TOXICOLOGICAL HIGHLIGHT

Arsenolysis and Thiol-Dependent Arsenate Reduction

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Conversion of arsenate to arsenite is a critical event in the pathway that leads from inorganic arsenic to a variety of methylated metabolites. The formation of methylated metabolites influences distribution and retention of arsenic and affects the reactivity and toxicity of these intermediates. Indeed, some of the toxic and carcinogenic effects associated with exposure to arsenate or arsenite are probably mediated by methylated arsenicals. Recent work has demonstrated a biologically plausible role for phosphorolytic-arsenolytic enzymes in a reaction scheme in which an “activated” arsenate ester is readily reduced by thiols to arsenite. Thiol-dependent reduction of arsenate esters formed by arsenolysis may be one of several functionally reductant processes that control the flux of arsenic into the cellular pathway for arsenic methylation. Integrating these reductive processes into a conceptual model for arsenic metabolism may provide new insights into the cellular machinery for handling this toxic metalloid.

Key Words: arsenate; arsenolysis; thiols; glutathione; arsenate esters.

PRÉCIS

The paper by Gregus et al. (2009) in this issue of Toxicological Sciences (Németi et al., 2010) culminates a series of studies showing that enzymatically catalyzed arsenolysis promotes thiol-dependent reduction of arsenate to arsenite. This group had earlier shown that phosphorolytic-arsenolytic enzymes facilitate reduction of arsenate to arsenite by catalyzing formation of a ribose-1-arsenate complex. In the presence of the monothiol glutathione (GSH) or other thiols, arsenate in this unstable species is quickly reduced to arsenite (see, Gregus et al., 2009).

Arsenolysis is a biochemical phenomenon with a distinguished lineage that reflects the chemical similarities of arsenate and phosphate. Early in the 20th century, biochemists elucidating the role of phosphorous in cellular energetics found that arsenate could disrupt phosphate metabolism. By midcentury, it was clear that arsenate could substitute for phosphate in many reactions, including formation of arsenate esters, which were far less stable than corresponding phosphate esters. For example, replacement of phosphate with arsenate in reactions catalyzed by bacterial sucrose phosphorylase converted sucrose to glucose not glucose-1-phosphate (Doudoroff et al., 1947). These investigators postulated that glucose-1-arsenate formed in this reaction was unstable; the rapid decomposition of this arsenate ester was termed arsenolysis. Subsequent studies of oxidative phosphorylation in partially purified rat liver mitochondria showed that arsenate reduced the rate of ATP generation by arsenolysis of an unstable ADP-arsenate complex and coincidentally provided the first indirect evidence that arsenate was reduced to arsenite in a mitochondrial-enriched assay system (Crane and Lipmann, 1953).

In the new paper, the investigators first examined the reactions catalyzed by recombinant Escherichia coli purine nucleoside phosphorylase (E.C. 2.4.2.1, PNPass). In the presence of polyA, arsenate and GSH, PNPass catalyzed a reaction in which arsenite was a final reaction product. Although formation of AMP-arsenate was unaffected by the presence of GSH, arsenite production depended on the presence of a mono- or dithiol. Experiments involving sequential addition of arsenate and GSH to reaction mixtures indicated that formation of AMP-arsenate facilitated thiol-dependent reduction of arsenate to arsenite.

These studies of the role of arsenolysis in the reduction of arsenate were extended to examine the reduction of arsenate in mitochondria. Németi and Gregus (2002) had reported that isolated rat liver mitochondria efficiently reduce arsenate to

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arsenite and extrude arsenite. In a series of studies that link back to the work of Crane and Lipmann (1953), they examined the role of ATP synthase activity of mitochondria in reduction of arsenate in this organelle. Because ATP synthase activity depends on the structural integrity of mitochondria, studies were performed in in vitro systems containing isolated rat liver mitochondria. Hence, these studies were not wholly amenable to the tools used by an enzymologist to study catalysis by a purified enzyme, and some conclusions must be qualified by the uncertainties surrounding results obtained in complex systems. Given these caveats, results reported in this paper are consistent with a prominent role for mitochondrial ATP synthase in reduction of arsenate. In particular, depletion of intra-mitochondrial GSH markedly reduced production of arsenite, suggesting that formation of ADP-arsenate and thiol-dependent reduction of arsenate to arsenite probably occurred in the organelle. As noted by the authors, other phosphorolytic-arsenolytic enzymes in mitochondria might also contribute to the organelle’s capacity to reduce arsenate, although their contribution to reductive capacity is likely to be small relative to the role of ATP synthase.

These findings merit consideration from several perspectives. First, what is the importance of arsenate reduction in the metabolism of arsenic? Second, how is thiol-dependent arsenolytic reduction of arsenate related to other pathways for reduction of arsenate? Third, how might this new information be incorporated into a conceptual model for arsenic metabolism?

WHY REDUCE ARSENATE?

