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Pentavalent methylated arsenicals are substrates of human AQP9

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Abstract

Liver aquaglyceroporin AQP9 facilitates movement of trivalent inorganic arsenite (As^{III}) and organic monomethylarsonous acid (MAs^{III}). However, the transport pathway for the two major pentavalent arsenic cellular metabolites, MAs^{V} and $DMAs^{V}$, remains unknown in mammals. These products of arsenic metabolism, in particular $DMAs^{V}$, are the major arsenicals excreted in the urine of mammals. In this study, we examined the uptake of the two pentavalent organic arsenicals by human AQP9 in *Xenopus laevis* oocytes. *Xenopus laevis* oocytes microinjected with AQP9 cRNA exhibited uptake of both MAs^{V} and $DMAs^{V}$ in a pH-dependent manner. The rate of transport was much higher at acidic pH (pH5.5) than at neutral pH. Hg(II), an aquaporin inhibitor, inhibited transport of As^{III}, MAs^{III}, MAs^{V} and $DMAs^{V}$ via AQP9. However, phloretin, which inhibits water and glycerol permeation via AQP9, can only inhibit transport of pentavalent MAs^{V} and $DMAs^{V}$ but not trivalent As^{III} and MAs^{III}, indicating the translocation mechanisms of these arsenic species are not exactly the same. Reagents such as FCCP, valinomycin and nigericin that dissipate transmembrane proton potential or change the transmemebrane pH gradient did not significantly inhibit all arsenic transport via AQP9, suggesting the transport of pentavalent arsenic

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is not proton coupled. The results suggest that in addition to the initial uptake of trivalent inorganic As^{III} inside cells, AQP9 plays a dual role in the detoxification of arsenic metabolites by facilitating efflux from cells.

Keywords

AQP9; Liver; Urine; Methylation; Arsenite; Arsenate; Monomethylarsonous acid; Monomethylarsonate; Dimethylarsinate

Introduction

Arsenic is an environmental pollutant and a human carcinogen. It is bioavailable in either of two oxidation states, As^{V} (arsenate) or As^{III} (arsenite). In hepatocytes As^{III} is methylated into a variety of species by a multistep pathway, producing organic trivalent and pentavalent mono-, di- and trimethylated arsenic species, including MAs^{III} (monomethylarsonous acid), MAs^{V} (monomethylarsonic acid), $DMAs^{III}$ (dimethylarsonous acid), $DMAs^{V}$ (dimethylarsinic acid), $TMAs^{V}O$ (trimethylarsine oxide) and $TMAs^{III}$ (Fig. 1) (Drobna et al. 2006; Lin et al. 2002; Thomas et al. 2007). Humans and most other mammals methylate inorganic arsenic and excrete the methylated species in urine, predominantly $DMAs^{V}$ followed by MAs^{V} (Alauddin et al. 2003). Other species such as the trivalent organic species MAs^{III} and $DMAs^{III}$ were not detected previously, possibly due to oxidation, either in vivo or during isolation (Xie et al. 2006). However, recently the detection of these trivalent species (MAs^{III} and $DMAs^{III}$) in urine from rodents and human has been reported (Aposhian et al. 2004; Kenyon et al. 2008).

Different arsenicals have different rates of uptake, which, as a rate limiting metabolic step, is related to their overall toxicity. For example, As^{III} is taken into cells much faster than As^{V}, which is a major reason why As^{III} is more toxic. The in vivo toxicity of inorganic and organic arsenicals is $DMA^{III} \approx MAs^{III} > As^{III} > As^{V} > DMAs^{V} \approx MAs^{V} > TMAs^{V}O$ (Schuhmacher-Wolz et al. 2009), with trivalent MAs^{III} and $DMAs^{III}$ being the most toxic (Drobna et al. 2005; Petrick et al. 2000). Pentavalent $DMAs^{V}$ and $TMAs^{V}O$ are one hundred- and one thousand-fold less toxic than As^{III}, respectively (Hirano et al. 2004). Therefore intracelluar transformation of organic pentavalent arsenicals such as MAs^{V}, $DMAs^{V}$ and TMAO is believed to be a detoxification process. In spite of the lower toxicity, $DMAs^{V}$ has been shown to be a carcinogen in rodents (Cohen et al. 2006). It was also found that $DMAs^{V}$ is carcinogenic at high doses to the rat urinary bladder, but not in mice (Cohen et al. 2007). However, it is still in debate as to whether arsenic methylation is a detoxification process since it produces the more toxic trivalent species as intermediates (Thomas et al. 2007). Individuals with genetic polymorphisms of the gene for the methylating enzyme, AS3MT, have a different profile of methylated species in their urine (Hernandez et al. 2008), indicating they have different arsenic methylation activities. These inter-individual variations may lead to differential arsenic toxicity and/or carcinogenesis.

In addition to in vivo synthesis from inorganic arsenic, humans are directly exposed to organic arsenicals in their food and water. MAs^{V} and $DMAs^{V}$ are widely used as herbicides and pesticides. Inorganic arsenic and $DMAs^{V}$ are also found in rice, which two-thirds of the

world's population consumes as a staple food (Meharg et al. 2008). Therefore, identification of the transport pathways for these species is of importance in elucidation of the health hazards of methylated arsenicals and in evaluation of their overall toxicity and carcinogenesis.

