As(III) and Sb(III) Uptake by GlpF and Efflux by ArsB in *Escherichia coli*

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The toxicity of the metalloids arsenic and antimony is related to uptake, whereas detoxification requires efflux. In this report we show that uptake of the trivalent inorganic forms of arsenic and antimony into cells of *Escherichia coli* is facilitated by the aquaglyceroporin channel GlpF and that transport of Sb(III) is catalyzed by the ArsB carrier protein; everted membrane vesicles accumulated Sb(III) with energy supplied by NADH oxidation, reflecting efflux from intact cells. Dissipation of either the membrane potential or the pH gradient did not prevent Sb(III) uptake, whereas dissipation of both completely uncoupled the carrier protein, suggesting that transport is coupled to either the electrical or the chemical component of the electrochemical proton gradient. Reciprocally, Sb(III) transport via ArsB dissipated both the pH gradient and the membrane potential. These results strongly indicate that ArsB is an antiporter that catalyzes metalloid-proton exchange. Unexpectedly, As(III) inhibited ArsB-mediated Sb(III) uptake, whereas Sb(III) stimulated ArsB-mediated As(III) transport. We propose that the actual substrate of ArsB is a polymer of (AsO)₃, (SbO)₃, or a co-polymer of the two metalloids.

Arsenic, one of the most prevalent toxic metals in the environment, derives primarily from geochemical origins but also from man-made sources. Consequently, nearly every organism has intrinsic or acquired mechanisms for arsenic detoxification (1). The arsenical resistance operon (*arsRDABC*) of the conjugative R-factor R773 confers resistance to inorganic As(III) and Sb(III) in *Escherichia coli*. The arsenic transport system exhibits a dual mode of energy coupling depending on the subunit composition (2). When both ArsA and ArsB are present, they form the As(III)-translocating ArsAB ATPase, which is independent of the electrochemical proton gradient (3). In contrast, in the absence of ArsA, ArsB catalyzes As(III) extrusion coupled to electrochemical energy, which suggests that ArsB is a uniporter that extrudes the arsenite anion in response to the positive exterior membrane potential (4). This dual mode of energy coupling led us to propose that the ArsAB pump evolved by association of a secondary carrier with a soluble ATPase (5).

Over a decade ago, we proposed that other primary ATP-coupled pumps such as ATP-binding cassette transporters evolved in similar ways (5, 6).

ArsB is the most widespread determinant of arsenic resistance in bacteria and archaea, yet its transport properties are not well characterized. It is a member of the ion transporter superfamily (7), with 12 membrane-spanning segments and a membrane topology that is similar to many carrier proteins (8). To date, it has been shown to transport only As(III) (4). Here we report for the first time that ArsB transports inorganic Sb(III) in *E. coli*, and we describe the relationship with As(III) transport. Considering that the pKₐ of Sb(III) is 11.8 and As(III) is 9.2, at cytosolic pH the concentration of the oxyanion of either metalloid is negligible. Thus, it is unlikely that ArsB could be an electrophoretic anion uniporter. Instead, we demonstrate here that ArsB is a trivalent metalloid/H⁺ antiporter. ArsB-catalyzed uptake of Sb(III) into everted membrane vesicles coupled to either the pH gradient or membrane potential components of the electrochemical proton gradient. Sb(III)/H⁺ exchange was monitored using a fluorescent reporter, acridine orange, for the pH gradient and oxonal V for the membrane potential. The nature of the substrate of ArsB was explored by competition and co-transport experiments with Sb(III) and As(III). Given their pKₐ values, the physiologically relevant species of Sb(III) and As(III) in a solution of neutral pH are hydroxides, but the nature of the true substrate of ArsB is not clear. As(III) inhibits ArsB-mediated Sb(III) transport, yet Sb(III) stimulates ArsB-mediated As(III) transport. From these results and the results of As(III) and Sb(III) co-transport experiments, we propose that the true substrate of ArsB is a polymer of As(III) or Sb(III), or a co-polymer of As(III) and Sb(III).

