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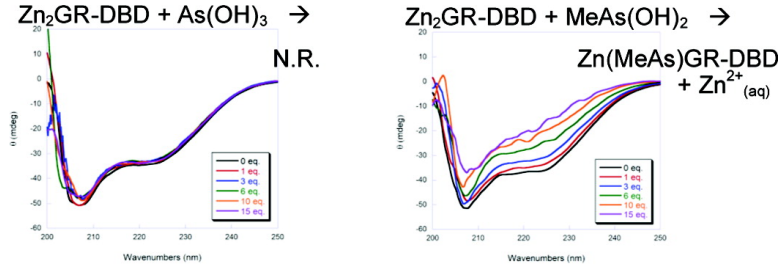

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Monomethylarsenite Competes with Zn^{2+} for Binding Sites in the Glucocorticoid Receptor

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Human diseases, including type II diabetes, cardiovascular disease, and cancer of the lung, skin, bladder, liver, and kidney, have been correlated with chronic exposure to arsenic,[1] primarily as arsenite (As(III)) in drinking water. The connection with such disparate diseases is somewhat puzzling, but there is growing evidence that arsenic affects pathways controlled by steroid hormones, thus behaving as an endocrine disruptor.[2] However, the molecular mechanism by which it disrupts hormone-regulated gene expression is not yet known.

Methylated arsenic species are found in the urine of individuals ingesting low levels of arsenic,[3] and intracellular methyltransferases that catalyze the oxidative addition of up to three methyl groups to arsenic have been identified.[4] Results from tissue culture[5] and animal[6] studies suggest that monomethylarsenite (MMA^{III}) is more toxic than arsenite, but methylation occurs within cells and this difference may relate to differential transport and uptake. We have shown, however, that MMA^{III} forms more stable complexes than arsenite with the biologically relevant thiol glutathione (GSH).[7] Further, recent studies suggest that individual differences in arsenic methylation may correlate with differences in health risks.[8]

Cell culture assays have shown that arsenic modulates transcription that is regulated by the glucocorticoid receptor (GR)[2] and other members of the hormone receptor family.[9] Systematic truncation of GR indicates that the conserved DNA-binding domain (DBD) is a likely target for arsenite.[10] This ~80 residue domain requires two Zn^{2+} ions, each coordinated to four Cys residues, to stabilize a protein structure[11] that is competent to bind as a head-to-head dimer to the near palindromic DNA sequence of the glucocorticoid response element (GRE).[12] Since arsenite has a known affinity for thiols, it is possible that competition with Zn^{2+} for the DBD Cys residues may be a key molecular event in arsenic disruption of GR-regulated pathways. It has been shown that Au^{+}, Cu^{+}, and Cd^{2+} inhibit GR binding to GRE;[13] however, these ions have a higher affinity than Zn^{2+} for thiolate coordination, and it is unclear whether arsenite can compete with Zn^{2+} at physiological concentrations.

We have quantified the affinity of GR-DBD[14] for Zn^{2+}, arsenite, and MMA^{III} with isothermal titration calorimetry (ITC),[15] and Figure 1 shows representative thermograms. The expected 2:1 stoichiometry of Zn^{2+} has been confirmed by ITC previously.[16] On the basis of the usual tristhiolate and bisthiolate coordination of arsenite and MMA^{III},[17] respectively, and the 10 Cys residues of GR-DBD, higher As(III) stoichiometries were expected. For example, arsenite and MMA^{III} bind to the 20 Cys residues of metallothionein with stoichiometries of ~6:1 and ~10:1, respectively.[18] Since both As(III) species also bind to GR-DBD with a 2:1 stoichiometry, the protein structure limits the domain to only two binding sites.