Uptake routes for trivalent inorganic arsenite have been identified in recent years. Arsenite uptake is mediated by aquaglyceroporins (AQPs), which is neutral solute channel in both prokaryotes and eukaryotes. In *Escherichia coli*, GlpF, a bacterial member of the AQP superfamily, conducts arsenite as $As(OH)_3$ (Meng et al. 2004; Sanders et al. 1997). AQPs are membrane channels that include water-selective pores (orthodox aquaporins) and multifunctional channels (aquaglyceroporins) (Agre et al. 1999, 2002). Recently the human aquaglyceroporins AQP7 and AQP9 were shown to conduct $As(OH)_3$ in *Xenopus* oocytes, with AQP9 catalyzing the highest rate (Liu et al. 2004). AQP9 is expressed predominately in the hepatocytes of the liver, the organ of arsenic detoxification (Tsukaguchi et al. 1998). Therefore, we have proposed that AQP9 is responsible for accumulation of inorganic arsenic in liver (Liu et al. 2002).

On the other hand, arsenate, with pKa values of 2.19, 6.94 and 11.5, is taken up via phosphate transporters in prokaryotes and eukaryotes (Bun-ya et al. 1996; Rosenberg et al. 1977; Yompakdee et al. 1996). In mammals, the type II Na^+/Pi co-transporter, SCL34a2a, has been shown to transport arsenate into renal cells (Hartmann et al. 1995; Xu et al. 2002), so it is reasonable to consider that phosphate transporters mediate uptake of As^V into liver as well. However, it is well know that As^V uptake in a varieties of prokaryotic and eukayotic cells are much slower than As^{III}, which explains its lesser toxicity.

We recently reported that the trivalent organic arsenical MAs^{III} is also transported by rat AQP9 three times faster than As^{III} (Liu et al. 2006b). Since AQP9 is a bi-directional channel, intracellular MAs^{III} would flow out of hepatocytes into the blood stream down its concentration gradient. In this study, we demonstrated that human AQP9 also transports MAs^{III} at a rate 5-times higher than As^{III}. In addition to AQP9, here we reported that despite their high sequence similarity (77%), mouse but not human AQP7 can transport MAs^{III}, which is similar to AQP9 in the rate of transport of MAs^{III}. Humans and rodents have different metabolism of arsenic and thus have different levels of methylated species such as MAs^{III} (Wang et al. 2002). Together the different methylation pattern and transport profile of these arsenicals constitute a large difference between human and mouse.

However, the identification of the transporters for the major arsenic metabolites in liver, MAs^V and $DMAs^V$, the species found in urine, remains unknown in mammals (Alauddin et al. 2003). Recently it was reported that a AQP9 homologue, Lsi1 in *Oryza sativa* can transport MAs^V and $DMAs^V$ in a pH dependent manner (Li et al. 2009). Here we report that AQP9 mediates transport of MAs^V and $DMAs^V$. This finding not only explains how arsenic metabolites can go from liver to other tissues but also highlights the multiple roles of AQP9 in both influx of inorganic arsenic and efflux of organic products. In addition, identification of the uptake pathway for pentavalent organoarsenicals indicates how environmental pollutants such as MAs^V and $DMAs^V$ may be taken into the human body.

Materials and methods

Strains and plasmids

Mouse and human AQP7 and AQP9 were cloned into pXβG-ev1, as described previously (Liu et al. 2002). *E. coli* strain JM109 [*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ*(lac-proAB) F′(traD36 proAB* $^{+}$ *lacI* ^{q} *lacZΔM15*] and JM110 [*rps* (Str^{r}) *thr leu thi-1 lacY galK galT ara tonA tsx dam dcm supE44* Δ*(lac-proAB)* [*F′traD36 proAB lacIqZΔM15*] was used for molecular cloning. *E. coli* cells were grown in LB medium supplemented when necessary with 125 μg/ml ampicillin.

Expression of AQP7/AQP9 in *Xenopus* oocytes

Capped cRNAs were synthesized in an in vitro reaction using mMessage mMachine T3 ultra kit (Applied Biosystem) with pXβG-ev1 plasmids linearized with *NotI* (Liu et al. 2006a). Oocytes from *Xenopus laevis* were defolliculated and injected with 25 ng of cRNA or with 50 nl of water. They were then incubated in complete ND96 buffer for 3 days at 16°C and used for uptake assays.

Transport assays

For assay of metalloid accumulation in oocytes, oocytes were incubated in 1 mM each of sodium arsenite (As^{III}, Sigma), sodium monomethylarsenite (MAs^{III}, a gift from Miroslav Styblo in University of North Carolina at Chapel Hill), monosodium acid methanearsonate (MAs^{V}, Chem Service) or cacodylic acid ($DMAs^{V}$, Sigma) at room temperature for 60 min. When necessary, oocytes were pretreated by 20 μM carbonyl cyanide 4-trifluoromethoxyphenylhydrazone (FCCP, Sigma), 10 μM phloretin (Sigma), 100 μM valinomycin (Sigma) or 100 μM nigericin (Sigma) for 30 min or 200 μM Hg(II) (mercury chloride, Sigma) for 5 min before arsenic transport assay. The oocytes were then collected and washed in ND96 buffer three times (Liu et al. 2006a). Oocytes were completely digested using 70% (vol/vol) HNO_3 for at least 2 h. The samples were then diluted with HPLC grade water, and total arsenic was determined by inductively coupled plasma mass spectroscopy (ICP-MS) (ELAN 9000, PerkinElmer, Norwalk, CT).