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes and nucleic acid-modifying enzymes were purchased from Invitrogen and New England Biolabs, Inc. Carrier-free "AsO₄" was obtained from Los Alamos National Laboratories. ¹²⁵SbCl₅ was produced by PerkinElmer Life Sciences. The pentavalent forms of the isotopes were reduced to ⁷³As(III) and ¹²⁵⁸Sb(III) by the method of Reay and Asher (9). All other chemicals were obtained from commercial sources.

Strains, Plasmids, and Media—*E. coli* strains and plasmids used in this study are listed in Table I. *E. coli* strains harboring the indicated plasmids were grown in Luria-Bertani medium (10) at 37 °C with 100 µg/ml ampicillin, 35 µg/ml chloramphenicol, or 50 µg/ml kanamycin as required. Protein expression was induced by addition of 0.1 mM isopropyl-1-thio-β-D-galactopyranoside.

Resistance Assays—For assays of resistance to arsenite and antimonite, cultures were grown overnight at 37 °C with shaking. The cells were diluted 100-fold into fresh, prewarmed medium with the indicated concentrations of As(III) in the form of sodium arsenite or Sb(III) in the form of potassium antimonyl tartrate and incubated at 37 °C with shaking for an additional 6 h. Growth was estimated from the absorbance at 600 nm.

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Transport Assays—For uptake assays in intact cells, cultures were grown to an A600 of 1 at 37 °C with aeration in Luria-Bertani medium. The cells were harvested, washed, and suspended in a buffer consisting of 75 mM HEPES-KOH, pH 7.5, containing 0.15 M KCl and 1 mM MgSO₄, brought to a concentration of 80 mg of wet cells/ml at room temperature. To initiate the transport assay, 0.1 ml of cells was diluted in 1 ml of the same buffer at room temperature containing 10 μM sodium arsenite and 0.4 μCl of ¹²⁵I arsenic (III) or 10 μM potassium antimonyl tartrate. Samples (0.1 ml) were withdrawn at the indicated times, filtered through 0.2-μm pore size nitrocellulose filters (Whatman), and washed with 15 ml of the same buffer, all at room temperature. The filters were dried and quantified by liquid scintillation counting.

Transport assays using everted membrane vesicles were performed as described (4). Everted membrane vesicles were prepared essentially as described previously (4). Unless otherwise noted, the reaction mixture contained 0.3 mg of membrane protein in a final volume of 0.6 ml of a buffer consisting of 75 mM HEPES-KOH, pH 7.5, containing 0.1 M K₂SO₄, 0.25 M sucrose, and 1.25 mM MgSO₄, brought to a concentration of 80 mg of wet cells/ml at room temperature. To initiate the transport assay, 0.1 ml of cells was diluted in 1 ml of the same buffer at room temperature containing 10 μM sodium arsenite and 0.4 μCl of ¹²⁵I arsenic (III) or 10 μM potassium antimonyl tartrate and 0.4 mM of ¹⁵²Sb(III). Samples (0.1 ml) were withdrawn at the indicated times, filtered through 0.2-μm pore size nitrocellulose filters (Whatman), and washed with 15 ml of the same buffer, all at room temperature. The filters were dried and quantified by liquid scintillation counting.

Assays were initiated by the addition of 5 mM NADH, final concentration. Fluorescence was measured in stirred cuvettes with an Aminco AB2 spectrofluorometer with excitation at 589 nm and emission at 616 nm.

