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KINETICS AND THERMODYNAMICS OF ZINC(II) AND ARSENIC(III) BINDING TO XPA AND PARP-1 ZINC FINGER PEPTIDES

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Abstract

Inhibition of DNA repair is an established mechanism of arsenic co-carcinogenesis, and may be perpetuated by the binding of As(III) to key zinc finger (zf) DNA repair proteins. Validated molecular targets of As(III) include the first zinc finger domain of Poly (ADP-Ribose) Polymerase 1 (PARP-1), and the zinc finger domain of Xeroderma Pigmentosum Complementation Group A (XPA). In order to gain an understanding of the thermodynamic and kinetic parameters of the interaction of As(III) with these two zinc finger motifs, a fluorescence based approach was used to investigate Zn(II) and As(III) binding to synthetic model peptides corresponding to the zf motif of XPA and first zf motif of PARP-1, referred to in this paper as XPAzf and PARP-1zf-1, respectively. While XPAzf and PARP-1zf-1 display similar relative affinities for As(III), PARP-1zf-1 shows a potential kinetic advantage over XPAzf for As(III) binding, with a rate constant for the fast phase of formation of As(III)-PARP-1zf-1 approximately 4-fold higher than for As(III)-XPAzf. However, the binding of Zn(II) with either peptide proceeds at a faster rate than As(III). Notably, XPAzf demonstrates comparable affinities for binding both metals, while PARP-1zf-1 shows a slightly higher affinity for Zn(II), suggesting that the relative concentrations of Zn(II) and As(III) in a system may significantly influence which species predominates in zinc finger occupancy. These results provide insight into the mechanisms underlying interactions between zinc finger structures and As(III), and highlight the potential utility of zinc supplementation in mitigating adverse effects of As(III) on zinc finger functions in vivo.

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AUTHOR CONTRIBUTIONS

KJL and LGH conceived and managed the study. JH performed the experiments, wrote the paper, and contributed to curve fitting and data analysis. XZ designed the experiments. CF, XZ and LC coordinated and contributed to curve fitting and data analysis. All authors have reviewed the results, contributed to manuscript revision, and approved the final manuscript.

GRAPHICAL ABSTRACT (SYNOPSIS)

As(III) and Zn(II) show comparable binding affinities for Xeroderma Pigmentosum Complementation Group A zinc finger peptide (XPAzf). Zn(II) shows a slightly higher affinity than As(III) for Poly (ADP-Ribose) Polymerase 1 zinc finger peptide (PARP-1zf-1). As(III) shows similar affinities for PARP-1zf-1 and XPAzf, but binds approximately 4 times faster to PARP-1zf-1.

Keywords

Arsenic(III); Zinc(II); XPA; PARP-1; kinetics; thermodynamics

1. INTRODUCTION

Arsenic is a naturally occurring toxic metalloid associated with numerous adverse health outcomes as a result of human exposure, including the development of liver, lung, urinary tract and skin cancers [1-7]. At low levels, trivalent arsenic (As(III)) has also been shown to act as a co-carcinogen, increasing the retention of DNA damage caused by other carcinogens, such as benzo(a)pyrene and ultraviolet radiation [8-12]. As(III) has been shown to modify or bind to certain key zinc finger DNA repair proteins [12-18], thus potentially contributing to this phenomenon.

After the exposure of organisms to arsenite or arsenic trioxide, the formed As(III) selectively targets zinc finger motifs containing either three or four cysteine residues (hereafter referred to respectively as C3H1 or C4 zinc finger motifs), but does not significantly bind to zinc finger proteins containing only two cysteine residues (referred to as C2H2 motifs) [19, 20]. The finding that As(III) has been demonstrated to complex with C3H1 and C4 zinc finger motifs by binding at least three cysteine residues [19, 20] suggests that the additional fourth cysteine residue in C4 motifs could confer greater stability for As(III) binding. However, little is known about the potential difference in As(III)-binding between the C3H1 and C4 motifs.