Statistical analysis

All experiments contain at least two batches of oocytes from two animals; at least 3 replicates are used each time. One batch of experiments is used to present in this paper.

Quantitative results are shown as means ± standard deviations. The statistical analysis was performed by ANOVA. $P < 0.05$ were considered significant, and all figures represent at least $P < 0.01$.

Results

MAs^{III} is differentially transported by mouse and human AQP7 in *Xenopus* oocytes

The ability of human and mouse AQP7 to conduct As^{III}, MAs^{III}, MAs^{V} or $DMAs^{V}$ at different pH levels in oocytes was examined. Mouse AQP7 transports both As^{III} and MAs^{III}, but not MAs^{V} and $DMAs^{V}$ at pH5.5, 6.5 and 7.5 (Fig. 2). MAs^{III} was transported at a five-

fold higher rate than that of As^{III}. However, human AQP7 transports only inorganic As^{III} (Fig. 2c). It is striking that the transport profile of arsenic is different in human and mouse, considering that mouse is often used as a model for arsenic-associated carcinogenesis (Wang et al. 2002).

MAs^{V} and $DMAs^{V}$ are transported by human AQP9

The ability of human AQP9 to increase As^{III}, MAs^{III}, MAs^{V} and $DMAs^{V}$ permeability at different pH levels in oocytes was examined. Transport of five different trivalent and pentavalent arsenicals, including As^{III}, MAs^{III}, As^{V}, MAs^{V} and $DMAs^{V}$, at pH5.5, 6.5 and 7.5, respectively was assayed (Fig. 3a, b). The results show that human AQP9 facilitates uptake of trivalent arsenicals (As^{III} and MAs^{III}) and organic pentavalent arsenicals (MAs^{V} and $DMAs^{V}$). However, under these conditions, hAQP9 did not conduct As^{V} (data not shown). We previously reported that rat AQP9 facilitates MAs^{III} uptake more efficiently than inorganic As^{III}. Here we show that human AQP9 also transports MAs^{III} more efficiently than As^{III}, with a rate five-fold higher than that of As^{III}. Within the pH range from 5.5 to 7.5, uptake of As^{III} and MAs^{III} were equivalent. Inorganic As^{V} is not transported in any of the pH conditions tested, which is consistent with our previous observation using rat AQP9 (Liu et al. 2002). However, MAs^{V} and $DMAs^{V}$ are conducted in a pH-dependent manner (Fig. 3c, d). Transport of $DMAs^{V}$ at pH 5.5 is more efficient than As^{III} and comparable to the efficiency of glycerol transport (data not shown). Transport of MAs^{V} is less efficient at physiological pH (Fig. 3c) and is observed mostly under acidic conditions. Both MAs^{V} and $DMAs^{V}$ exhibit much lower transport at higher pH, indicating the neutral forms of these compounds are substrates for AQP9, as discussed below.

Effect of mercury and phloretin on arsenic transport by AQP9

Hg(II) is an inhibitor of aquaporins that inhibits water permeation by binding to a cysteine thiol group, blocking the water permeable channel (Savage and Stroud 2007), To examine whether Hg(II) inhibits transport of all arsenicals, mercury chloride was added to the transport buffer at a final concentration of 200 μM (Fig. 4a). Transport of all tested trivalent and pentavalent arsenicals was inhibited significantly by Hg(II), indicating that binding of mercury blocks a common permeation pathway for both trivalent and pentavalent arsenicals. Phloretin, another inhibitor of AQP9 that inhibits both water and glycerol permeation (Ishibashi et al. 1998; Tsukaguchi et al. 1999), completely inhibited transport of pentavalent arsenicals but not transport for trivalent As^{III} and MAs^{III} (Fig. 4b). These results suggest that, although those substrates likely share a single translocation pathway, their mechanisms of transport are not entirely identical.

Effect of carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP), valinomycin and nigericin on arsenic transport via AQP9

To examine whether transport of pentavalent arsenic via AQP9 is coupled to the proton motive force, the effect of valinomycin, nigericin, and FCCP (Collins and Larson, 2005; Hagos et al. 2007; Sweet and Pritchard 1999), which dissipate the transmembrane potential, or changes the transmembrane pH gradient, or both, was examined. After 30 min of incubation, arsenicals were added each at a final concentration of 1 mM. Valinomycin, nigericin or FCCP did not significantly inhibit the trivalent or pentavalent arsenic transport

(Fig. 5a–c), indicating transport of none of the tested arsenicals occurs in a proton-coupled manner.

Discussion

Arsenic is a major environmental toxin, mostly due to the combination of its ubiquitous environmental presence and toxicity. Inorganic As^{III} is taken into most organisms adventitiously by aquaglyceroporin channels and is subsequently methylated to more toxic trivalent forms and to less toxic pentavalent forms (Fig. 1). In this study, we demonstrated that human AQP9 facilitates transport of pentavalent products MAs^{V} and $DMAs^{V}$.

