Differential Binding of Monomethylarsonous Acid Compared to Arsenite and Arsenic Trioxide with Zinc Finger Peptides and Proteins

Xixi Zhou,‡ Xi Sun,‡ Charlotte Mobarak,‡ A. Jay Gandolfi,§ Scott W. Burchiel,† Laurie G. Hudson,*‡ and Ke Jian Liu,*‡

‡Department of Pharmaceutical Sciences, College of Pharmacy, University of New Mexico Health Sciences Center, Albuquerque, New Mexico 87131, United States
§Department of Pharmacology & Toxicology, College of Pharmacy, University of Arizona, Tucson, Arizona 85004, United States

ABSTRACT: Arsenic is an environmental toxin that enhances the carcinogenic effect of DNA-damaging agents, such as ultraviolet radiation and benzo[a]pyrene. Interaction with zinc finger proteins has been shown to be an important molecular mechanism for arsenic toxicity and cocarcinogenesis. Arsenicals such as arsenite, arsenic trioxide (ATO), and monomethylarsonous acid (MMA(III)) have been reported to interact with cysteine residues of zinc finger domains, but little is known about potential differences in their selectivity of interaction. Herein we analyzed the interaction of arsenite, MMA(III), and ATO with C2H2, C3H1, and C4 configurations of zinc fingers using UV−vis, cobalt, fluorescence, and mass spectrometry. We observed that arsenite and ATO both selectively bound to C3H1 and C4 zinc fingers, while MMA(III) interacted with all three configurations of zinc finger peptides. Structurally and functionally, arsenite and ATO caused conformational changes and zinc loss on C3H1 and C4 zinc finger peptide and protein, respectively, whereas MMA(III) changed conformation and displaced zinc on all three types of zinc fingers. The differential selectivity was also demonstrated in zinc finger proteins isolated from cells treated with these arsenicals. Our results show that trivalent inorganic arsenic compounds, arsenite and ATO, have the same selectivity and behavior when interacting with zinc finger proteins, while methylation removes the selectivity. These findings provide insights on the molecular mechanisms underlying the differential effects of inorganic versus methylated arsenicals, as well as the role of in vivo arsenic methylation in arsenic toxicity and carcinogenesis.

INTRODUCTION

Arsenic is a significant public health concern due to its toxicity and carcinogenesis. Chronic arsenic exposure is related to many adverse health effects,1 such as increased risk of cancers of the skin, lung, and urinary tract.1−3 More than 140 million people worldwide are believed to be exposed to arsenic levels above the World Health Organization maximum contaminant level of 10 ppb.6 Arsenic exists in inorganic and organic forms. Environmental routes of exposure to arsenic include ingestion and inhalation of inorganic arsenic. Arsenite is an inorganic trivalent arsenic compound widely present in water, soil, and food.1 In contrast, arsenic trioxide (ATO, As2O3), another trivalent arsenic compound, is the most common inorganic arsenical in airborne dust.1 Inhalation of ATO has been shown to alter immune function.7 Organic arsenicals mainly consist of mono- and dimethylated arsenic metabolites, derived from biomethylation of inorganic arsenicals in cellular environment.8−11 A trivalent monomethylated arsenic metabolite, monomethylarsonous acid (MMA(III)), has been shown to display greater toxicity and/or carcinogenic potential than inorganic arsenite.12−14

Interaction with zinc finger proteins is considered to be an important mechanism of arsenic toxicity and carcinogenesis. Substitution of zinc with another metal, such as arsenic, is believed to disrupt the coordination sphere in the zinc finger environment and consequently the zinc finger function.15 Furthermore, both inorganic and organic trivalent arsenic compounds interact with zinc finger proteins. Zinc finger proteins, poly (ADP-ribose) polymerase 1 (PARP-1), and xeroderma pigmentosum group A (XPA) are both involved in DNA repair and have been validated as direct molecular targets for arsenite and MMA(III).1−5,16−18 ATO is also known to interact with cysteine-rich zinc finger proteins.6,19 We have investigated extensively the interaction of arsenite with zinc finger proteins in recent years. Our findings demonstrate that arsenite can replace zinc in the zinc finger moiety, leading to changes of structure and loss of protein function.20 In addition, we found that arsenite selectively interacts with zinc finger motifs with C3H1 or C4 configurations by coordinating with three cysteine residues.1,21 This suggests that subsets of zinc finger proteins are more sensitive molecular targets of arsenite than others. Since a methyl group already occupies one of the three covalent bonds in MMA(III), it is likely that MMA(III)
will not be able to bind with three cysteine residues as arsenite does. Therefore, we hypothesize that binding selectivity of MMA(III) will be different from that of arsenite.

