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An Overview of Arsenic Metabolism and Toxicity

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Abstract

It is likely that at least some of the toxic and carcinogenic effects associated with exposure to inorganic arsenic are, in fact, due to actions of its methylated metabolites. Here, we provide an overview of current models for the biological methylation of arsenicals. This information provides a context for understanding the chemical, biochemical, and genetic approaches to elucidation of the formation and function of methylated arsenicals which are presented in the following manuscripts.

Introduction

The toxic properties of arsenic (As) were recognized long before Albertus Magnus in the 13th century prepared its elemental form (Buchanan, 1962). Its use as a poison has played lethal and decisive roles in domestic and dynastic intrigues throughout history (Cullen, 2008). Inorganic As (iAs) remained a mainstay of the poisoner's art until methods for its detection were developed in the 19th century (Jolliffe, 1993). The potency of As as a carcinogen in humans has also been apparent since the beginning of its use in industrial processes. Studies in workers exposed to iAs as arsenite (iAs^{III}) or arsenate (iAs^V) have found increased prevalences of various internal cancers (Enterline et al., 1995; Lubin et al., 2000). In addition, arsenicals have a long history of medicinal use. Fowler's solution (an alcoholic solution of potassium arsenite) was widely used for several centuries as a tonic for dermatological, hematological, and respiratory ailments. Synthesis of Salvarsan (arsenphenamine, compound 606) by Paul Ehrlich and its use as a treatment for syphilis marked the beginning of modern chemotherapeutics (Schwarz, 2004). Organoarsenicals

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were widely used as antibiotics until the 1940s when they were displaced by sulfa drugs or penicillin and other microbial antibiotics. In recent years, iAs as arsenic trioxide has returned to the therapeutic armamentarium as a treatment for acute promyelocytic leukemia (Douer and Tallman, 2005).

In the past half century, iAs has emerged as a significant environmental contaminant. Exposure to iAs as a contaminant of drinking water was first recognized as a major public health problem in Taiwan. In certain regions of that island, individuals who chronically consumed drinking water containing iAs displayed a range of skin and vascular lesions that were attributed to As exposure (Tseng et al., 1968; Tseng, 1977). Studies in Taiwan and elsewhere have demonstrated that chronic exposure is associated with increased risk of vascular disease, diabetes, and internal cancers (Smith et al., 1992; Navas-Acien et al., 2005, 2008; Chen et al., 2007; Wang et al., 2007). Through the years, other populations elsewhere in the world have also been identified as chronic users of drinking water supplies that are contaminated with iAs. There is abundant evidence of adverse health effects in all of these populations. Indeed, the plight of tens of millions of individuals exposed to iAs in Bangladesh and West Bengal has been described as a “public health emergency” (Smith et al., 2000) and as “the largest poisoning of a population in history” (Bhattacharjee, 2007), assessments that are difficult to discount given the number of affected individuals.

Given the strong epidemiological evidence of myriad adverse health effects that are attributable to chronic exposure to iAs, there has been a growing interest in understanding of the distribution and metabolism of this metalloid in humans. Coupled with a renewed interest in potential modes of actions of arsenicals as toxicants or carcinogens, this has led to a rapid expansion of our knowledge in these fields. In this chapter, attention is devoted to the study of metabolism of As in *in vitro* assays and in cultured cells. In particular, the enzymatic basis of As methylation is considered. In the following paragraphs, we provide a brief summary of current models for the biological methylation of arsenicals.

The nomenclature for arsenicals used here follows common usage in the toxicological literature. Because the oxidation state of arsenic in various compounds is of interest, this distinction is indicated in the naming of compounds. Systematic names and abbreviations used for arsenicals: arsenite (arsenous acid) $\text{As}^{\text{III}}(\text{OH})_3$, iAs^{III} ; arsenate (arsenic acid) $\text{As}^{\text{V}}(\text{O})(\text{OH})_3$, iAs^{V} ; (mono)methylarsonous acid $\text{CH}_3\text{As}^{\text{III}}(\text{OH})_2$, MAs^{III} ; (mono)methylarsonic acid $\text{CH}_3\text{As}^{\text{V}}(\text{O})(\text{OH})_2$, MAs^{V} ; dimethylarsinous acid $(\text{CH}_3)_2\text{As}^{\text{III}}\text{OH}$, DMAs^{III} ; dimethylarsinic acid $(\text{CH}_3)_2\text{As}^{\text{V}}(\text{O})\text{OH}$, DMAs^{V} ; trimethylarsine oxide, $(\text{CH}_3)_3\text{As}^{\text{V}}(\text{O})$, TMAO. In cases where the oxidation state of arsenic cannot be determined or is not germane, the generic abbreviations iAs, MAs, and DMAs are used.