How are these predominantly anionic pentavalent species recognized by neutral solute channels? AQP1 and GlpF have been demonstrated to transport only neutral species but not proton and ions (Fu and Lu 2007; Saparov et al. 2005). Since AQP9 has corresponding NPA (Asp-Pro-Ala) and Arginine ring regions that have been identified as proton filters (Fu and Lu 2007), it is likely that AQP9 also conducts only neutral species. Trivalent arsenite, with a pKa value of 9.1, exists primarily as $As(OH)_3$ at physiological pH (Ramirez-Solis et al. 2004). In contrast, at physiological pH, arsenate, with pKa values of 2.2, 7 and 11.6, is a mixture of the anions $H_2AsO_4^-$ and $HAsO_4^{-2}$, and these are not transported by hAQP9 (data not shown). The organic pentavalent arsenical MAs^{V} has two pKa values, 3.6 and 8.2, and $DMAs^{V}$ has a single pKa of 6.5. Therefore, MAs^{V} and $DMAs^{V}$ exist in equilibrium between the neutral undissociated and anionic species at physiological pH and below. Both MAs^{V} and $DMAs^{V}$ were transported at pH 7.5, and the rates were higher at pH 5.5. The lower pH would alter the transmembrane gradient and membrane potential, but the dissipation of these gradients by FCCP, nigericin, and valinomycin did not affect transport (Fig. 5a–c). Thus we reason that both pentavalent methylated species are substrates of AQP9 as uncharged molecules. At pH 6.5, transport of both MAs^{V} and $DMAs^{V}$ was 50% slower than at pH 5.5. At pH 7.5, which is close to physiological pH, transport of MAs^{V} was limited, presumably because most of the molecules were dissociated to anionic species. At pH 7.5, where 10% of the $DMAs^{V}$ can be calculated to be undissociated, significant uptake by AQP9 remained.

What is the physiological relevance of transport of As^{III} and its products MAs^{III}, MAs^{V} and $DMAs^{V}$ by hAQP9? Arsenic accumulates in multiple tissues including liver, and methylation of arsenic is to found to be mainly in liver, whereas other organs are not excluded (Lin et al. 2002). As^{III} can enter the hepatocyte through AQP9 and following methylation flows out of liver via AQP9 down its concentration gradient into the blood stream via AQP9 and ends up in urine (Fig. 6). Even though the rate of transport of the pentavalent arsenicals is not as high at physiological pH as at pH 5.5, the equilibrium between the undissociated and anionic species ensures that, by mass action, the pentavalent species will continuously flow downhill from the hepatocyte into the blood stream. These findings addressed the multiple roles of AQP9 in the uptake of more toxic trivalent inorganic species and efflux of much less toxic organic pentavalent species and are consistent with the unique role of hAQP9 in liver arsenic detoxification.

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References

Agre P, Mathai JC, Smith BL, Preston GM. Functional analyses of aquaporin water channel proteins. Methods Enzymol. 1999; 294:550–572. [PubMed: 9916248]

Agre P, King LS, Yasui M, Guggino WB, Ottersen OP, Fujiyoshi Y, Engel A, Nielsen S. Aquaporin water channels—from atomic structure to clinical medicine. J Physiol. 2002; 542:3–16. [PubMed: 12096044]

Alauddin M, Alauddin ST, Bhattacharjee M, Sultana S, Chowdhury D, Bibi H, Rabbani GH. Speciation of arsenic metabolite intermediates in human urine by ion-exchange chromatography and flow injection hydride generation atomic absorption spectrometry. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2003; 38:115–128. [PubMed: 12635822]

Aposhian HV, Zakharyan RA, Avram MD, Sampayo-Reyes A, Wollenberg ML. A review of the enzymology of arsenic metabolism and a new potential role of hydrogen peroxide in the detoxication of the trivalent arsenic species. Toxicol Appl Pharmacol. 2004; 198:327–335. [PubMed: 15276412]

Bun-ya M, Shikata K, Nakade S, Yompakdee C, Harashima S, Oshima Y. Two new genes, PHO86 and PHO87, involved in inorganic phosphate uptake in Saccharomyces cerevisiae. Curr Genet. 1996; 29:344–351. [PubMed: 8598055]

Cohen SM, Arnold LL, Eldan M, Lewis AS, Beck BD. Methylated arsenicals: the implications of metabolism and carcinogenicity studies in rodents to human risk assessment. Crit Rev Toxicol. 2006; 36(2):99–133. [PubMed: 16736939]

Cohen SM, Ohnishi T, Arnold LL, Le XC. Arsenic-induced bladder cancer in an animal model. Toxicol Appl Pharmacol. 2007; 222:258–263. [PubMed: 17109909]

Collins A, Larson M. Regulation of inward rectifier K + channels by shift of intracellular pH dependence. J Cell Physiol. 2005; 202:76–86. [PubMed: 15389543]

Drobna Z, Waters SB, Devesa V, Harmon AW, Thomas DJ, Styblo M. Metabolism and toxicity of arsenic in human urothelial cells expressing rat arsenic (+3 oxidation state)-methyltransferase. Toxicol Appl Pharmacol. 2005; 207:147–159. [PubMed: 16102566]

Drobna Z, Xing W, Thomas DJ, Styblo M. shRNA silencing of AS3MT expression minimizes arsenic methylation capacity of HepG2 cells. Chem Res Toxicol. 2006; 19:894–898. [PubMed: 16841956]

Fu D, Lu M. The structural basis of water permeation and proton exclusion in aquaporins. Mol Membr Biol. 2007; 24:366–374. [PubMed: 17710641]

Hagos Y, Stein D, Ugele B, Burckhardt G, Bahn A. Human renal organic anion transporter 4 operates as an asymmetric urate transporter. J Am Soc Nephrol. 2007; 18:430–439. [PubMed: 17229912]