In this study, we tested the differential binding selectivity hypothesis by investigating interactions of arsenite, ATO, and MMA(III) with three different configurations of zinc finger peptides and proteins: C2H2 (apraxin, APTX), C3H1 (PARP-1), and C4 (XPA). A variety of analytical approaches were utilized to determine whether the three arslenicals display differential binding selectivity toward these zinc finger configurations, and potential consequences of these interactions in terms of structural or functional changes. Our results demonstrate that the binding selectivity indeed differs among the methylated versus nonmethylated arslenicals, which provides insightful understanding for the molecular mechanisms underlying the differential effects of inorganic versus organic arslenicals in arsenic toxicity and carcinogenesis.

**EXPERIMENTAL PROCEDURES**

**Chemicals.** Peptides derived from the finger motifs of APTX, XPA, and the first zinc finger motif of PARP-1 (sequences in Table 1) were commercially synthesized by Genemed Synthesis Inc. (San Antonio, TX). Purity confirmed by HPLC was greater than 95%. Diiodo-ethan (MMIII iodide, CD2I2>98% pure) was prepared by the Synthetic Chemistry Facility Core (Southwest Environmental Health Sciences Center, Tucson, AZ) and kindly provided by Dr. A. Jay Gandolfi, University of Arizona. As2O3 (ATO, >99.95%) was obtained from Mallinckrodt Chemical Works (St. Louis, MO). Cobalt chloride, zinc chloride, and sodium arsenite were obtained from Fluka Chemie. All other chemicals were obtained from Sigma-Aldrich.

**Cobalt Spectrometry Analysis of Free Metal Binding Sites on Zinc Finger Peptides.** Lyophilized zinc finger peptides were incubated at 1 mM in 20 mM Tris (pH 7.8) containing 0.1 mM Tris(2-carboxyethyl)phosphine (TCEP) to protect the cysteine residues from oxidation prior to incubations. Solutions of arslenic compounds were freshly prepared containing 0.1 mM TCEP to protect the cysteine residues from oxidation. 

**UV–Vis Spectrometry Analysis of As–S Bond Formation on Zinc Finger Peptides.** Aliquots of 100 µM zinc finger peptides were incubated with 100 µM arsenic compounds for 30 min at 25 °C. The samples were then diluted 50 times in 5 mg/mL α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich) in a 1:1 (v/v) water/acetonitrile solution, and 1 µL of each sample was deposited in duplicate on the MALDI plate, allowed to dry at 37 °C, and MALDI-TOF-MS analyses performed on an Applied Biosystems 4700 Proteomics Analyzer (TOF/TOF) operating in MS reflector positive ion mode. The total acceleration voltage was 20 kV. Desorption was performed using a neodymium/yttrium–aluminum–garnet laser (355 nm, 3 ns pulse width, and 200 Hz repetition rate). Mass spectra were acquired with laser pulses over a mass range of m/z from 1000 to 5000 Da using focus mass of 3500. Final mass spectra were the summation of 10 subectra, each acquired with 200 laser pulses.

**Intrinsic Fluorescence Analysis of Arsenical Binding to Zinc Fingers.** Aliquots of 100 µM zinc finger peptides were incubated with different concentrations of arslenic compounds or 100 µM zinc chloride for 30 min at 25 °C. After that, the emission fluorescence spectra from 300 to 400 nm were collected at 25 °C on a SpectraMax M2 fluorescence spectrophotometer (Molecular Devices, LLC, Sunnyvale, CA). The excitation wavelength was 280 nm. The intensity of fluorescence is related to the chemical environments of phenylalanine, tyrosine, and tryptophan. The intrinsic fluorescence intensity of zinc finger peptides undergoes a dramatic change on folding/unfolding. This allows for the tertiary structure change of zinc finger peptides to be monitored by fluorescence spectroscopy. Fluorescent intensity at 350 nm was used to represent the status of the tertiary structure of zinc finger peptides with different treatments.

**Cell Culture and Zinc Finger Protein Isolation by Immunoprecipitation.** The human keratinocyte cell line (HaCaT) was a kind gift from Dr. Mitch Denning (Loyola University Medical Center, Maywood, IL). Cells were maintained as described previously. After exposure to 2 µM arsenic compounds for 24 h, cells were harvested in RIPA cell lysis buffer (25 mM Tris·HCl at pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS), sonicated, and centrifuged at 14,000 rpm for 15 min at 4 °C to remove cellular debris. Protein (500 µg in 500 µL) was incubated with 5 µL of rabbit polyclonal antibody (APTX, Abcam #31841; PARP-1, Cell Signaling #9542 or XPA, Abcam ab85914) for at least 2 h at 4 °C. Protein A beads (Invitrogen) were added in a 1:1 slurry, and samples were incubated for an additional 2 h at 4 °C. The beads were recovered by centrifugation at 10,000 rpm for 5 min at 4 °C and washed five times with 1 mL of lysis buffer. To elute protein, the pellets were incubated with 100 µL of 100 mM citric acid (pH 3.0) for 30 min, followed by centrifugation at 14,000 rpm for 5 min at 4 °C. The supernatant was adjusted to pH 7 with 10 M NaOH.