RESULTS

Uptake of As(III) and Sb(III) in E. coli—We have previously shown that disruption of the aquaglyceroporin GlpF confers resistance to Sb(III) (13). This disruption was interpreted as a loss of the uptake pathway for Sb(III); however, transport of Sb(III) has never been directly demonstrated in E. coli. Moreover, the GlpF disruption did not gain resistance to Ars(III), so it was unclear whether GlpF is an uptake pathway for Ars(III). Uptake of either ¹²⁵IAs(III) or ¹⁵²Sb(III) was measured in the E. coli strains AW3110 or AW10, in which the chromosomal arsRDABC operon was deleted (4, 14), and OSBR1, which was created from AW3110 by inserting TphA into glpF (13). Because AW3110 lacks arsB, it was unable to extrude metalloids and accumulated ¹⁵²Sb(III) (Fig. 1A) or ¹²⁵IAs(III) (Fig. 1B). Disruption of glpF greatly reduced the level of uptake of both metalloids. This clearly demonstrates that GlpF is the major uptake pathway for both As(III) and Sb(III). Aquaglyceroporins are channels that facilitate the movement of neutral substrates such as glycerol and other polyols but not ions (15). Considering that the pKₐ value of trivalent arsenic and antimony is 9.2 and 11.8, respectively, there is essentially no arsenite or antimonite anion at physiological pH levels. To be substrates of aquaglyceroporins, the metalloids would be expected to be the neutral hydroxides As(OH)₃ and Sb(OH)₃, which are the inorganic equivalents of polyols (13, 16). However, as discussed in more detail below, other possibilities exist, so, for the purposes of this study they are designated As(III) and Sb(III).

ArsB Confers Sb(III) and As(III) Resistance—Intracellular As(III) and Sb(III) are toxic to most cells, including E. coli (1). E. coli has a chromosomal arsB gene in the three-gene arsRBC operon that confers moderate levels of resistance to these metalloids (14). Plasmids such as R773 have five-gene arsRDABC operons that confer high levels of resistance (17). The R773 and chromosomal ArsBs share 90% identity at the amino acid level, and the R773 arsB gene complemented both Sb(III) (Fig. 2A) and As(III) (Fig. 2B) hypersensitivity resulting from deletion of the chromosomal arsB gene. Expression of arsA in trans increased resistance. These results demonstrate that ArsB can function either alone or as a complex with ArsA to confer resistance to Sb(III), and importantly, they demonstrate the interchangeability of the chromosomal and plasmid ArsB carriers.

ArsB Catalyzes ¹⁵²Sb(III) Uptake in Everted Membrane Vesicles—ArsB has been shown to transport As(III) (4), but Sb(III)
transport has not been demonstrated. Uptake into everted membrane vesicles prepared from cells expressing the R773 ArsB is the equivalent of efflux from cells. Everted membrane vesicles from strain AW10 (∆ars::cam) expressing arsB from plasmid pKMB1 accumulated 125Sb(III) when NADH was used as a respiratory substrate (Fig. 3). No uptake was observed without NADH or in vesicles from cells with vector only. Addition of the uncoupler carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) produced a rapid loss of accumulated Sb(III). These results demonstrate that Sb(III) uptake is coupled to the electrochemical proton gradient. Similar results were obtained if 125Sb(III) was prepared with unlabeled potassium antimonyl tartrate or SbCl₃ (data not shown), showing that only the oxidation state of antimony is relevant. The effects of oxyanions on ArsB-catalyzed 125Sb(III) uptake into everted membrane vesicles were examined (Fig. 4). Among the oxyanions tested, the sodium or potassium salts of AsO₄³⁻, Sb(OH)₅⁻, PO₄³⁻, NO₃⁻, NO₂⁻, SO₄²⁻, SeO₃²⁻, and BO₃⁻ had little effect on 125Sb(III) transport. Only As(III) (added as sodium arsenite) inhibited transport; therefore ArsB appears to be specific for Sb(III) and As(III).

