 μM arsenate; conditions same as for Figure 1. (B) Inhibition by oxyanions of arsenate reductase-dependent NADPH oxidation. Various concentrations of arsenite (○), antimonite (△), or tellurite (□) were added with 50 μM arsenate, and the rates of NADPH oxidation were normalized to control values.

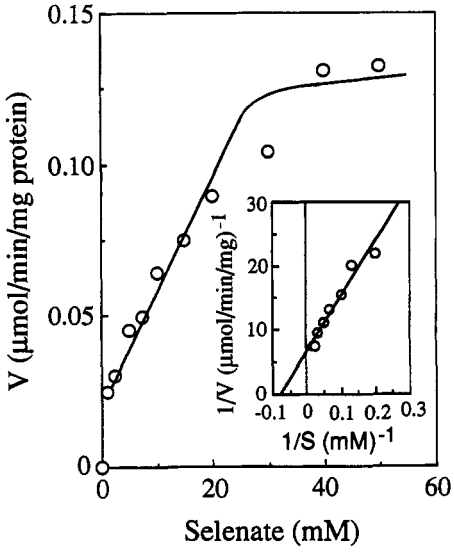

FIGURE 4: Selenate-dependent, arsenate reductase-dependent NADPH oxidation. Conditions same as for Figure 2, except selenate was added instead of arsenate.

mass of arsenate reductase from the DNA sequence translation product is 14 813 Da, and loss of the first three amino acids (Met-Asp-Lys) would reduce the mass by 374 Da to 14 439 Da. The loss of the first three amino acid residues from part of the arsenate reductase may have occurred intracellularly or extracellularly during the purification process. The 2.5–3-Da difference between the expected and measured masses of the larger and smaller protein forms is consistent with the loss of four hydrogens during the formation of two intramolecular disulfide linkages. These small mass differences are within the analytical capacity of the mass spectrometer.

Purified arsenate reductase was characterized by analysis of the products of endoproteinase Glu-C (which cuts specifically after glutamate residues under the conditions used; Figure 5). Glu-C cleavage after glutamates was expected to yield 12 peptides from 3 to 45 amino acids in length. Partial digestion would yield additional peptides. The peptides that were identified by electrospray mass spectrometry of the HPLC peaks from part B of Figure 5 are marked in part A.

For HPLC analysis of peptides, purified arsenate reductase (containing both of the subsequently recognized forms) was denatured in 6 M guanidine hydrochloride and reduced with DTT, and the cysteine residues were alkylated with 4-vinylpyridine [yielding (pyridylethyl)cysteine], as described in Materials and Methods. Following digestion by endoproteinase Glu-C and separation of the peptides by HPLC (Figure 5B), the individual peptide peaks were identified by mass spectrometry. The cysteine-containing peaks (E4, E5, E7a, and E11, Figure 5A) had molecular masses consistent with the complete alkylation of the DTT-reduced protein. The tetrapeptide Ala-Met-Lys-Glu (positions 51–54) eluted from the HPLC column at 1.6 min, prior to the start of the mass spectral analysis, but were found in a subsequent peptide map analysis. Thus, the entirety of the sequence (from DNA analysis) was confirmed (by electrospray mass spectrometry of the Glu-C products).

Secondary Structure Changes Monitored by CD Spectroscopy. The CD spectrum of arsenate reductase was measured from 190 to 300 nm along with the effects of arsenate and arsenite on the spectrum and the lack of effect of phosphate (data not shown). Whereas the addition of 2 mM phosphate had no measurable effect on the CD spectrum of arsenate reductase, the addition of 2 mM arsenate or arsenite reduced the magnitude of the ellipticity of arsenate reductase from 203 to 240 nm (data not shown), indicative of a loss of secondary structure. An isobestic point occurred around 203 nm. Comparisons of CD difference spectra showed similar effects of arsenate and arsenite and showed that the CD spectral effects were partial at 0.1 mM oxyanion, but complete at 1 mM oxyanion (data not shown).

DISCUSSION

This is only the second report on the properties of arsenate reductase, following the initial discovery of enzyme activity (Ji & Silver, 1992b). The protein from the *arsC* gene of plasmid pI258 has been purified, and its basic enzymological properties have been studied. Arsenate reductase of plasmid pI258 purified in the presence of DTT is a monomeric protein of 131 amino acids, including four cysteine residues (Ji &