**Measurement of Zinc Content in Protein.** Proteins obtained from cells by immunoprecipitation were incubated with 10 mM H2O2 for at least 2 h at 4 °C to release zinc from proteins. Zinc content was measured by adding 10 µL of 1 mM 4-(2-pyridylazo)resorcinol to 100 µL of protein sample followed by scanning the UV–vis spectra at 350 to 550 nm on a SpectraMax M2 spectrophotometer (Molecular Devices, LLC, Sunnyvale, CA). The absorbance of resorcinol shifts from 411 to 493 nm in the presence of zinc, and the 493 nm peak is recorded and compared with a standard curve for calculation of zinc content in protein samples.

**RESULTS**

**Cobalt Spectrometry Analysis of the Differential Selectivity of Occupying Metal Binding Sites on Zinc Fingers.** Zinc binding within zinc finger motifs is critical for the maintenance of the tertiary structure and activity of zinc finger proteins. Occupation of metal binding sites by arsenic is an indicator of arsenic interaction with zinc fingers. To determine whether an arsenic compound is capable of occupying the metal binding sites and to investigate differences in metal binding site occupation by MMA(III), arsenite or ATO, we used cobalt as a probe to detect available metal
binding sites on zinc finger motifs after incubation with arsenic compounds. Cobalt binding with zinc finger motifs generates absorbance at 660 nm, which is used to quantitatively determine the remaining free metal binding sites after arsenic occupation. The zinc finger peptides derived from the zinc finger domains of C2H2 (APTX), C3H1 (PARP-1), and C4 (XPA) were incubated with arsenicals, and then the cobalt spectrum was analyzed. In PARP-1 and XPA peptides, arsenite incubation decreased subsequent cobalt binding in a concentration-dependent manner but not in APTX peptides (Figure 1A). This indicates that arsenite selectively occupies the metal binding site on C3H1 and C4 but not the C2H2 zinc fingers. This result is consistent with our published findings. In contrast, incubation of peptide with MMA(III) led to a concentration-dependent decrease in cobalt binding to all three peptides indicating that MMA(III) occupied the metal binding site on C3H1 and C4 but not the C2H2 zinc fingers. This result is consistent with our published data. MMA(III) induced a +88 m/z shift, showing that MMA(III) used 2 cysteine residues on the zinc fingers, as expected from our previous report. In contrast, in MMA(III) treated samples, A270 values increased in a concentration-dependent manner for PARP-1 and XPA zinc fingers but not for APTX (Figure 2A), showing that arsenite selectively forms an As–S bond with C3H1 and C4 zinc fingers, as expected from our previous report. In contrast, MMA(III) treated samples, A270 values increased for all three types of zinc fingers in a MMA(III) concentration-dependent manner (Figure 2B), indicating that MMA(III) could form an As–S bond with each zinc finger. ATO formed As–S bonds with PARP-1 and XPA zinc fingers but not APTX (Figure 2C), showing the same binding selectivity for C3H1 and C4 configurations as arsenite in terms of forming As–S bonds. These results indicate that interaction with Cys residues by forming As–S bonds is the molecular mechanism for zinc binding site occupation by arsenic. Among the three arsenicals, arsenite and ATO showed the same selectivity in forming As–S bonds with C3H1 and C4 zinc fingers, but MMA(III) could form As–S bonds with all three configurations of zinc finger peptides.