The best fit of Zn^{2+} binding isotherms requires two sites with different thermodynamics, which are evaluated in detail elsewhere.[19] In the case of arsenite and MMA^{III}, however, both ions bind with indistinguishable fit parameters. Since ITC measures the overall solution equilibrium and Zn^{2+} has significantly different aqueous chemistry than that of As(III) species,[17] it is necessary to evaluate the overall binding equilibrium in each case (eqs 1–3)[20] to obtain affinities and thermodynamics that can be compared (Supporting Information). Zn^{2+} has a quantifiable interaction with the buffer, which also becomes protonated when Zn^{2+} binds to the four Cys and displaces four protons. Arsenite and MMA^{III}, however, exist as hydroxo species in neutral aqueous solution, so there is negligible interaction with the buffer, and binding to Cys thiols leads to thiolate−As(III) coordination and formation of water.

$$2\mathrm{Zn(buff)}_x^{2+} + \mathrm{GR\text{-}DBD} + (8-2X)\mathrm{buff} \rightleftarrows [\mathrm{Zn_2GR\text{-}DBD}]^{4-} + 8\mathrm{buffH}^{+} \quad (1)$$

$$2\mathrm{As(OH)_3} + \mathrm{GR\text{-}DBD} \rightleftarrows [\mathrm{As_2GR\text{-}DBD}] + 6\mathrm{H_2O} \quad (2)$$

$$2\mathrm{MeAs(OH)_2} + \mathrm{GR\text{-}DBD} \rightleftarrows [(\mathrm{MeAs})_2\mathrm{GR\text{-}DBD}] + 4\mathrm{H_2O} \quad (3)$$

Analysis of the average best fit ITC values from ≥3 good data sets leads to buffer-independent stability constants for the two Zn^{2+} ions, which can be compared to those of $As(OH)_3$ and $MeAs(OH)_2$, binding to GR-DBD at pH 7.2 (Table 1).

The lower affinity for arsenite than MMA^{III} is consistent with previous results with small thiols.[7] Comparison with Zn^{2+} reveals that the GR-DBD affinity for MMA^{III} is only an order of magnitude lower than its affinity for the less tightly bound Zn^{2+}, allowing MMA^{III} to compete with one Zn^{2+} ion. Comparison of the binding thermodynamics at pH 7.2 (Table 1) reveals that Zn^{2+}, particularly the first one, binds with less net enthalpy than arsenite and MMA^{III}, but the As(III) species have a significant entropic penalty.

Circular dichroism (CD) in the near-UV can be used to detect Zn^{2+} stabilization of GR-DBD secondary structure. However, neither arsenite nor MMA^{III} stabilize secondary structure, as indicated by CD spectral data (Supporting Information), even though they bind to GR-DBD with the same stoichiometry as Zn^{2+}. Therefore, arsenite and MMA^{III} competition with Zn^{2+} for GR-DBD has been monitored by CD under strictly anaerobic conditions (Figure 2). These data indicate that excess arsenite has no effect on the Zn-stabilized structure but that MMA^{III} causes a loss of secondary structure, consistent with partial or complete displacement of at least one Zn^{2+} ion and, therefore, loss of competence to bind to GRE.[12]

Finally, we consider other factors in a cellular competition between MMA^{III} and Zn^{2+} for GR-DBD, including the significant concentration of GSH that results in its complexes with As(III) and Zn^{2+} in intracellular equilibria (e.g., eq 4).[20]

$$\mathrm{MeAs(GS)_2} + [\mathrm{Zn_2GR\text{-}DBD}]^{4-} + 2\mathrm{H}^{+} \rightleftarrows [\mathrm{Zn(MeAs)GR\text{-}DBD}]^{2-} + \mathrm{Zn(GS)_2} \quad (4)$$

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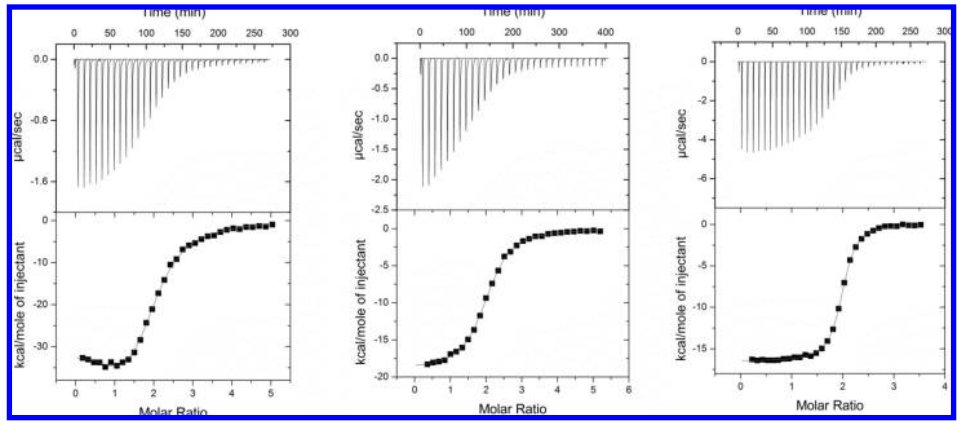