Hartmann CM, Wagner CA, Busch AE, Markovich D, Biber J, Lang F, Murer H. Transport characteristics of a murine renal Na/Pi-cotransporter. Pflugers Arch. 1995; 430:830–836. [PubMed: 7478940]

Hernandez A, Xamena N, Sekaran C, Tokunaga H, Sampayo-Reyes A, Quinteros D, Creus A, Marcos R. High arsenic metabolic efficiency in AS3MT287Thr allele carriers. Pharmacogenet Genomics. 2008; 18:349–355. [PubMed: 18334919]

Hirano S, Kobayashi Y, Cui X, Kanno S, Hayakawa T, Shraim A. The accumulation and toxicity of methylated arsenicals in endothelial cells: important roles of thiol compounds. Toxicol Appl Pharmacol. 2004; 198:458–467. [PubMed: 15276427]

Ishibashi K, Kuwahara M, Gu Y, Tanaka Y, Marumo F, Sasaki S. Cloning and functional expression of a new aquaporin (AQP9) abundantly expressed in the peripheral leukocytes permeable to water and urea, but not to glycerol. Biochem Biophys Res Commun. 1998; 244:268–274. [PubMed: 9514918]

Kala SV, Neely MW, Kala G, Prater CI, Atwood DW, Rice JS, Lieberman MW. The MRP2/cMOAT transporter and arsenic-glutathione complex formation are required for biliary excretion of arsenic. J Biol Chem. 2000; 275:33404–33408. [PubMed: 10938093]

Kenyon EM, Hughes MF, Adair BM, Highfill JH, Crecelius EA, Clewell HJ, Yager JW. Tissue distribution and urinary excretion of inorganic arsenic and its methylated metabolites in C57BL6 mice following subchronic exposure to arsenate in drinking water. Toxicol Appl Pharmacol. 2008; 232:448–455. [PubMed: 18706920]

Li RY, Ago Y, Liu WJ, Mitani N, Feldmann J, McGrath SP, Ma JF, Zhao FJ. The rice aquaporin Lsi1 mediates uptake of methylated arsenic species. Plant Physiol. 2009; 150(4):2071–2080. [PubMed: 19542298]

Lin S, Shi Q, Nix FB, Styblo M, Beck MA, Herbin-Davis KM, Hall LL, Simeonsson JB, Thomas DJ. A novel S-adenosyl-L-methionine:arsenic(III) methyltransferase from rat liver cytosol. J Biol Chem. 2002; 277:10795–10803. [PubMed: 11790780]

Liu Z, Shen J, Carbrey JM, Mukhopadhyay R, Agre P, Rosen BP. Arsenite transport by mammalian aquaglyceroporins AQP7 and AQP9. Proc Natl Acad Sci U S A. 2002; 99:6053–6058. [PubMed: 11972053]

Liu Z, Carbrey JM, Agre P, Rosen BP. Arsenic trioxide uptake by human and rat aquaglyceroporins. Biochem Biophys Res Commun. 2004; 316:1178–1185. [PubMed: 15044109]

Liu Z, Sanchez MA, Jiang X, Boles E, Landfear SM, Rosen BP. Mammalian glucose permease GLUT1 facilitates transport of arsenic trioxide and methylarsonous acid. Biochem Biophys Res Commun. 2006a; 351:424–430. [PubMed: 17064664]

Liu Z, Styblo M, Rosen BP. Methylarsonous acid transport by aquaglyceroporins. Environ Health Perspect. 2006b; 114:527–531. [PubMed: 16581540]

Meharg AA, Lombi E, Williams PN, Scheckel KG, Feldmann J, Raab A, Zhu Y, Islam R. Speciation and localization of arsenic in white and brown rice grains. Environ Sci Technol. 2008; 42:1051–1057. [PubMed: 18351071]

Meng YL, Liu Z, Rosen BP. As(III) and Sb(III) uptake by GlpF and efflux by ArsB in Escherichia coli. J Biol Chem. 2004; 279:18334–18341. [PubMed: 14970228]

Petrick JS, Ayala-Fierro F, Cullen WR, Carter DE, Vasken Aposhian H. Monomethylarsonous acid (MMA(III)) is more toxic than arsenite in Chang human hepatocytes. Toxicol Appl Pharmacol. 2000; 163:203–207. [PubMed: 10698679]

Ramirez-Solis A, Mukopadhyay R, Rosen BP, Stemmler TL. Experimental and theoretical characterization of arsenite in water: insights into the coordination environment of As–O. Inorg Chem. 2004; 43:2954–2959. [PubMed: 15106984]

Rosenberg H, Gerdes RG, Chegwidden K. Two systems for the uptake of phosphate in Escherichia coli. J Bacteriol. 1977; 131:505–511. [PubMed: 328484]

Sanders OI, Rensing C, Kuroda M, Mitra B, Rosen BP. Antimonite is accumulated by the glycerol facilitator GlpF in Escherichia coli. J Bacteriol. 1997; 179:3365–3367. [PubMed: 9150238]

Saparov SM, Tsunoda SP, Pohl P. Proton exclusion by an aquaglyceroprotein: a voltage clamp study. Biol Cell. 2005; 97:545–550. [PubMed: 15850456]