Mass Spectrometry Analysis of Arsenicals Interacting with C2, C3, and C4 Zinc Fingers. To further understand the differences in binding selectivity, MALDI-TOF mass spectrometry was utilized to analyze the precise molecular weights of the arsenic–zinc finger complex. Zinc finger peptides with different configurations (100 μM) were treated with 100 μM arsenic compounds. The mass spectra of apo-zinc finger peptide for APTX, PARP-1, and XPA are shown in Figures 3A, B, and C, respectively. Arsenite showed no binding to the APTX zinc finger (Figure 3D) but bound with PARP-1 and XPA zinc finger peptides, both giving +72 m/z shift against the apo-peptide signals (Figures 3E and F), indicating that arsenite coordinates with zinc fingers with the arsenic atom alone (m/z = 75), releasing three hydrogen atoms (m/z = −3) at the same time. This result is consistent with our previous published data. MMA(III) induced a +88 m/z shift to the APTX zinc finger peptide (Figure 3G). The interpretation of a +88 m/z shift is that MMA(III) bound to the APTX zinc finger peptide with As–CH3 (m/z: 75, As+12, C+3, 3H = 90), losing 2H (m/z: −2) from Cys residues on zinc finger peptides. MMA(III) also bound to the PARP-1 zinc finger (Figure 3H) with a +88 m/z shift, showing that MMA(III) used 2 cysteine residues.
residues for binding. For XPA, 1 molecule of MMA(III) bound to the XPA zinc finger, giving a +88 m/z shift to the aptopeptide. At the same time, we detected the signal of 2 molecules of MMA(III) bound to the same XPA zinc finger peptide (Figure 3I), giving a +88 m/z shift for each. Since the XPA zinc finger has 4 cysteine residues, when 1 molecule of MMA(III) bound to the zinc finger peptide, occupying 2 cysteine residues, there were still 2 free cysteine residues available for another molecule of MMA(III) coordination. This result further confirms that, unlike arsenite, MMA(III) only occupies 2 Cys during binding with zinc fingers. The mass spectra for ATO was the same as arsenite; it did not bind to the APTX zinc finger (Figure 3J), but bound with PARP-1 or XPA zinc fingers, giving a +72 m/z shift (Figure 3K and L). Therefore, the selectivity of ATO binding with zinc fingers was the same as arsenite. Furthermore, the +72 m/z shift indicates that ATO bound with zinc fingers in the same manner as arsenite, i.e., coordinating with 3 Cys residues. Together, the mass spectrometry results show that MMA(III) coordinated with 2 Cys but that arsenite and ATO both occupy 3 Cys when binding with zinc fingers.

Intrinsic Fluorescence Analysis of the Alteration of Tertiary Structure of Zinc Fingers. Next, we investigated whether arsenic binding could lead to structural changes of the zinc finger peptides. Zinc finger motifs of DNA repair proteins are frequently responsible for DNA recognition and DNA binding. Maintaining a correct tertiary structure is critically important for DNA binding and DNA repair capability. In order to investigate conformational changes due to arsenic binding to the zinc fingers, intrinsic fluorescence was used to analyze tertiary structure alteration on zinc finger peptides after treatments with arsenic compounds, as compared to zinc incubation. Intrinsic fluorescence is primarily generated from tryptophan and tyrosine residues (phenylalanine also contributes a small portion), representing the chemical environment of these amino acids. The intensity of fluorescence usually increases while peptides fold and side chains of Trp and Tyr are located in a relatively hydrophilic environment. We treated different configurations of zinc finger peptides (100 μM) for 30 min at room temperature with varying concentrations of arsenic compounds. After that, we collected the fluorescent spectra of each sample under the excitation wavelength of 280 nm and emission from 300 to 400 nm. Treatment with 100 μM zinc chloride was used as a control to show the natural folded conformation of the zinc finger peptides. Finally, treatment intensity at 350 nm was used to represent the tertiary structure change of zinc fingers. As shown in Figure 4A, the fluorescent signal of the APTX zinc finger could be decreased in a concentration-dependent manner only by MMA(III), but not arsenic or ATO, while zinc treatment generated the highest fluorescent signal (shown as a single data point in the top left corner of Figure 1A, B, and C). This result indicates that the APTX zinc finger forms a defined structure with zinc ions but that MMA(III) treatment could unfold the structure in a concentration-dependent manner. Arsinite or ATO showed no effect, which is consistent with the lack of binding based on the selectivity data (Figures 1, 2, and 3). For the PARP-1 zinc finger peptide, all 3 arsenic compounds decreased the fluorescent intensity in a concentration-dependent manner (Figure 4B). Results on the XPA zinc finger exhibited a trend similar (Figure 4C) to that of PARP-1 (Figure 4B). Together, these results indicate that MMA(III) alters the tertiary structure of all 3 conformations of zinc fingers, while arsenic and ATO selectively disrupted the tertiary structure of C3H1 and C4 zinc fingers. Furthermore, the findings demonstrate that the alteration in the tertiary structure of the zinc finger is a direct consequence of arsenic binding and that the selectivity of structural changes induced by arsenicals is consistent with the binding selectivity.

Selective Loss of Zinc from Zinc Finger Proteins in Cells Exposed to Arsenicals. Finally, in order to test whether the selectivity and behavior of MMA(III), arsenite, and ATO binding with zinc finger proteins are applicable in cells, zinc content in DNA repair proteins from cells treated with arsenicals was analyzed. We have reported that zinc loss from zinc finger proteins is a direct consequence of arsenic binding and a key event for protein function loss and arsenic toxicity in cells. Human keratinocyte (HaCat) cells were treated with 2 μM arsenite, ATO, or MMA(III) for 24 h. APTX, PARP-1, and XPA protein were immunoprecipitated from cell extracts using
corresponding antibodies, and the zinc content in each protein sample was determined. As shown in Figure 5, MMA(III) caused zinc loss from all three configurations of zinc finger proteins, while arsenite and ATO selectively displaced zinc from PARP-1 and XPA proteins isolated from cells. These results demonstrate that arsenite and ATO selectively interacted with C3H1 and C4 zinc fingers in the context of native protein but that MMA(III) interacted with all 3 configurations of zinc finger proteins in HaCat cells.