**ArsB Catalyzes Sb(III)/H⁺ Exchange**—We previously proposed that ArsB is a uniporter for the arsenite anion, AsO₂⁻, which was thought to be the form of As(III) in solution (4). This idea is consistent with the uncoupling effect of FCCP on NADH-driven As(III) uptake in everted membrane vesicles that shows that ArsB-catalyzed As(III) uptake is driven by the electrochemical proton gradient, which is acid and positive interior in these vesicles (Fig. 3). However, as discussed above, the metalloid substrates of ArsB are not anions at physiological pH levels. An alternative for coupling transport of a neutral solute into the acid and positive interior of the everted vesicles is by exchange with a cation or proton, but dependence on a specific cation such as Na⁺, K⁺, Mg²⁺, or Ca²⁺ was not observed (data not shown). To examine the possibility that ArsB is an electrohydrostatic Sb(III)/H⁺ antiporter, the effects of a permeant anion (SCN⁻) and weak base (NH₄⁺) on Sb(III) uptake into everted membrane vesicles were measured (Fig. 5). In an attempt to limit the use of exotic isotopes, the amount of antimony taken up in this and other assays, as noted, was determined by ICP-MS. When SCN⁻ and NH₄⁺ were added together, they uncoupled Sb(III) transport from NADH oxidation as well as did FCCP. However, neither SCN⁻, which dissipates the positive interior Δφ but does not dissipate ΔpH, nor NH₄⁺, which dissipates the acid interior ΔpH but does not dissipate Δφ, by themselves had any effect on NADH-driven accumulation of Sb(III). These results indi-
cate that either Δψ or ΔpH alone is sufficient to energize Sb(III) transport. Because dissipation of only Δψ does not inhibit transport, ArsB cannot be an electrogenic anion uniporter. Instead, these results are consistent with metalloid-proton exchange.

The effect of Sb(III) on ΔpH was examined. Solute/proton exchange can be assayed by the effect of the co-transported species on ΔpH using a fluorescent, weak base (18, 19). The fluorescence of the weak base acridine orange is quenched on formation of a pH gradient in everted membrane vesicles (Fig. 6) (11). Addition of 10 mM (NH₄)₂SO₄ completely reversed fluorescence quenching, consistent with dissipation of ΔpH by a weak base (data not shown). Addition of Sb(III) in the form of potassium antimonyl tartrate reversed quenching in a concentration-dependent manner; potassium tartrate alone had no effect. Membranes from cells lacking an arsB gene showed no fluorescence enhancement, demonstrating that this assay measures ArsB activity.

The effect of Sb(III) on Δψ was examined. Membrane potential formation in everted membrane vesicles can be visualized from the quenching of the permeant dye oxonol (12). Addition of NADH quenched oxonol fluorescence (Fig. 7).

Fig. 3. Energy-dependent accumulation of ¹²⁵Sb(III) in everted membrane vesicles. Accumulation of ¹²⁵Sb(III) in everted membrane vesicles prepared from strain AW10, harboring either plasmid pKK223-3 or pKMB1 with 100 μM ¹²⁵Sb(III), was measured as described under “Experimental Procedures.” ○, pKK223-3 with 5 mM NADH; ▲, pKMB1 without NADH; ♦, pKMB1 with 5 mM NADH; ◀, pKMB1 with 5 mM NADH to which 10 μM FCCP was added after 2 min (arrow).

Fig. 5. Energetics of ArsB-mediated ¹²⁵Sb(III) transport. Accumulation of ¹²⁵Sb(III) was assayed in everted membrane vesicles prepared from strain AW10 harboring pKMB1 with 5 mM NADH and 100 μM ¹²⁵Sb(III). At 1.5 min (arrow) the following additions were made: no addition (●); 10 mM NH₄Cl (●); 10 mM KSCN (○); 10 mM (NH₄)₂SO₄ (●); 10 mM NH₄Cl + 10 mM KSCN (□); 10 mM (NH₄)₂SO₄ + 10 mM KSCN (♦); 10 μM FCCP (■).