Two important molecular targets of As(III) are the C3H1 zinc finger protein Poly (ADP-Ribose) Polymerase-1 (PARP-1) [12, 21, 22], and the C4 zinc finger protein Xeroderma Pigmentosum Complementation Group A (XPA) [13, 17], whose activities are central to base excision repair and nucleotide excision repair, respectively [23-25]. In the base excision repair pathway, PARP-1 binds to single-strand DNA breaks and catalyzes the addition of branched poly(ADP-ribose) (PAR) chains to recruit additional key DNA repair proteins such as DNA ligase III to the site of damage, thus facilitating completion of DNA repair [23]. PARP-1 contains three zinc finger motifs within its N-terminal DNA binding domain. The first of these, a C3H1 zinc finger motif, is believed to be essential for DNA binding, and

susceptible to loss of function as a result of As(III) binding or modification [12, 21, 22, 26]. In model peptide studies, As(III) binding results in the replacement of divalent zinc (Zn(II)) within the first zinc finger motif of PARP-1, and as a result, conformational changes within the peptide [26]. In cellular studies, As(III) displacement of Zn(II) correlates with reduction in PARP-1 activity and increased retention of DNA damage [12, 26, 27]. This binding has been observed in tagged PARP-1 DNA binding domain isolated from As (III) exposed cells, demonstrating that it occurs in the presence of physiological levels of glutathione [18].

In the nucleotide excision repair pathway, XPA serves a somewhat analogous function to PARP-1 in base excision repair, through recognizing and binding to bulky DNA damage lesions such as cyclopurimidine dimers and 6'4-photoproducts, and facilitating the recruitment of additional DNA repair proteins to the site of damage [28, 29]. In humans, mutations to XPA result in a rare, autosomal recessive genetic disorder of DNA repair known as Xeroderma Pigmentosum [28, 29]. The C4 zinc finger motif of XPA is believed to be essential for its proper folding and function in nucleotide excision repair [30-32]. Previous studies have demonstrated that this motif may be targeted for binding and modification by As(III) [13, 14, 16-18]. Indirect evidence of As(III) binding, i.e. loss of Zn(II) from the zinc finger motif, has been observed in both PARP-1 and XPA isolated from As(III) treated cells [18, 19]. Direct evidence for As(III) displacement of Zn(II) has been demonstrated through mass spectrometric approaches using peptides based upon the zinc finger motifs of both PARP-1 [12, 18-20], and XPA [17, 20]. Evidence for As(III) occupation of these same zinc finger peptides has also been provided through cobalt spectrometry [20]. In a previous study, pre-incubation of these peptides with As(III) resulted in a decrease in the characteristic absorbance at 660 nm which indicates the formation of a complex between cobalt and zinc finger motifs [33, 34], indicating a decrease in available metal-binding sites within the peptides [20].

In order to uncover the precise mechanisms underlying As(III)'s impact in a cellular or *in vivo* context, it is necessary to improve our understanding of the physical chemistry underlying Zn(II) and As(III) interactions with zinc finger motifs. The purpose of this study is to investigate the kinetic and thermodynamic properties of the interactions of As(III) and Zn(II) with model peptides corresponding to the first C3H1 zinc finger motif of PARP-1 and the C4 zinc finger motif of XPA. These peptides are hereafter referred to as PARP-1zf-1 and XPAzf respectively, while the terms "XPA" and "PARP-1" refer to full length proteins. This work seeks to investigate whether the rate of binding to and/or affinity for these model peptides is greater for Zn(II) or As(III), as well as whether As(III) may show greater affinity for and/or faster binding rate to a C4 zinc finger peptide over a C3H1 peptide in an *in vitro* system.

2. MATERIALS

2.1. Caution

Inorganic arsenic is a Group 1 human carcinogen and should be handled with care. Tris (2-carboxyethyl) phosphine hydrochloride is corrosive and hazardous if inhaled or ingested. Urea is mutagenic, an irritant, and may cause cardiac disturbances.

2.2 Materials

Synthetic peptides corresponding to the first zinc finger motif of PARP-1 [GRASCKKCSIPKDKVPHWYHFSCFWKV], a C4 mutant of the first zinc finger motif of PARP-1 [GRASCKKCSIPKDKVPHWYCFSCFWKV], and the zinc finger motif of XPA [DYVICEECGKEFMDSYLMNHFDLPTCDNCRDADDKDK] were obtained from Genemed Synthesis Inc. (San Antonio, TX), at a purity > 95%. Sodium meta-arsenite (NaAsO_2) was obtained from Fluka Chemie (Buchs, Germany) at a purity 99%. Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), and urea were obtained from Sigma-Aldrich (St. Louis, MO). HEPES (1M), was obtained from Life Technologies (Carlsbad, CA).