**DISCUSSION**

Targeted interaction with zinc finger domains is considered an important mechanism for arsenic toxicity and cocarcinogenesis. In the present study, by using cobalt spectrometry, we demonstrated that both inorganic and organic arsenicals interacted with zinc fingers by direct occupation of metal binding sites. UV−vis spectra demonstrated that all three arsenicals formed As−S bonds with Cys residues on zinc fingers, illustrating the importance of Cys residues for arsenic binding. Mass spectrometry analysis further confirmed the formation of As−S covalent bond. In addition, loss of hydrogen
change on the XPA zinc manner. (C) All three arsenic compounds could cause conformational change. Data were presented as the mean ± SD, *p < 0.05 vs corresponding [As] = 0 group, n = 3.

Figure 4. Conformational changes of zinc fingers induced by arsenic binding. Intrinsic fluorescence analysis was performed as described in the Experimental Procedures section. Intensities of fluorescence at 350 nm were used to represent the conformation/folding status of zinc finger peptides. The fluorescent intensities of 100 μM zinc treatment on zinc finger peptides are shown (top left corner) as controls. (A) Natural conformation of APTX could be altered by MMA(III) in a concentration-dependent manner, while arsenic and ATO showed no effect. (B) All three arsenic compounds could cause conformational change on the PARP-1 zinc finger in a concentration-dependent manner. (C) All three arsenic compounds could cause conformational change on the XPA zinc finger in a concentration-dependent manner. Data were presented as the mean ± SD, *p < 0.05 vs corresponding [As] = 0 group, n = 3.

For the two trivalent inorganic arsenicals, arsenite and ATO, their patterns of interaction with zinc fingers are the same. This conclusion is drawn from the cobalt spectra showing the occupation of metal binding sites, the UV-vis spectra showing the formation of As–S bonds, and the mass spectra showing the coordination with 3 Cys residues on zinc fingers. As shown by mass spectrometry, arsenite and ATO gave exactly the same +72 m/z shift to C3H1 or C4 zinc fingers, indicating that both arsenite and ATO bind to zinc fingers using the arsenic atom only and coordinate with 3 Cys on zinc finger motif, with the release of three hydrogens. This behavior may explain the selectivity in binding with C3H1 and C4 zinc fingers (as illustrated in Figure 6, top row). Kitchin and Wallace reported that arsenite bound C3H1 and C4 complexes are over 2 orders of magnitude more stable than a C2H2 complex (155 versus 1.29 min) in kinetic studies.28 Therefore, it is reasonable to suggest that trivalent arsenite or ATO may form two As–S bonds with C2H2 zinc fingers while leaving the third bond unoccupied but that the resulting product is too unstable to accumulate to high enough a concentration to be detected by our analytical approaches due to the presence of an unoccupied bond. This binding selectivity toward zinc fingers with 3 or more Cys could have potential biological significance. In terms of structural/functional consequences, arsenite and ATO led to selective conformational changes of C3H1 and C4 zinc fingers and induced zinc loss selectively in PARP-1 and XPA proteins in cells. There was no significant difference in the efficiency of arsenite and ATO in changing the structure of zinc fingers as well as zinc release from proteins. Circular dichroism (CD) and nuclear magnetic resonance (NMR) spectra indicate that arsenic binding to a C3H1 or C4 zinc finger motif result in an unfolded structure.29,30 These results, together with the findings here, provide evidence to the alteration of zinc finger structure by arsenic binding and further support that arsenic interaction with zinc finger proteins will likely disrupt protein function. Since C3H1 and C4 zinc finger proteins are a minority in the whole zinc finger protein family (less than 20%), inorganic arsenicals could target some C3H1 and C4 proteins but not the zinc fingers.