Fig. 6. Effect of Sb(III) on ΔpH. The transmembrane pH gradient was estimated from the quenching of acridine orange fluorescence by everted membrane vesicles of strain AW10 pKMB1 (curves 1–8) or AW10 pKK223-3 (curve 9) as described under “Experimental Procedures.” Formation of ΔpH was initiated by addition of 5 mM NADH (first arrow). Potassium tartrate or potassium antimonyl tartrate was added at the indicated times (second and third arrows). Additions: curve 1, 10 μM potassium antimonyl tartrate; curve 2, 50 μM potassium antimonyl tartrate; curve 3, 100 μM potassium antimonyl tartrate; curve 4, 200 μM potassium antimonyl tartrate; curve 5, 500 μM potassium antimonyl tartrate; curve 6, 1000 μM potassium antimonyl tartrate; curve 7, 2000 μM potassium antimonyl tartrate; curve 8, 500 μM potassium antimonyl tartrate; curve 9, vector + 500 μM potassium antimonyl tartrate.
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Fig. 7. Effect of Sb(III) on \( \Delta \psi \). The membrane potential was estimated from the quenching of oxonol fluorescence by everted membrane vesicles of strain AW10 pKMB1 (\( \text{curve } 1 \) and 5) or AW10 pKK223-3 (\( \text{curve } 4 \) and 6) as described under “Experimental Procedures.” Formation of \( \Delta \psi \) was initiated by addition of 5 mM NADH (first arrow). Potassium tartrate or potassium antimonyl tartrate was added at the indicated times (second arrow). Addition of curve 1, ArsB + 500 \( \mu \)M potassium antimonyl tartrate; curve 2, ArsB + 1000 \( \mu \)M potassium antimonyl tartrate; curve 3, ArsB + 2000 \( \mu \)M potassium antimonyl tartrate; curve 4, ArsB + 2000 \( \mu \)M potassium antimonyl tartrate; curve 5, ArsB + 2000 \( \mu \)M potassium antimonyl tartrate; curve 6, vector + 100 \( \mu \)M potassium antimonyl tartrate.

Fig. 8. Sb(III) stimulates ArsB-catalyzed \( ^{73} \text{As(III)} \) transport. Uptake of \( ^{73} \text{As(III)} \) by everted membrane vesicles of strain AW10 pKMB1 (\( \text{ArsB} \)) or AW10 pKK223-3 (\( \text{vector} \)) was assayed with 100 \( \mu \)M \( ^{73} \text{As(III)} \) as described under “Experimental Procedures.” \( \text{ ArsB} \), no ArsB + 5 \text{m M NADH}; \( \text{ArsB} \), ArsB without NADH; \( \text{ArsB} \), ArsB + 5 \text{m M NADH}; \( \text{ArsB} \), ArsB + 5 \text{m M NADH} + 100 \mu \text{M potassium antimonyl tartrate.}

In contrast, As(III) inhibited the uptake of \( ^{125} \text{Sb(III)} \) (Fig. 4). To examine this apparently paradoxical result in more detail, the rates of As(III) and Sb(III) uptake were determined simultaneously using ICP-MS (Fig. 9). In this experiment, the concentration of one metalloid was fixed at five different concentrations: 10, 50, 100, 500, and 1000 \( \mu \)M. At each of these concentrations of one metalloid, the concentration of the other metalloid was varied between 0 and 1000 \( \mu \)M. At every concentration of As(III), Sb(III) stimulated uptake of As(III), and at every concentration of Sb(III), As(III) inhibited uptake of Sb(III). When the two metalloids were present at approximately equal concentrations, the rates of uptake of the two were approximately the same. The most parsimonious explanation for these results is that ArsB catalyzes co-transport of the two metalloids.

