

ArsD: A Novel Metallochaperone for an Arsenic Detoxification Pump

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Arsenic is a metalloid toxicant that is widely distributed throughout the earth's crust and causes a variety of health and environment problems. As an adaptation to arsenic-contaminated environments, organisms have developed resistance systems. E. coli plasmid R773 carries the well-studied arsRDABC operon. ArsA is an ATPase that is the catalytic subunit of the ArsAB As(III) extrusion pump. ArsD was shown to have weak repressor activity, but this may not be its physiological function. Most ars operons contain only three genes. arsRBC. Five gene operons have two additional genes, arsD and arsA, and these are usually adjacent to each other. Obviously arsD and arsA were co-evolved, suggesting a related function for the two gene products.

Recently metallochaperones have been identified for a number of metals. Metallochaperones prevent inappropriate metal interactions with other cellular components. Thus, these ubiquitous proteins have a critical biological function: to deliver metals in the cytoplasm to the site of utilization or export. We report here that ArsD is an arsenic chaperone that transfers trivalent metalloids to the ArsA ATPase. Through protein-protein interactions, ArsD increases the affinity of the ATPase for As(III) and results in increased efflux and resistance. This is the first report of an arsenic chaperone and suggests that cells can regulate the intracellular concentration of free arsenite to prevent toxicity. Supported by NIH grant Al45428.

1: Evolution of ars operons prokarvotes. In five-gene ars operons, the arsD and arsA genes are always found together. This observation led us to propose that five-gene arsRDABC operons evolved from three-gene arsRBC operons by insertion of the arsD and arsA genes as a unit. The linkage of these two genes leads us to consider the possibility that ArsD and ArsA might have associated functions in arsenio detoxification.

> Fig2: Model of ArsD-ArsA interaction As(III) enters cells such as GlpF aquaglyceroporins where it is bound by ArsD through two or three cysteine residues (Cys12 Cys13 and/or Cys18). As(III) is then transferred to Cys113, Cys172 and Cys422 in the metal binding domain of ArsA in a sten-wise manner ArsD and ArsB are proposed to bind to the same site on ArsA sequentially in a cycle of metal transfer from ArsD to ArsA to ArsB concomitant with ATP binding and then hydrolysis by ArsA.

I			η,	
I			12/13 18	
	Escherichia	1	METLAVEDRAMCCSTEVCETDVDQALVDESTDVQWERQC-SVQIBRENLAQQBMSEVQN	
I	Salmonella	1	MKTLA VEDRAMCCSTEVCETDVD OAR VDESADV OWEKOC-EVO I BRENLAQORMSEVON	
l	Klebsiella	1	MKT I T VEDRAMCOSTEVCESDVDQVH VDSSADV OWASGR-EVOVER VNHAQOEMSEVON	
I	Acidiphilium	1	MKT TUSDRAMCOSYCVOCSDVDQVHUDSSAD: QWASGR-CVOVBRUNHAQQEMSSVHN	
I	IncN	1	MKM T ISDRAMCOSYCHOCSDVDQVH MS-AD, QWASGR-CHO BR AM, HERMSRADA	
ł	Shewanella	1	MTHESTEDRAL COSTOVOCADVD OT LATSAAD COMMOO - STAVER STUBREND SOUBMAEVEN	Fig. 2: Multiple alignment
I	Rhodospirillum	60	INK DIVIDEN CCS SVCEPDVDENH ASAAD LA AEQ-SVSVTRVNH SOOBOASAAN	rig. 5. multiple angriment
	Rhodopirellula	1	MSH (QUYDRAMCCSTCVCCPOVDET#PREAAD) DWAYOO-CHRVDR: NDAC BEABEAGN	of ArsD nomologues.
	Listeria	1	MSKVSLVEPAMCODYCVCCPGVDTBH RVSSI OTVEKVDCVEVER NUTG PAARVEN	ArsD homologues are
l	Bacteroides	1	KK BINDRAGOPRE CET. PBF RVAVV ETHARO-EVIVTREAFRDBOV ASA	shown from 12 archeal
I	Sinorhizobium	1	NKK E ISDRAGCSTOVCEPSVEPER RV VA NNHANK-E DVTR NH SEEDASANN	and bacterial organisms.
I	Halobacterium	1	VTO TATE BANCOSYCHOOPEPEDER EV HA DOWENEFDYDWSRAM OF IEOSIET	Cysteine residues are
I				indicated The multiple
ł			112/113 119/120	alignment was calculated
I	Escherichia	59	EKAKA JI ASEAD ING ING DEDTU AERVERRADIAR FE PL K. GLAPSGOOGEN SOC	with CLUSTAL M27
	Salmonella	59	EKAKA JI ASEAD ING ING DEDTY AERVERRADIAR FE PL K. GLAPSGOOGEN SOC	WILLIGLUSTAL W27.
l	Klebsiella	59	EKAKA # ASCADOM MA DEDTW ACRYERRADIAR FOUPL K GLAPIGOOGGN SOC	
I	Acidiphilium	59	EKAKA 17 ASEAS IN INCOMP. AGRICATION AGRICATION FOR PL K. GLAPTGAOGGN SOC	
I	IncN	59	EKAKA IF ASCHOOL IN DESTY ACRYERRADIAR FOURL K GLASTGOOGEN SOC	
	Shewanella	59	ALAKR H. TSHADSHELLIN, GO. ARRY ROBARAK TL APATEATGAOGGN SCO	
I	Rhodospirillum	120	PAUVKEP AG-I REPUBLIC STELVERGUEAKL. SPSPAPAAAGS OSPR GOO	
l	Pirellula	59	ATTOOM SEESVECTO VITY OR WSRSDAR RENALT TATOMEPHLPTADGGOOGG-SCO	
l	Listeria	60	EKNGELFQSKEN ING WINDED WKM GYP NEEP VITE NF-SEDKKEEQSNS OF SPS GOO	
I	Bacteroides	59	KTWNE IF OKNEARS WE THAN DESINAL SKATS IN MUSEN TO INLUL PAK	
I	Sinorhizobium	59	VVHSQLUTDKEP VHSUTHUTEK WEEKSHLUNESTQLTDUTE E SQKPVVRLKLNVKK	
н	Halobacterium	60	OOUNDLAS FREDSTERNITURE DOWNARE THE STERDSDUDORN	