It has been reported that MMA(III) binds to PARP-1 and XPA zinc finger peptides.31,32 Here, we further demonstrated that MMA(III) could occupy metal binding sites on zinc fingers with Cys residues but not His residues on zinc fingers, which is different from the mechanism of zinc binding with zinc fingers. This may also be one of the reasons that arsenic binding changes conformation of zinc fingers, which we demonstrated by intrinsic fluorescent analysis. Collectively, from the data offered by these diverse techniques, we show that arsenite, MMA(III), and ATO occupy metal binding sites on zinc fingers by directly coordinating with Cys residues to form As–S bonds, leading to conformational change as well as zinc loss from the zinc fingers.
sites (Figure 1A) and form As–S bonds (Figure 2A) on all 3 configurations of zinc fingers, nonselectively. This nonselective interaction is also confirmed structurally and functionally using conformation and zinc content analysis (Figures 4 and 5). The molecular mechanism behind the nonselectivity is that MMA(III) covalently binds to two Cys residues, releasing two hydrogen, as shown in mass spectrometry (Figures 3G, H, and I). In contrast to arsenite and ATO binding with three Cys, MMA(III) only binds with two Cys on zinc fingers. This is demonstrated by the +88 m/z shift, as well as the binding of two MMA(III) molecules to the C4 zinc finger (Figure 3I). The +88 m/z shift and loss of 2H is consistent with previous findings by Wnek et al.\(^{31}\) In addition, a complex of two MMA(III) molecules with the C4 XPA zinc finger peptide has been detected by Piatek et al.\(^{32}\) In this work, we confirmed these findings using MALDI-MS and put these together to explain the mechanism of differential binding of MMA(III) to zinc finger peptides at the molecular level. Although arsenic in MMA(III) is still trivalent, one bond is already occupied by the methyl group, leaving the remaining two bonds for binding with Cys. The +88 m/z shift in mass spectrometry studies with C2H2, C3H1, and C4 zinc fingers confirm that when MMA(III) binds to zinc fingers, the methyl group is still bound to arsenic. In other words, the presence of the methyl group on MMA(III) changed the binding behavior and selectivity of this trivalent arsenical (as illustrated in Figure 6, bottom row), enabling it to bind with two Cys on zinc fingers. Importantly, unlike the situation with inorganic arsenite, the product derived from MMA(III) binding with two Cys is stable since there is no longer an unoccupied bond existing on the molecule. This result indicates that the methylation of arsenic could dramatically change the preference and profile of arsenic interaction with zinc finger proteins. It is possible that the greater breadth of zinc finger protein binding may lead to differences in toxicity and carcinogenic potential. Some recent studies showed that MMA(III) is more toxic than inorganic arsenic in terms of certain parameters of carcinogenesis, such as cell transformation.\(^{33,34}\) As for the possible molecular mechanism, Piatek et al. demonstrated that MMA(III) acts more effectively than arsenite in destroying the structure of C4 zinc fingers.\(^{32}\) Meanwhile, in studies of PARP activity, up to 90% inhibition is readily evident at submicromolar concentration of arsenite in human keratinocytes,\(^{16,21}\) but exposure to 1 μM MMA(III) caused about 30% PARP activity inhibition in urothelial cells.\(^{31}\) These findings might suggest that arsenite causes a greater magnitude of PARP inhibition than MMA(III) or that the findings may simply reflect cell type differences. Apparently, further research is needed to investigate the relationship between binding selectivity and toxicity/carcinogenesis, i.e., whether the change of selectivity enhances the effect of arsenic in vivo or simply dilutes arsenic interaction across the large family of zinc finger proteins.

In conclusion, this work demonstrates that arsenite and ATO have the same selective effect in binding with C3H1 and C4 zinc finger proteins, whereas MMA(III) interacts with all three configurations of zinc finger proteins. Methylation of trivalent inorganic arsenicals is responsible for the change in binding selectivity. These findings provide insightful understanding of the molecular mechanisms underlying the differential effects of inorganic versus methylated arsenicals, as well as the role of in vivo arsenic methylation in arsenic toxicity and carcinogenesis.

**AUTHOR INFORMATION**

**Corresponding Authors**
* (L.G.H.) College of Pharmacy, MSC09 5360, 1 University of New Mexico, Albuquerque, NM 87131-0001. Tel: 505-272-2482. Fax: 505-272-0704. E-mail: lhudson@salud.unm.edu.
* (K.J.L.) College of Pharmacy, MSC09 5360, 1 University of New Mexico, Albuquerque, NM 87131-0001. Tel: 505-272-9546. Fax: 505-272-0704. E-mail: kliu@salud.unm.edu.

**Funding**
This work was supported by grants from the U.S. National Institutes of Health (R01ES15826 and R01ES021100). Support was also provided by the UNM Cancer Center P30CA118100 through a postdoc matching grant 1127 and a pilot award 1118. The MMA(III) was prepared in the Synthetic Core of the Southwest Environmental Health Sciences Center (P30ES006694) and supplied by the University of Arizona Superfund Program (ES 04940).

**Notes**
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**
We thank Dr. Karen L. Cooper for valuable discussions.
ABBREVIATIONS

MMA(III), monomethylarsonous acid; ATO, arsenic trioxide; PARP-1, poly(ADP-ribose) polymerase-1; APTX, apurinic; XPA, Xeroderma pigmentosum group A; UV—vis spectroscopy, ultraviolet—visible spectroscopy; MALDI-TOF-MS, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

REFERENCES

(1) Agency for Toxic Substances Disease Registry (ATSDR) (2007) Toxicological Profile for Arsenic, U.S. Department of Health and Human Services, Public Health Service, Washington, DC.