3. EXPERIMENTAL

3.1. Preparation of peptide and metal solutions

TCEP was dissolved in 20 mM HEPES buffer to a final concentration of 0.25 mM TCEP. HEPES buffer was chosen for the purposes of facilitating a comparison between the results of the present study and a previous investigation by Bossak et al. [35] into the affinity between PARP-1zf-1 and Zn(II). TCEP is a reducing agent used to prevent oxidation of cysteine in the peptide. The resulting HEPES-TCEP stock solution (pH 7.4) was used to dissolve PARP-1zf-1 and XPAzf peptide. Peptide concentration was determined using an extinction coefficient at 280 nm of $12490 \text{ M}^{-1} \text{ cm}^{-1}$ for PARP-1zf-1 and PARP-1zf-1 C4 mutant, and of $2980 \text{ M}^{-1} \text{ cm}^{-1}$ for XPAzf, respectively, which were calculated in accordance with a previously reported methodology [36]. 5 μL of peptide solution was incubated with 10 μL of 12 M urea for 30 minutes, diluted with nanopure water to a final volume of 100 μL , and absorbance at 280 nm was measured. Peptide stocks were then serially diluted to appropriate concentration, as described in sections 3.2 and 3.3. Stock solutions of As(III) and Zn(II) were prepared through dilution in HEPES-TCEP to 100 mM, 10 mM, 1 mM, and 250 μM for NaAsO_2 , and to 50 mM, 5 mM, 500 μM and 125 μM for ZnSO_4 .

3.2. Kinetic experiments

Solutions of ZnSO_4 and NaAsO_2 were prepared through serial dilution of 4 mM stocks to yield final concentrations ranging from 50 to 200 μM when combined with the peptide solution. Peptide solutions were prepared through serial dilution of 0.3 mM or 0.6 mM stock to give final concentrations of 15 μM , 30 μM , 45 μM or 60 μM when combined with As(III) or Zn(II) solutions. For each experiment, 90 μL of peptide solution was introduced to a cuvette containing 10 μL of Zn(II) or As(III) solution. Fluorescence readings were collected using a SpectraMax M2 Multi-Mode Microplate Reader (Molecular Devices, Sunnyvale, CA) at room temperature. Kinetics mode was used to monitor real-time changes in peptide conformation due to As(III) or Zn(II) binding, using an average of 6 readings at 5 second intervals for 5-10 minutes, until a plateau in fluorescence level was achieved. 274/303 nm and 280/350 nm were used as excitation/emission wavelengths for experiments involving XPAzf peptide, and PARP-1zf-1 peptide, respectively. These wavelengths were selected based upon the excitation and emission maxima for tyrosine and tryptophan residues, and confirmed by collecting fluorescence spectra for the XPAzf and PARP-1zf-1 model peptides as described in section 3.4.

3.3. Thermodynamic experiments

Samples were prepared from stock solutions of HEPES-TCEP, peptide, NaAsO₂ and ZnSO₄, to contain final peptide concentrations of 15 or 30 μM, and either Zn(II) at concentrations ranging from 0.65 μM to 0.48 mM, or As(III) at concentrations ranging from 1.3 μM to 36 mM. Based upon preliminary kinetics experiments, it was observed that reaction equilibrium corresponding with a plateau in fluorescence is typically reached in less than 10 minutes; however, in the interest of ensuring that the very broad range of metal concentrations used in the thermodynamic experiments could react completely with the available peptide, we extended the incubation time to 3 hours at room temperature prior to assaying endpoint fluorescence and absorbance levels. Fluorescence readings were collected using a SpectraMax M2 Multi-Mode Microplate Reader at room temperature. Endpoint mode was used to measure changes in peptide fluorescence intensity due to Zn(II) or As(III) binding after reaching equilibrium. 274/303 nm and 280/350 nm were used as excitation/emission wavelengths for experiments involving XPAzf and PARP-1zf-1 peptide, respectively.

3.4. Collection of fluorescence spectra

Samples were prepared as described in section 3.3, to give a final peptide concentration of approximately 30 μM, and metal concentrations ranging from 1.875 μM to 262.5 μM for Zn(II) and from 3.75 μM to 525 μM for As(III). Fluorescence readings were collected using a SpectraMax M2 Multi-Mode Microplate Reader at room temperature. Spectrum mode was used to scan fluorescence spectra from 290 nm to 390 nm for both peptides with and without the addition of As(III) or Zn(II). 274 and 280 nm were used as excitation wavelengths for XPAzf and PARP-1zf-1, respectively.