However, co-transport does not explain the converse effect of one metalloid on the rate of uptake of the other. If it were simply co-transport, with separate sites on ArsB for As(III) and Sb(III), then As(III) would be expected to stimulate Sb(III) transport as Sb(III) stimulates As(III) transport. If As(III) and Sb(III) were simply alternate substrates for the same site on ArsB, then Sb(III) would be expected to compete with the uptake of As(III) as As(III) competes for Sb(III) uptake. Moreover, the effect of As(III) on Sb(III) uptake does not appear to be simple competitive inhibition. When the concentration dependence of Sb(III) uptake was analyzed as a function of As(III), increasing sigmoidicity was observed (Fig. 10). In the absence of As(III), the data from two separate experiments could be reasonably fitted to the Michaelis-Menten relationship, generating apparent \( K_m \) and \( V_{max} \) values of 43 \( \mu \)M and 182 nmol/mg protein/min, respectively. When the data were analyzed using the Hill relationship, the apparent \( K_m \) value was 44 \( \mu \)M, with a \( V_{max} \) of 172 nmol/mg protein/min and a Hill coefficient of 1.6 (Table II). As the concentration of As(III) increased, the apparent \( K_m \) increased, the \( V_{max} \) decreased, and the Hill coefficient in-
creased to a value of 2.5 at 1000 μM As(III). These results strongly suggest some sort of interaction of As(III) and Sb(III) associated with transport by ArsB.

**DISCUSSION**

Reflecting the pervasiveness of environmental arsenic (20), ArsB is a ubiquitous transport protein found in the genomes and plasmids of most bacteria and archaea (1). ArsB is unusual in that it is either a secondary carrier coupled to the electrochemical proton gradient or the translocation subunit of the As(III)/Sb(III)-translocating ArsAB ATPase (2, 3). Based on this novel dual mode of energy coupling, we had proposed that not only the ArsAB pump but other solute-translocating ATPases such as the F₀ − F₁ and ATP-binding cassette transporters evolved from the association of carriers or channels with soluble ATPases (5, 6).

Yet, both the mechanism and substrate of ArsB are probably different from those that were previously conceived (4). Based on a dependence on the electrochemical proton gradient, we had proposed that ArsB is a uniporter that catalyzes electrophoretic efflux of the arsenite anion out of cells in response to the outside positive membrane potential. In this report we demonstrate for the first time translocation of Sb(III) by ArsB. Because the pKₐ value of inorganic trivalent antimony is 11.8, the concentration of the antimonite anion at a cytosolic pH level of 7.5 (21) is 4 orders of magnitude lower than the total Sb(III) concentration. The paucity of the intracellular antimonite anion would make a uniport mechanism improbable; rather, As(III) and Sb(III) are protonated neutral molecules at cytosolic pH level, and mechanisms that couple efflux of a neutral substrate to the electrochemical proton gradient must be considered. Extrusion of a neutral molecule from cells into the acid and positive exterior could be accomplished by exchange with a cation. Because dependence on an inorganic cation was not observed, protons are a reasonable alternative. This hypothesis was tested in two ways. First, the electrochemical proton gradient was applied as only a membrane potential or only a pH gradient. Either was capable of supporting Sb(III) uptake into everted membrane vesicles. These results are inconsistent with either a uniporter for a neutral solute, which would catalyze only facilitated diffusion, or a uniporter for an anion, which would be able to couple only to the membrane potential and not the pH gradient. Second, Sb(III)/H⁺ exchange can be inferred by the dissipation of ΔpH concomitant with the addition of Sb(III). That exchange is electrophoretic is shown by the ability of Sb(III) to dissipate Δψ. The most reasonable explanation for these results is that ArsB is a metalloid-proton antiporter. From the reported topological determination of ArsB (8), two glutamate and four aspartate residues can be predicted to be located in transmembrane domains of ArsB, some of which may be involved in H⁺ translocation.

What is the nature of the trivalent metalloid substrate of ArsB? We have recently shown by extended x-ray absorption fine structure spectroscopy (EXAFS) that in solution at a neutral pH the predominant arsenic species is As(OH)₃²⁻ and by analogy the antimony species would be Sb(OH)₃⁻. Indeed, we postulate that the trihydroxides are the forms of the metalloids that are translocated by GlpF and the eukaryotic aquaglyceroporin channels (16).