(2) Schuhmacher-Wolz, U., Dieter, H. H., Klein, D., and Schneider, K. (2009) Oral exposure to inorganic arsenic: evaluation of its carcinogenic and non-carcinogenic effects. Crit. Rev. Toxicol. 39, 271–298.

(3) Rahman, M. (2006) Prevalence of arsenic exposure and skin lesions. A population based survey in Matlab, Bangladesh. J. Epidemiol. Community Health 60, 242–248.

(4) Schoen, A., Beck, B., Sharma, R., and Dubé, E. (2004) Arsenic toxicity at low doses: epidemiological and mode of action considerations. Toxicol. Appl. Pharmacol. 198, 253–267.

(5) Yoshida, T., Yamauchi, H., and Fan Sun, G. (2004) Chronic health effects in people exposed to arsenic via the drinking water: dose-response relationships in review. Toxicol. Appl. Pharmacol. 198, 243–252.

(6) World Health Organization (2006) Guidelines for Drinking-Water Quality, World Health Organization, Geneva, Switzerland.

(7) Burchiel, S. W., Mitchell, L. A., Lauer, F. T., Sun, X., McDonald, J. D., Hudson, L. G., and Liu, K. J. (2009) Immunotoxicity and biodistribution analysis of arsenic trioxide in C57Bl/6 mice following a 2-week inhalation exposure. Toxicol. Appl. Pharmacol. 241, 253–259.

(8) Aposhian, H. V., Zakharyan, R. A., Avram, M. D., Sampayo-Reyes, A., and Wollenberg, M. L. (2004) 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. 198, 327–335.

(9) Dopp, E., Kligerman, A. D., and Diaz-Bone, R. A. (2010) Organoselenarsenic. Uptake, metabolism, and toxicity. Met. Ions Life Sci. 7, 231–265.

(10) Drobná, Z., Walton, F. S., Harmon, A. W., Thomas, D. J., and Styblo, M. (2010) Interspecies differences in metabolism of arsenic by cultured primary hepatocytes. Toxicol. Appl. Pharmacol. 245, 47–56.

(11) Hirner, A. V., and Rettenmeier, A. W. (2000) Methylated metalloid species in humans. Met. Ions Life Sci. 7, 465–521.

(12) Styblo, M., Del Razo, L. M., Vega, L., Germolec, D. R., LeCluyse, E. L., Hamilton, G. A., Reed, W., Wang, C., Cullen, W. R., and Thomas, D. J. (2000) Comparative toxicity of trivalent and pentavalent inorganic and methylated arsenicals in rat and human cells. Arch. Toxicol. 74, 289–299.

(13) Kligerman, A. D., Doer, C. L., Tennant, A. H., Harrington-Brock, K., Allen, J. W., Winkfeld, E., Poorman-Alen, P., Kundu, B., funasaka, K., Roop, B. C., Mass, M. J., and DeMarini, D. M. (2003) Methylated trivalent arsenicals as candidate ultimate genotoxic forms of arsenic: induction of chromosomal mutations but not gene mutations. Environ. Mol. Mutagen. 42, 192–205.

(14) Eblin, K. E., Bredfeldt, T. G., and Gandolfi, A. J. (2008) Immortalized human urothelial cells as a model of arsenic-induced bladder cancer. Toxicology 248, 67–76.

(15) Quintal, S. M., dePaula, Q. A., and Farrell, N. P. (2011) Zinc finger proteins as templates for metal ion exchange and ligand reactivity. Chemical and biological consequences. Metallomics 3, 121–139.

(16) Ding, W., Liu, W., Cooper, K. L., Qin, X. J., de Souza Bergo, P. L., Hudson, L. G., and Liu, K. J. (2008) Inhibition of poly(ADP-ribose) polymerase-1 by Arsenite interferes with repair of oxidative DNA damage. J. Biol. Chem. 284, 6809–6817.

(17) Asmuss, M. (2000) Differential effects of toxic metal compounds on the activities of Pfg and XPA, two zinc finger proteins involved in DNA repair. Carcinogenesis 21, 2097–2104.

(18) Hartwig, A., Blessing, H., Schwedtke, T., and Walter, I. (2003) Modulation of DNA repair processes by arsenic and selenium compounds. Toxicology 193, 161–169.