3.5. Calculation and Curve-fitting

3.5.1 Kinetic experiments—For As(III) binding kinetics, kinetic fluorescent curves were fit with 1- and 2-phase exponential decay equations using GraphPad Prism 6 data analysis software. Residual values for these fits were plotted, and show that the 2-phase decay equation provided a superior fit for the data. The 2-phase decay model provided by Graphpad is as follows:

$$\text{SpanFast} = (Y_0 - \text{Plateau}) * \text{PercentFast} * .01$$

$$\text{SpanSlow} = (Y_0 - \text{Plateau}) * (100 - \text{PercentFast}) * .$$

$$Y = \text{Plateau} + \text{SpanFast} * \exp(-k_{\text{fast}} * X) + \text{SpanSlow} * \exp(-k_{\text{slow}} * X).$$

This model represents the sum of a fast and a slow process of exponential decay. The change in fluorescence intensity is assumed to correlate with extent of binding process. Y_0 thus corresponds to completely unbound peptide, while Y is fluorescent intensity, and the plateau in fluorescence corresponds to reaction completion. X corresponds with metal concentration.

3.5.2. Thermodynamic experiments—For Zn(II) and As(III) binding thermodynamics, fluorescent signal was normalized from 0 to 100%. The data were fit with the equations

$$I = (A_1 [M] + b_1) * 50 \left(\left([M] + [Pep]_{Total} + K_d \right) \pm \frac{\sqrt{\left([M] + [Pep]_{Total} + K_d \right)^2 - 4 [M] [Pep]_{Total}}}{[Pep]_{Total}} \right) + (A_0 [M] + b_0),$$

for zinc, and

$$I = (A_1 [M] + b_1) * \left(100 - 50 \left(\left([M] + [Pep]_{Total} + K_d \right) \pm \frac{\sqrt{\left([M] + [Pep]_{Total} + K_d \right)^2 - 4 [M] [Pep]_{Total}}}{[Pep]_{Total}} \right) \right) + (A_0 [M] + b_0),$$

for arsenite, where I is relative fluorescent intensity, $[M]$ is the concentration of free metal, $[Pep]_{Total}$ is total peptide concentration, and K_d is the reaction dissociation constant. The terms $(A_1 [M] + b_1)$ and $(A_0 [M] + b_0)$ have been introduced to account for the potential influence of metal concentration on the fluorescence quantum yield of bound and unbound peptide respectively, where A_1 , b_1 , A_0 , and b_0 are constants related to this phenomenon. Notably, at $[M] = 0$, all peptide will be in an unbound state corresponding with $I = 100$ or $I = 0$, for As(III) or Zn(II) respectively. Therefore, b_0 is constrained to zero, in order to ensure that these values remain constant. Relative fluorescent intensity, I , is assumed to be related to metal-bound peptide, $[Pep - M]$, as follows: $I \propto [Pep - M]/[Pep]_{Total}$ for Zn(II), and $I \propto 100 - [Pep - M]/[Pep]_{Total}$ for As(III), where $[Pep - M]$ represents metal-bound peptide. The reaction dissociation constant K_d relates to the concentrations of free metal, unbound and metal bound peptide as follows: $K_d = [Pep][M]/[Pep - M]$, where $[M]$ is free metal, and $[Pep]$ is unbound peptide. The basic relationships $[Pep] = [Pep]_{Total} - [Pep - M]$, and $[M] = [M]_{Total} - [Pep - M]$ are substituted into the basic equation for K_d in order to facilitate the derivation of the final equation.