General Aspects of Arsenic Methylation

Elucidation of the pathway for As methylation has its roots in studies in the 19th century that demonstrated that microorganisms could convert iAs to a volatile methylated product (Gosio gas, trimethylarsine). Studies of metabolism of As in microbes culminated in two comprehensive reviews of biological methylation by Frederick Challenger (Challenger, 1945, 1951) in which a pathway with steps leading from iAs to methylated products was

proposed (Figure 1a). In this scheme, As in the pentavalent oxidation state is reduced to trivalency and the resulting trivalent arsenical then undergoes oxidative methylation. This model has proven sufficient to explain much of the available data on the formation of methylated arsenicals in biological systems. In subsequent years, much effort has been expended understanding the mechanistic basis for reduction of pentavalent As and for oxidative methylation of trivalent As (see Cullen et al., 1984a, b). An alternative model for As methylation (Figure 1b) has been proposed (Hayakawa et al., 2005, Naramandura et al., 2006). Here, As bound to a cellular thiol is the substrate for methylation. This thiol group could be a component of a cellular protein or the reactive moiety in glutathione (GSH), the most abundant low molecular weight thiol compound in cells. In this model, methylation of trivalent As is not oxidative; formation of pentavalent arsenicals is attributed to oxidation of As after hydrolysis of As-thiol complexes. Although this alternative model is consistent with many experimental details of the methylation process, there are data suggesting that the presence of thiol species is not an absolute requirement for biological methylation of As (see Thomas et al., 2007).

Recognition of the importance of methylation in the metabolism and toxicity of As in humans has its roots in the report by Crecelius (1977) that humans convert ingested iAs^V into methylated (MAs) and dimethylated arsenicals (DMAs) that are excreted in urine. In the past three decades, data have accumulated showing that the methylation of iAs is a nearly universal phenomenon in vertebrates. In most species examined, DMAs is the predominant urinary metabolite after exposure to iAs , accounting for 60 to 80% of the As in urine. Both iAs and MAs each typically account for 5 to 20% of the As in urine after exposure to iAs .

Linkage of Arsenic Methylation and Toxicity

Because methylated arsenicals containing pentavalent As (MAs^V and $DMAs^V$) were less acutely cytotoxic than iAs^{III} , it was initially assumed that biological methylation was a process that detoxified As as a prelude to its excretion (Yamauchi and Fowler, 1994). However, early work showed $DMAs^V$, a methylated metabolite of iAs , to be a teratogen, a nephrotoxin, a tumor promoter, and complete carcinogen in the rat (Rogers et al., 1981; Murai et al., 1993; Yamamoto et al., 1995; Wei et al., 1999). These findings raised the possibility that formation of methylated arsenicals produced compounds with unique and potentially higher toxicity. Subsequent studies identified methylated arsenicals containing trivalent As, MAs^{III} and $DMAs^{III}$, to be urinary metabolites after exposure to iAs (Aposhian et al., 2000; Le et al., 2000; Del Razo et al., 2001) and that these compounds were more potent cytotoxins, genotoxins, and enzyme inhibitors than was iAs^{III} (for a review see Thomas et al., 2001). Taken together, these findings argued that the methylation of iAs should be regarded as an activation process that forms more reactive species which exert unique toxic effects. Therefore, understanding the molecular basis of As methylation may be the key to understanding its actions as a toxicant and a carcinogen.

Enzymatic Basis of Arsenic Methylation in the Context of Cellular Metabolism

Based on evidence that iAs is extensively methylated in humans and many other species, understanding the biological basis of As methylation became a prime research area. As described in the following sections of this chapter, it has been possible to demonstrate that methylation of As in many species is an enzymatically catalyzed process that is consistent with Challenger's original scheme for this process. A single protein, arsenic (+3 oxidation state) methyltransferase (AS3MT), catalyzes both reduction and methylation reactions for As shown in Figure 2. As is typical for methyltransferases, AdoMet is the methyl donor for these reactions. Hence, the activity of this As methylating enzyme can be linked to a multiple-step process in cells that controls the availability of this prime methyl group donor. Furthermore, the activity of AS3MT is linked with that of thioredoxin reductase (TR) which catalyzes reduction of thioredoxin (Tx), a small dithiol containing protein that provides reducing equivalents to AS3MT (Waters et al., 2004a). This linkage is strengthened because MA^{III} , an intermediate in the AS3MT-catalyzed pathway for As methylation, is a potent inhibitor of the activity of thioredoxin reductase (Lin et al., 1999, 2001). Integrating these different metabolic processes into a network of interconnected reactions will provide a more comprehensive understanding of the link between the metabolism of As in the cell and the effect of arsenicals on the molecular processes that underlie its actions as a toxin or carcinogen.