(19) Zhang, X. W., Yan, X. J., Zhou, Z. R., Yang, F. F., Wu, Z. Y., Sun, H. B., Liang, W. X., Song, A. X., Lallemand-Breitenbach, V., Jeanne, M., Zhang, Q. Y., Yang, H. Y., Huang, Q. H., Zhou, G. B., Tong, J. H., Zhang, Y., Wu, J. H., Hu, H. Y., de, H., Chen, S. J., and Chen, Z. (2010) Arsenic trioxide controls the fate of the PML-RAR oncprotein by directly binding PML. Science 328, 240–243.

(20) Sun, X., Zhou, X., Du, L., Liu, W., Liu, Y., Hudson, L. G., and Liu, K. J. (2013) Arsenite binding-induced zinc loss from PARP-1 is equivalent to zinc deficiency in reducing PARP-1 activity, leading to inhibition of DNA repair. Toxicol. Appl. Pharmacol. 274, 313–318.

(21) Zhou, X., Sun, X., Cooper, K. L., Wang, F., Liu, K. J., and Hudson, L. G. (2011) Arsenite interacts selectively with zinc finger proteins containing C3H1 or C4 motifs. J. Biol. Chem. 286, 22855–22863.

(22) Payne, J. C., Rous, B. W., Tenderholz, A. L., and Godwin, H. A. (2003) Spectroscopic determination of the binding affinity of Zinc to the DNA-binding domains of nuclear hormone receptors. Biochemistry 42, 14214–14224.

(23) Kopper, E., Schwedtke, T., Hartwig, A., and Bal, W. (2004) Co(II) and Cd(II) substituting for Zn(II) in the zinc finger derived from the DNA repair protein XPA, demonstrating a variety of potential mechanisms of toxicity. Chem. Res. Toxicol. 17, 1452–1458.

(24) Spuches, A. M., Krszynska, H. G., Rich, A. M., and Wilcox, D. E. (2005) Thermodynamics of the As(III)—thiol interaction: Arsenite and Monomethylarsenite complexes with glutathione, dihydrolipoic acid, and other thiol ligands. Inorg. Chem. 44, 2964–2972.

(25) Asahina, H., Kuraoka, I., Shirakawa, M., Morita, E. H., Miura, N., Miyamoto, I., Ohtsuka, E., Okada, Y., and Tanaka, K. (1994) The XPA protein is a zinc metalloprotein with an ability to recognize various kinds of DNA damage. Mutat. Res. 315, 229–237.

(26) Ali, A. A. E., Timinszky, G., Arribas-Bosacoma, R., Kozlowski, M., Hassa, P. O., Hassler, M., Ladurner, A. G., Pearl, L. H., and Oliver, A. W. (2012) The zinc-finger domains of PARP1 cooperate to recognize DNA strand breaks. Nat. Struct. Mol. Biol. 19, 685–692.

(27) Fersht, A. (1999) Structure and Mechanism in Protein Science, W. H. Freeman & Company, New York.

(28) Kitchin, K. T., and Wallace, K. (2006) Dissociation of arsenite-peptide complexes: triphasic nature, rate constants, half-lives, and biological importance. J. Biochem. Mol. Toxicol. 20, 48–56.

(29) Demicheli, C., Frézard, F., Pereira, F. A., Santos, D. M., Mangrum, J. B., and Farrell, N. P. (2011) Interaction of arsenite with a zinc finger CCHC peptide: evidence for formation of an As-Zn-peptide mixed complex. J. Inorg. Biochem. 105, 1753–1758.

(30) Zhao, L., Chen, S., Jia, L., Shu, S., Zhu, P., and Liu, Y. (2012) Selectivity of arsenite interaction with zinc finger proteins. Metallomics 4, 988–994.

(31) Wnek, S. M., Kuhlman, C. L., Camarillo, J. M., Medeiros, M. K., Liu, K. J., Lau, S. S., and Gandolfi, A. J. (2011) Interdependent genotoxic mechanisms of monomethylarsonic acid: role of ROS-induced DNA damage and poly(ADP-ribose) polymerase-1 inhibition in the malignant transformation of urothelial cells. Toxicol. Appl. Pharmacol. 257, 1–13.

(32) Piatek, K., Schwedtke, T., Hartwig, A., and Bal, W. (2008) Monomethylarsonic acid destroys a tetrathiolate zinc finger much more efficiently than inorganic arsenite: mechanistic considerations and consequences for DNA repair inhibition. Chem. Res. Toxicol. 21, 600–606.

(33) Bredfeldt, T. G., Jagdish, B., Eblin, K. E., Mash, E. A., and Gandolfi, A. J. (2006) Monomethylarsonic acid induces transformation of human bladder cells. Toxicol. Appl. Pharmacol. 216, 69–79.
(34) Stýblo, M., Drobná, Z., Jaspers, I., Lin, S., and Thomas, D. J. (2002) The role of biomethylation in toxicity and carcinogenicity of arsenic: a research update. *Environ. Health Perspect.* 110, 767–771.