Figure 1. Representative ITC data for Zn^{2+} (left), arsenite (middle), MMA^{III} (right) titrations of 30, 40, and 45 μM GR-DBD, respectively; 25 °C, 0.10 M bis-Tris, pH 7.2; best fit values for Zn^{2+} (two-site model): $K_1 = 7 \pm 1 \times 10^6$, $\Delta H_1 = -33.3 \pm 0.2$ kcal/mol, $K_2 = (4.7 \pm 0.2) \times 10^5$, $\Delta H_2 = -46.9 \pm 0.1$ kcal/mol; arsenite (one-site model): $n = 2.0$, $K = (4.3 \pm 0.1) \times 10^5$, $\Delta H = -19.0 \pm 0.1$ kcal/mol; MMA^{III} (one-site model): $n = 2.0$, $K = (1.6 \pm 0.1) \times 10^6$, $\Delta H = -16.5 \pm 0.1$ kcal/mol.

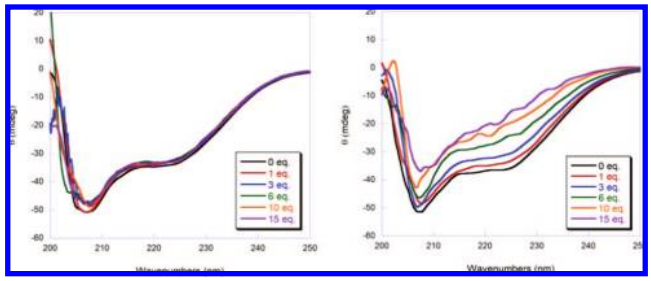

Figure 2. CD spectra of 30 μM Zn_2GR-DBD with increasing arsenite (left) and MMA^{III} (right); 37 °C, 25 mM phosphate, pH 7.2.

Table 1. Thermodynamics of Zn^{2+}, Arsenite, and MMA^{III} Binding to GR-DBD at pH 7.2 and 25 °C

	$Zn^{2+}{}_1$	$Zn^{2+}{}_2$	$As(OH)_3$	$MeAs(OH)_2$
log K	9 ± 2	7.5 ± 0.5	5.6 ± 0.6	6.2 ± 0.3
$\Delta G°$ (kcal/mol)	-12 ± 2	-10.2 ± 0.7	-7.6 ± 0.8	-8.5 ± 0.5
$\Delta H°$ (kcal/mol)	-5.7 ± 0.1	-13.0 ± 0.6	-20.3 ± 0.7	-16.5 ± 0.1
$\Delta S°$ (cal/mol·K)	22 ± 8	-9 ± 3	-43 ± 1	-27 ± 1

Using pH 7.2 stability constants determined in this study, the known stability constants for $MeAs(GS)_2$ (1.3×10^7)[7] and $Zn(GS)_2$ (3.9×10^{13})[21] and a reported value for the cellular concentration of free Zn^{2+} (0.4 fM),[22] it can be estimated that ~0.5 μM MMA^{III} is required for 50% formation of functionally compromised $[Zn(MeAs)GR\text{-}DBD]^{2-}$ at pH 7.2. Since arsenic effects on GR-regulated transcription are found in cells exposed to low micromolar levels of arsenite,[9,10] it is quite plausible that this cellular concentration of MMA^{III} can be achieved.

Thus, thermodynamic and structural results indicate that MMA^{III}, but not arsenite, is able to compete with Zn^{2+} ions that are required to stabilize the functionally competent structure of the GR DNA-binding domain. Whether this is general for Cys_4 coordination sites remains to be determined, although a recent report suggests that this may be the case for the Cys_4 Zn-binding site in the DNA repair protein XPA.[23]

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Supporting Information Available: Analysis of Zn^{2+} ITC data to remove buffer effects; CD spectra of GR-DBD species. This material is available free of charge via the Internet at http://pubs.acs.org.

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