Savage DF, Stroud RM. Structural basis of aquaporin inhibition by mercury. J Mol Biol. 2007; 368:607–617. [PubMed: 17376483]

Schuhmacher-Wolz U, Dieter HH, Klein D, Schneider K. Oral exposure to inorganic arsenic: evaluation of its carcinogenic and non-carcinogenic effects. Crit Rev Toxicol. 2009; 39:271–298. [PubMed: 19235533]

Sweet DH, Pritchard JB. rOCT2 is a basolateral potential-driven carrier, not an organic cation/proton exchanger. Am J Physiol. 1999; 277:F890–898. [PubMed: 10600936]

Thomas DJ, Li J, Waters SB, Xing W, Adair BM, Drobna Z, Devesa V, Styblo M. Arsenic (+3 oxidation state) methyltransferase and the methylation of arsenicals. Exp Biol Med (Maywood). 2007; 232:3–13. [PubMed: 17202581]

Tsukaguchi H, Shayakul C, Berger UV, Mackenzie B, Devidas S, Guggino WB, van Hoek AN, Hediger MA. Molecular characterization of a broad selectivity neutral solute channel. J Biol Chem. 1998; 273:24737–24743. [PubMed: 9733774]

Tsukaguchi H, Weremowicz S, Morton CC, Hediger MA. Functional and molecular characterization of the human neutral solute channel aquaporin-9. Am J Physiol. 1999; 277:F685–696. [PubMed: 10564231]

Wang JP, Qi L, Moore MR, Ng JC. A review of animal models for the study of arsenic carcinogenesis. Toxicol Lett. 2002; 133:17–31. [PubMed: 12076507]

Xie R, Johnson W, Spayd S, Hall GS, Buckley B. Arsenic speciation analysis of human urine using ion exchange chromatography coupled to inductively coupled plasma mass spectrometry. Anal Chim Acta. 2006; 578:186–194. [PubMed: 17723711]

Xu H, Inouye M, Missey T, Collins JF, Ghishan FK. Functional characterization of the human intestinal NaPi-IIb cotransporter in hamster fibroblasts and Xenopus oocytes. Biochim Biophys Acta. 2002; 1567:97–105. [PubMed: 12488042]

Yompakdee C, Bun-ya M, Shikata K, Ogawa N, Harashima S, Oshima Y. A putative new membrane protein, Pho86p, in the inorganic phosphate uptake system of Saccharomyces cerevisiae. Gene. 1996; 171:41–47. [PubMed: 8675028]

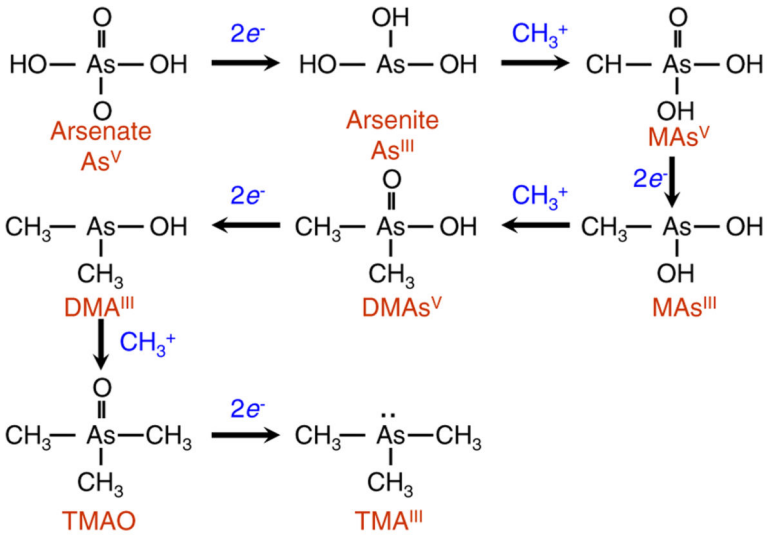

Fig. 1.

Pathway of arsenic methylation. The arsenic step-by-step menthylation pathway is shown with intermediates indicated. $DMAs^V$ is the dominant arsenic product that is detected in human urine of many endemic areas. MAs^V and TMAO are always found in human urine. MAs^{III} and $DMAs^{III}$ are also found in urine of human and rodents with relatively fewer amounts