The nomenclature for genes encoding arsenic (+3 oxidation state) methyltransferase follows recommendations of the Human Genome Organization Gene Nomenclature Committee (HGNC) for the naming of genes and for the use of symbols to identify genes and proteins in the genome of humans and other species (Wright and Bruford, 2006). Symbols for genes are always italicized. Hence, the symbol for the gene encoding this protein in humans is AS3MT; in all other species, it is As3mt. Symbols for protein products of genes are not italicized. Therefore, the protein product of the human gene is AS3MT; for all other species, it is As3mt.

Identifying the Products of Arsenic Metabolism

A continuing challenge in research on metabolism of As has been development and application of analytical techniques for determination of arsenicals in biological samples. As understanding of the complexities of As metabolism has evolved, researchers have responded to the challenges of identifying the full range of metabolites and accounting quantitatively for As as the administered compound or as a metabolite by adapting and refining methods. For example, from old spot tests for the detection of As (e.g., Marsh's or Gutzeit's test) to current analytical techniques (e.g., hydride generation-cryotrapping-atomic absorption spectrometry), researchers have commonly exploited the formation of volatile arsines from arsenicals as a means to separate and quantify arsenicals. Indeed, there has been a continuing dialogue between analytical chemists and biologists in which results of studies of As metabolism spur development and application of new methods to determine whether previously undetected metabolites are present. For example, recognition that recombinant rat As3mt could catalyze formation of trimethylarsine oxide (TMAO) as the

penultimate metabolite of iAs^{III} was spurred by the observation that the sum of the concentrations of MAs and DMAs in reaction mixtures did not quantitatively account for As added to reaction mixtures (Waters et al., 2004b). Application of new methods for collection and analysis permitted identification and quantitation of these metabolites. New pathways for As metabolism are presently under investigation and stimulate the development of new analytical approaches. The conversion of oxoarsenical species to thioarsenicals (for example, conversion of $DMAs^V$ to dimethylthioarsenic) occurs via a reaction with hydrogen sulfide generated in tissues (Naranmandura et al., 2007). Dimethylthioarsenic has been detected in urine of individuals chronically exposed to iAs in drinking water (Raml et al., 2007) and in urine of rats exposed to iAs^V or $DMAs^V$ in drinking water (Adair et al., 2007). Mass spectrometric-based methods for quantitation of members of this class of metabolites are under development in many laboratories. These analytical methods will greatly assist in elucidating the pathway for formation of thioarsenicals and in understanding the linkage between the pathway for formation of methylated oxoarsenicals and the pathway for formation of thioarsenicals.

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Figure 1.1.

Two proposed pathways for the conversion of inorganic arsenic into mono-, di-, and trimethylated products. a) The Challenger scheme with alternating steps of oxidative methylation and reduction. b) An alternative pathway in which trivalent arsenic bound to glutathione (GSH) or another cellular thiol is methylated without a change in the oxidation state of arsenic. Abbreviations; iAs^{III}, arsenite; MAs^V, methylarsonic acid; MAs^{III}, methylarsonous acid; DMAs^V, dimethylarsinic acid; DMAs^{III}, dimethylarsinous acid; TMA^V, Trimethylarsine oxide; AdoMet, S-adenosylmethionine; AdoHcy, S-adenosylhomocysteine; GSH, glutathione.

Figure 1.2.

Interconnections between the cycle for arsenic methylation catalyzed by arsenic (+3 oxidation state) methyltransferase, the enzymatic cycle for regeneration of S-adenosylmethionine (AdoMet) from S-adenosylhomocysteine (AdoHcy) and the cycle for regeneration of reduced thioredoxin (Tx_{red}) from oxidized thioredoxin (Tx_{ox}) in a NADPH-consuming reaction catalyzed by thioredoxin reductase (TR).