Cells of yeast strain AH109 bearing both GAL4 AD and BD fusion plasmids were inoculated on histidine drop-out SD plates with 10fold series dilutions. B: Dibromobimane crosslinking. Proteins were incubated with 1 mM potassium antimonyl tartrate, MgCl₂ ATP, and 0.5 mM bBBr as indicated. Samples were analyzed by 8+16% SDS-PAGE. The gels were analyzed by immunoblotting ArsD-ArsA complexes are circled



Fig. 5: ArsD-ArsA metal transfer. A-B: Sb(III) transfer. Sb(III)-bound

MBP-fused ArsD was bound to

an amylose column which was

then loaded with purified BSA (A)

or ArsA (B) + 1 mM ATP and

MgCl₂, as indicated. After

thorough washing, ArsD was

eluted with 10 mM maltose, and

the fractions were analyzed for

the proteins and Sb(III). C: Effect

of nucleotides. The transfer

assay was performed in the

presence of the indicated

nucleotides. Transfer activity was calculated as ([Sb(III) ArsA]/[ArsA])

/([Sb(III)ArsD]/[ArsD]). The values

were normalized to BSA D

As(III) transfer. Binding of As(III)

to ArsA and ArsD was assayed

in the presence of MgATP γ S

without the partner protein (black

bar): and after interaction with

the partner protein (white bar).

C

Anti-Are A

Anti-ArsD



Fig. 7: Effect of the arsD gene on arsenical transport and resistance confered by the arsAB genes. Cells of E. coli strain AW3110 $(\Delta arsRBC)$ harboring vector plasmids pSE380 and pACYC184 (Ω) or plasmids with arsB (∇) , arsAB (\Box) , arsDAB (\Diamond) or arsDc12AIC13AAB (Δ) were grown and assayed. A: As(III) transport. The cells were assayed with 10 µM sodium arsenite in varying incubation time. B: As(III) resistance. The cells were assayed for resistance at various concentrations of sodium arsenite. C: ArsD does not affect expression of ArsA. Protein expression levels were determined by nunoblotting using anti-ArsA and anti-ArsD.



Fig. 8. The arsD gene confers a competitive advantage for cells with arsAB genes. A: Plasmic analysis. Cells of E. coli strain AW3110 bearing either arsAB or arsDAB were grown in a mixture culture. The mixture was 1000-fold diluted daily in LB medium containing 10 μ M sodium arsenite. The plasmids were extracted and analyzed by restriction analysis with Xbal and BamHI B: Cells with only arsAB are lost from the population. The percentage of the cells with each plasmid were calculated as following: arsDAB: X/(vector + Z): arsAB: Y/(vector + Z).

- We have identified the first chaperone for metalloids, the product of the arsD gene of the Escherichia coli plasmid R773 arsRDABC operon.
- Through protein-protein interactions, ArsD transfers As(III) to ArsA, increasing the affinity of the ATPase for As(III). Thus, at low concentrations of As(III), cells with arsDAB have increased efflux of and resistance to As(III).
- Cells with arsDAB have increased ability to regulate intracellular free As(III), preventing toxicity and thus providing a competitive advantage.



ألتحم

may= 97, Km= 110 μN

2000 3000 4000

[ATP] µM

<u>i trti</u>

04 K −21 µM

400 [NaAsO₂] µM

200 300

Fig. 6. ArsD increases the affinity of ArsA for metalloid. A: ArsD increases the stimulation of ArsA ATPase activity by As(III) ATPase activities were measured in different combinations of 3 μ M ArsD, 0.3 μ M ArsA, 10 μ M DTT and 100 μ M sodium arsenite, B: Stimulation by Sb(III) 10 µM potassium antimony tartrate was used to replace sodium arsenite in "A". C: Effect of ArsD on the Km for As(III). ATPase activities were measured in the absence and presence of ArsD at varying concentrations of sodium arsenite. D: Effect of ArsD on Km for ATP. ATPase activity was assayed at 0.5 mM sodium arsenite and varying concentrations of ATP.