On the other hand, As(OH)₃⁻ and Sb(OH)₃⁻ are not likely to be the substrates of ArsB because these forms cannot explain the interactions observed between As(III) and Sb(III): 1) As(III) inhibits uptake of Sb(III); 2) Sb(III) stimulates As(III) uptake; 3) the rates of uptake of the two metalloids are approximately equal when the two are present at roughly equivocal concentrations; and 4) the kinetics of Sb(III) uptake become increasingly sigmoidal in the presence of As(III). One possibility is that ArsB oligomerizes, with subunit-subunit interaction; another possibility is that ArsB has separate binding sites for As(III) and Sb(III). Neither possibility easily explains the stimulation of uptake of one substrate by the other and yet reciprocal inhibition of the first by the second. A third possibility is that the substrate polymerizes analogously to phosphate, pyrophosphate, and polyphosphate. In fact, trivalent As(III) is known to readily form oxo-bridged polymers. The crystal structure of arsenious oxide, As₃O₅, is a six-membered (As-O)₃ ring with the fourth As(III) coordinated to the three axial oxygens (22). In addition, a search of the Cambridge Structural Database identifies 109 oxo-bridged As-O⁻₃ rings, nearly all of which are cyclic, including 10 with hexose-like six-membered (As-O₅)₃ rings. (Note that polymerization of (AsO)₃⁻₃ creates even-numbered rings, and six- and eight-membered rings are the common in the data base, whereas pentose-like five membered rings are not formed.) If these are physiologically relevant forms, why are they not visible in the EXAFS spectra? First, EXAFS is remarkably accurate in determining bond length, but it is quite insensitive to the presence of minor species. If the As(OH)₃⁻ equilibrium with polymeric forms favors the monomeric form, the polymeric forms would probably not be observed. Second, an EXAFS assay was not performed because Sb(III) requires higher energy x-rays than were available, so it is possible that oxo-bridged Sb(III) species would be observed. Admittedly, this proposition relies on the existence of solution structures that have not yet been identified. However, we have recently found that As(III) is transported by most of the hexose transporters in Saccharomyces cerevisiae and have proposed that the substrate is a hexose-like six-membered (As-O₅)₃ ring (24). In yeast transport of As(III) via hexose, permeases are inhibited by hexoses, and glucose transport is inhibited by As(III). In contrast, ArsB-mediated transport of either As(III) or Sb(III) is not inhibited by a 1000-fold excess of glucose, mannose, galactose, or fructose.

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1. A. Ramírez-Solís, R. Mukopadhyay, B. P. Rosen, and T. L. Stemmler, unpublished observations.
(data not shown). Thus, ArsB is a specific metalloid carrier and not a sugar carrier.

A proposal for the substrate of ArsB that explains the present results similarly involves six-membered rings composed of As(III), Sb(III), and co-polymers of the two metalloids (Fig. 11). If the equilibrium favors rings containing Sb(III) over those containing As(III), the apparent preference for Sb(III) in ArsB catalysis may in actuality be simply the higher concentration of the (SbO)₃ substrate compared with the (AsO)₂ substrate at the same total amount of metalloid. The apparent stimulation of As(III) by Sb(III) is simply the mass action effect of Sb(III) in formation of the true substrate of ArsB. Similarly, the apparent inhibition of Sb(III) uptake by As(III) is also the result of mass action. The results of the co-transport experiment (Fig. 9) appear to require 1:1 co-transport, but these results could also be explained by formation of the two co-polymer forms (Fig. 11B) that would appear as an average of 1:1 co-transport. The most parsimonious explanation for the apparent cooperativity that As(III) imposes on Sb(III) uptake is that the rate-limiting step in uptake is formation of the substrate. As the concentration of As(III) increases, formation of the preferred (SbO)₃ is impeded. The increase in the Hill coefficient with increasing concentration of As(III) to values approaching 3 is consistent with a progression of six-membered rings from (SbO)₃ to (AsO)(SbO)₂ to (AsO)₂(SbO) to (AsO)₃. Thus, rather than interactions of sites within the ArsB carrier, we propose that cooperativity is imparted by interaction of substrates.

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