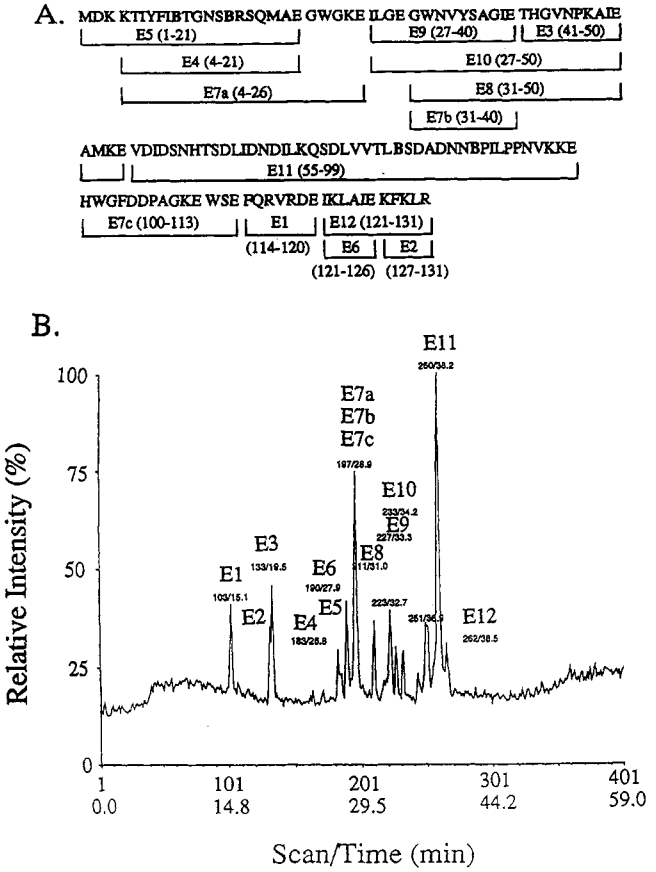

FIGURE 5: (A) Amino acid sequence of arsenate reductase from the DNA sequence (Ji & Silver, 1992a), as confirmed by analysis of the Glu-C peptide products (see B). The bars under the sequences (and numbers) indicate the starting and ending positions of predicted peptide products (from complete and some from incomplete digestion at glutamate positions) that were confirmed by mass spectral analysis. The standard amino acid one-letter abbreviations are used except that cysteine is represented by B, indicating the (pyridylethyl)cysteine product of the 4-vinylpyridine treatment. The first three residues, Met-Asp-Lys, do not end with a Glu-C site, but are missing from 70% of the purified protein. (B) HPLC analysis of the endoproteinase Glu-C digest of arsenate reductase. The x axis is in minutes of chromatography (and 8.8 s per mass spectral scan); the y axis is in total ion current rather than UV absorption.

Silver, 1992a). The protein sequence was confirmed by electrospray mass spectroscopy (Figure 5). Arsenate reductase has a high affinity for arsenate with a K_m = 0.8 μM AsO_4^{3-} (Figure 2). At substrate concentrations above 1 mM, a further increase in the apparent V_{max} occurred. This may be a general oxyanion effect since high phosphate and nitrate also increased the reaction rate (Figure 3A and data not shown), but sulfate did not.

The *arsC* genes from both *S. aureus* plasmid pI258 and *E. coli* plasmid R773 *ars* operons encode arsenate reductase. Both enzymes reduce arsenate to arsenite *in vivo*, but in crude cell-free extracts of *E. coli*, the R773 enzyme activity was 50 times lower than that of pI258 (Ji & Silver, 1992b). The DNA sequence of plasmid R773 *arsC* (Chen et al., 1986) predicts a 141 amino acid long protein that is less than 20% identical in sequence to ArsC of plasmid pI258 and contains only two cysteines, one each at approximately the locations of Cys_{12} and Cys_{82} in pI258 ArsC (Ji & Silver, 1992a). The accompanying paper by Gladysheva et al. (1994) reports properties for the arsenate reductase of plasmid R773 that are remarkably different from those reported here, perhaps because the proteins themselves are so very different in sequence. Gladysheva et al. (1994) found a K_m of 8 mM arsenate for the enzyme from plasmid R773, which is 10 000 times that from plasmid pI258 (Figure 2), and a surprisingly high K_m for an enzyme required for a toxic ion resistance mechanism. This may not reflect the intracellular K_m, or it may be related to the secondary rise in V_{max} at high substrate concentration shown in Figure 2. The apparent V_{max} in Figure 2 of 0.45 μmol/min per mg of protein corresponds to 6.4 turnovers per minute, an unusually low number for a enzyme; the comparable value for the R773 arsenate reductase is 19 turnovers per minute, a similarly low value. In addition to the lack of optimal *in vitro* assay conditions, there are two possible "biological" explanations for these low numbers: Firstly, arsenite is more toxic than arsenate, and it may be necessary to keep the arsenate reductase activity rate below that for arsenite efflux by the ArsB membrane protein (Ji & Silver, 1992b; Bröer et al., 1993). Alternatively, we have hypothesized that arsenite will not be released efficiently from arsenate reductase, but rather delivered directly to the membrane efflux protein (Silver et al., 1993).

The pI258 arsenate reductase is stimulated by phosphate and nitrate (Figure 3A and data not shown), whereas the R773 arsenate reductase is inhibited by phosphate and nitrate was without effect (Gladysheva et al., 1994). The most interesting difference between the enzymological properties of the two arsenate reductases is the obligatory coupling to thioredoxin (but not glutaredoxin) by the pI258 enzyme [Ji and Silver (1992b) and additional data not shown], whereas the R773 arsenate reductase couples with glutaredoxin (but not thioredoxin; Gladysheva et al., 1994).

With a new enzyme whose primary sequence (and secondary structure predictions) has been deduced from the DNA sequence (Ji & Silver, 1992a) and analyzed with computer consensus algorithms, it is important to establish protein characteristics directly. Firstly, the correspondence of the primary amino acid sequence to that expected was confirmed by electrospray mass spectrometry, which is a more powerful technique than the more familiar N-terminal cleavage methods (Jardine, 1990; Johnson & Walsh, 1992; Parveen et al., 1993). Two forms of arsenate reductase were found with 70% of the protein (as purified) missing the three N-terminal amino acids, Met-Asp-Lys, which were found in the remaining 30% of the protein. The arsenate reductase protein appeared as a monomer in the mass spectrometer, confirming the conclusion from gel filtration column chromatography (data not shown). All of the 131 amino acids of pI258 arsenate reductase were confirmed in analysis of the peptides generated by endoproteinase Glu-C. In addition, the mass spectrometry values for the full and processed forms of arsenate reductase were smaller than expected by about 3 m/e units, suggestive of an intrapolypeptide disulfide bond.

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