The reduction of arsenate to arsenite is intimately linked to distribution, metabolism, and fate of arsenic in organisms. Since the late 19th century, it has been clear that ingested inorganic arsenic undergoes extensive biotransformation. In microorganisms, biotransformation ultimately leads to formation of volatile trimethylarsine (Cullen, 2008). In humans and many other mammals, biotransformation of inorganic arsenic results in the presence of inorganic, mono-, and dimethylated arsenicals in urine. Based on the study of arsenic metabolism in microorganisms, Challenger (1951) devised a reaction scheme:

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\begin{align*}
\text{As}^{V}O_4^{3-} & \rightarrow 2\text{As}^{III}O_3^{3-} + \text{CH}_3 \rightarrow \text{CH}_3\text{As}^{V}O_2^{-} \\
2e & \rightarrow \text{CH}_3\text{As}^{III}O_2^{-} + \text{CH}_3 \rightarrow (\text{CH}_3)_2\text{As}^{V}O^{-} \\
2e & \rightarrow (\text{CH}_3)_2\text{As}^{III}O^{-} + \text{CH}_3 \rightarrow (\text{CH}_3)_3\text{As}^{V}O \\
2e & \rightarrow (\text{CH}_3)_3\text{As}^{III}.
\end{align*}
\]

In the Challenger (1951) scheme, arsenic occurs in two oxidation states, trivalent (AsIII) and pentavalent (AsV). The initial step in the pathway is reduction of arsenate to arsenite. Thereafter, the pathway consists of alternating steps in which AsIII-containing species are converted to methylated AsV-containing products. Arsenic in methylated products is reduced from AsV to AsIII before the next round of methylation. Identifying the molecular machinery for arsenic methylation provides insights into AsV reduction. For example, recombinant rat arsenic (+3 oxidation state) methyltransferase (As3mt) catalyzes each step in a pathway starting with arsenite and ending with formation of a volatile trimethylated arsenical species (Thomas et al., 2007). Based on the Challenger (1951) scheme, As3mt is postulated to catalyze reactions in which AsIII-containing species are oxidatively methylated and reactions in which AsV in methylated intermediates is reduced. Thioredoxin (Tx) or glutaredoxin (Gx), dithiol reductants that support activity of prokaryotic arsenate reductases (Messens and Silver, 2006), also support As3mt catalysis (Thomas et al., 2007). Coupling of oxidative methylation and arsenate reduction in arsenic metabolism has a striking effect on the distribution and clearance of this metalloid. In arsenite-treated As3mt knockout mice, absence of the capacity to methylate and reduce arsenic profoundly alters arsenic distribution and its clearance (Drobna et al., 2009).

FUNCTIONAL REDUNDANCY OF ARSENATE REDUCTION?

In addition to phosphorolytic-arsenolytic enzymes that catalyze thiol-dependent arsenate reduction, other cellular processes convert arsenate to arsenite. Figure 1 shows a pathway in which GSH reduces arsenate to arsenite and forms an AsIII(GS)3 complex (Delnomdedieu et al., 1994). Three families of prokaryotic arsenate reductases with distinct
catalytic features have been identified (Silver and Phung, 2005). Within each family, arsenate reductases share common active site amino acid sequences and a requirement for a specific reductant, such as Tx or Gx. Several eukaryotic arsenate reductases have active site amino acid sequences similar to those found in protein tyrosine phosphatases (PTPases) (Bhattacharjee et al., 2010). For example, with Gx as reductant, the catalytic domain of human Cdc25B PTPase isoforms functions as arsenate reductase (Fig. 1).

Multiplicity of pathways for arsenate reduction may be an example of functional redundancy in which a cell possesses several independent processes that mediate a single biochemical reaction (Wang and Zhang, 2009). Functional redundancy in capacity to reduce arsenate assures that arsenate that enters the cell will quickly be converted to arsenite, the favored substrate for As3mt. Facilitation of entry into the methylation pathway assures formation of metabolites that are quickly distributed and excreted. Each pathway for arsenate reduction requires the presence of a mono- or dithiol reductant and the cellular machinery needed to produce these reductants and to regenerate reactive thiol(s). Hence, arsenate reduction is linked to the capacity of cells to maintain appropriate redox status. In the case of Cdc25 PTPases and phosphorolytic-arsenolytic enzymes that function as arsenate reductases, it is unclear whether this activity should be considered adventitious or inherent. That is, is an enzyme’s arsenate reductase activity a consequence of the chemical similarity of arsenate to phosphate, its usual substrate, or has its function as an arsenate reductase evolved under selective pressure for a process that facilitates the removal of arsenic from cells?

**LOOKING FORWARD**

A conceptual model for the cellular metabolism of arsenic attempts to integrate information on critical determinants of arsenic accumulation and transform into a biologically plausible framework (Thomas, 2007). As our understanding of the steps in arsenic metabolism has increased, it has been possible to construct models with greater degrees of detail. For example, with our greater understanding of the role of reduction of AsV to AsIII, it is possible to identify reduction as a critical step in the metabolic process and to link this metabolic step to influx and efflux processes, to the methylation pathway, and to the binding of AsIII-containing species to intracellular targets (Fig. 2). In the case of arsenate reduction, we can now identify functionally redundant reactions that are involved in conversion of arsenate to arsenite. This allows the black box of intracellular arsenate reduction to be opened and its contents to be examined and manipulated. This approach allows the model to be refined, incorporating kinetic constants for individual reactions involved in arsenate reduction as well as relevant data on cellular distribution and concentrations of reactants (e.g., thiols) and enzymes (e.g., ATP synthase). Further study of arsenate reduction and other molecular phenomena involved in arsenic metabolism will change the model from strictly descriptive to a tool for quantitative analysis.

**REFERENCES**