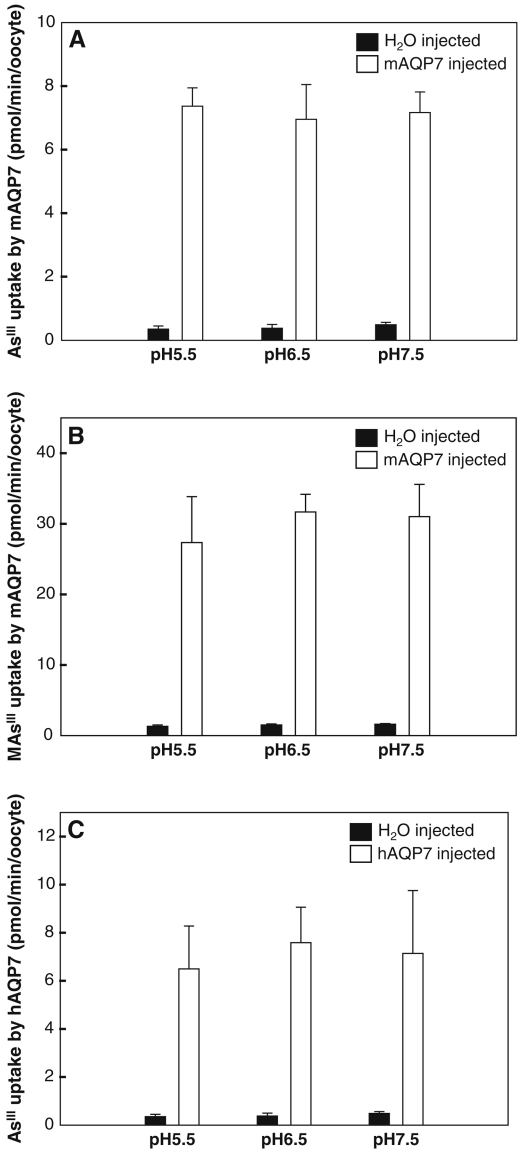

Fig. 2.

Arsenical transport by mouse and human AQP7. Transport of trivalent species by human and mouse AQP7 was examined under different pH conditions. **a:** As^{III} uptake via mouse AQP7. **b.** MAs^{III} uptake via mouse AQP7. **c:** As^{III} uptake via human AQP7. The open bars represents arsenic uptake via AQP7, and the solid bars are the water injected controls

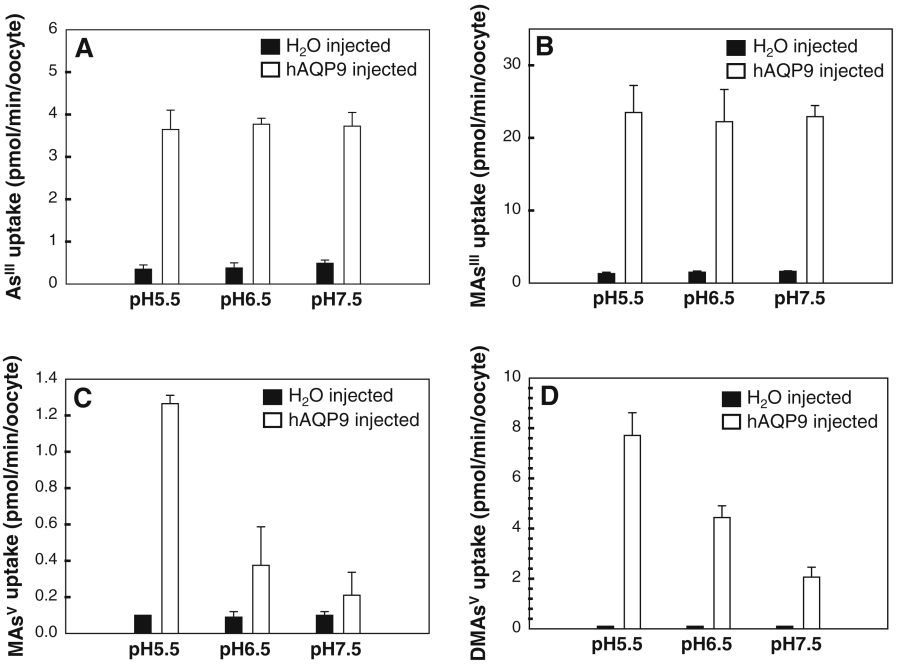

Fig. 3.

Arsenic transport by AQP9. Transport of arsenicals by hAQP9 was examined under different pH conditions. **a:** As^{III} uptake. **b.** MAs^{III} uptake. **c:** MAs^{V} uptake. **d.** $DMAs^{V}$. The open bars represents arsenic uptake via hAQP9, and the solid bars are the water injected controls

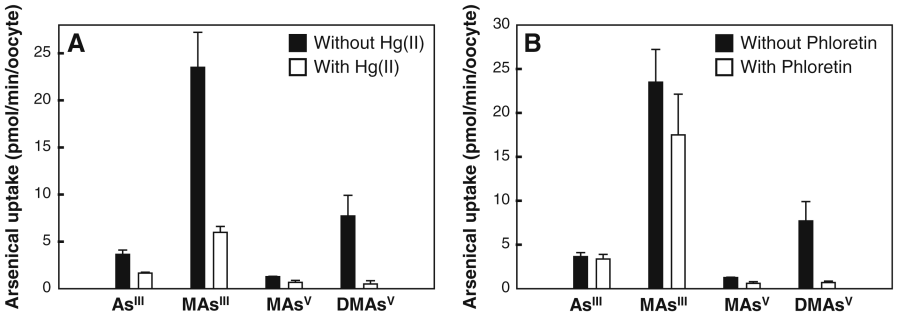

Fig. 4.

Effect of Hg(II) and phloretin on arsenic transport by hAQP9. **a:** Arsenic uptake inhibited by mercury. **b.** Arsenic uptake inhibited by phloretin. Oocytes were pretreated with $HgCl_2$ (200 μM) for 5 min or phloretin (100 μM) for 60 min at pH 5.5. To initiate transport, the indicated arsenicals were added to a final concentration of 1 mM. The oocytes were washed three times using the same buffer and digested for metalloid quantification. The solid bars represents arsenic uptake via hAQP9 without treatment, while the open bars are treated by either mercury or phloretin

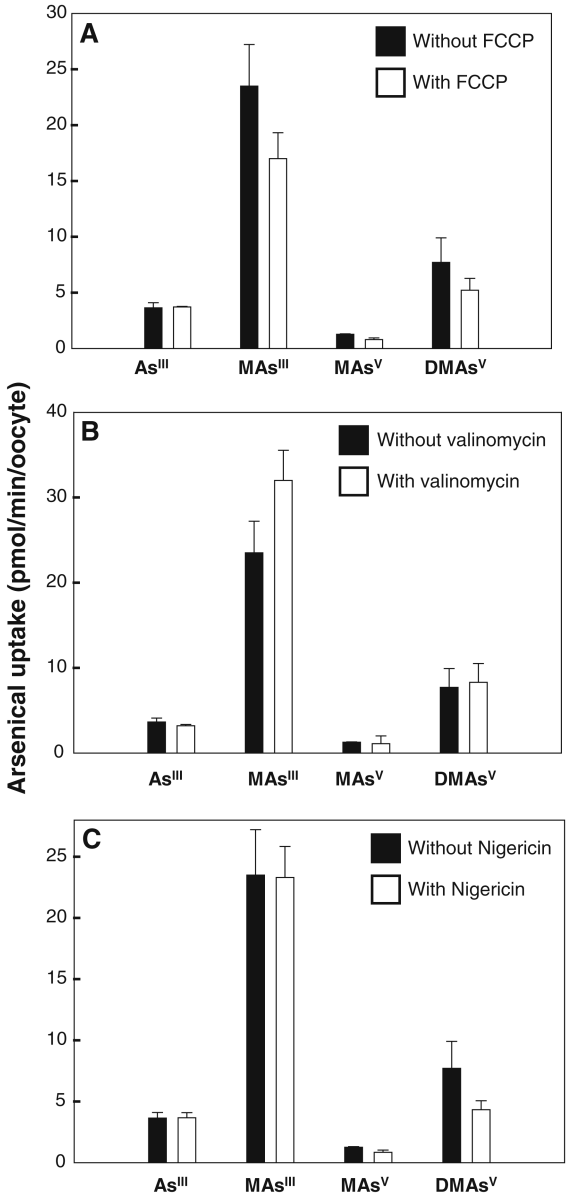

Fig. 5.

Effect of FCCP, valinomycin and nigericin on arsenical transport by hAQP9. **a** Arsenic uptake inhibited by FCCP. **b** Arsenic uptake inhibited by valinomycin. **c** Arsenic uptake inhibited by nigericin. Oocytes were pretreated with FCCP (20 μM), valinomycin (100 μM), or nigericin (10 μM) for 60 min at pH 5.5. To initiate transport, the indicated arsenicals were added to a final concentration of 1 mM. The oocytes were washed 3 times using the same buffer and digested for metalloid quantification. The solid bars represents arsenic uptake via AQP9 without treatment, while the open bars are treated

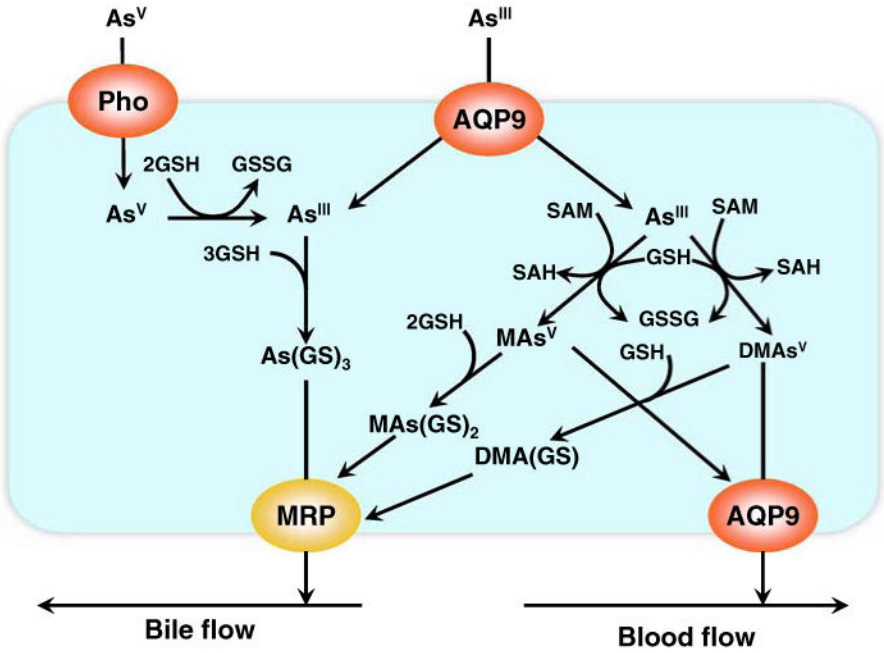

Fig. 6.

Hypothetical pathway of arsenical transport and cellular metabolism in heptocytes. As^{III} enters the hepatocyte down its concentration gradient via hAQP9, and it can also be generated by reduction of As^{V}, which enters through phosphate transporters. Methylation of As^{III} produces MAs^{V} and $DMAs^{V}$, as well as other species not shown. Both inorganic and organic trivalent species can be glutathionated, and are pumped into the bile by MRP2 (multiple-drug resistant protein 2) (Kala et al., 2000). MAs^{V} and $DMAs^{V}$ flow into the blood stream down their concentration gradients via AQP9. GSH, glutathione; SAM, s-adenosylmethionine; SAH, s-adenosylhomocysteine; GSSG, glutathione-disulfide