4. RESULTS

4.1. As(III) or Zn(II) binding results in concentration-dependent altered intrinsic fluorescence of C3H1 or C4 zinc finger peptide

Concentration-dependent alterations in intrinsic fluorescence due to Zn(II) or As(III) binding were measured by collecting fluorescence spectra, from 290 nm to 390 nm, for both peptides in the presence and absence of Zn(II) or As(III). As no tryptophan residues are present in XPAzf, tyrosine fluorescence must be measured instead for this peptide, and the measured emission maximum of the XPAzf apo-peptide lies at 305 nm (Figure 1A), consistent with the emission maximum of 303 for tyrosine. Metal binding induces a change in fluorescence intensity at 303 nm for XPAzf (Figure 1C). For PARP-1zf-1, the emission maximum of the apo-peptide lies at 350 nm (Figure 1B), and a similar binding-induced change in fluorescence intensity was observed (Figure 1D). Consistent with previous reports, an increase in the fluorescence intensity of the peptide is correlated with Zn(II) binding [26, 35, 37], and is negatively correlated with As(III) binding [26] (Figures 1C and 1D). Notably,

these changes correspond to alterations in tertiary structure of the peptide, which in the context of full-length protein may result in altered binding ability and protein function [26]. These data validate fluorescence spectroscopy as a useful methodology for investigating Zn(II) and As(III) binding of XPAzf and PARP-1-zf-1 peptides.

4.2. XPAzf peptide shows similar affinities to Zn(II) and As(III), while PARP-1zf-1 shows a slightly higher affinity for Zn(II) over As(III)

It has been observed that in a cellular environment, As(III) may replace bound Zn(II) in certain zinc finger motifs, including the first C3H1 motif of PARP-1 [12, 21, 22] and the C4 zinc finger of XPA [13, 17]. However, the mechanisms underlying this replacement are not well understood. To answer the question of whether the relative affinity of As(III) is higher than that of Zn(II) for either or both of the model peptides, each peptide was titrated with a broad range of Zn(II) and As(III) concentrations. Sample titration curves are shown in Figures 1C and 1D, which demonstrate concentration-dependent changes in peptide fluorescence intensities upon As(III) and Zn(II) binding.

As described in section 3.5, endpoint titration curves were normalized and the resulting plots were fitted to determine values for observed dissociation constant, or K_d , for each reaction. Table 1 lists the K_d values for each peptide, showing that, at each utilized peptide concentration, Zn(II) and As(III) possess similar affinities for XPAzf peptide while Zn(II) demonstrates a somewhat higher affinity for PARP-1zf-1 peptide than As(III). These findings suggest that from the perspective of thermodynamic control, the relative concentrations of As(III) and Zn(II) are likely to strongly influence zinc finger occupancy in the presence of As(III). Significantly, As(III) shows no thermodynamic advantage over Zn(II) for binding either XPAzf or PARP-1-zf-1 peptide.

4.3. Zn(II) binds faster than As(III) with both XPAzf and PARP-1zf-1 peptides

Given that As(III) may compete with Zn(II) in cells to bind zinc finger motifs, the kinetics of As(III)-zinc finger interactions were compared with the kinetics of Zn(II)-zinc finger interactions. To determine whether the rate of As(III)-peptide binding is faster than Zn(II) binding, a series of kinetic experiments was performed to determine the apparent binding constants of each binding process. The experiments were designed to observe reaction progression in real-time by following the peptide fluorescence intensities. In this assay, the fast phase of Zn(II) binding to either PARP-1zf-1 or XPAzf proceeded at a rate too rapid to be measured with the current experimental setup. The initial fluorescence readings after introducing peptide solution to the Zn(II) containing cuvette are significantly higher than that of the apo-peptide, thus providing insufficient data points for reliable curve fitting (Figures 2A and 2B). Figure 2 shows representative curves comparing the Zn(II) and As(III) binding data, demonstrating that the rate of Zn(II) binding is qualitatively much faster than the rate of As(III) binding for both PARP-1zf-1 and XPAzf peptides. Thus, As(III) shows no kinetic advantage over Zn(II) in binding to the model peptides.

4.4. The fast phase of As(III) binding to PARP-1zf-1 is more rapid than that of As(III)-XPAzf binding

In order to determine whether either peptide might exhibit a kinetic advantage for As(III) binding, kinetic fluorescent curves were fit with both one and two-phase exponential decay models in Graphpad Prism 6, as described in section 3.5.1. Representative curves are shown in Figure 3. Examination of the residual plots (data not shown) reveals that the two-phase decay is the better of the two fitting models. Half-lives for the fast and slow phases of the association are calculated as $0.6932/k_{fast}$ and $0.6932/k_{slow}$ respectively, and are thus inversely proportionate to the observed association constants. Results from the two phase model are compared here.

While the values of k_{slow} are similar for PARP-1zf-1 and XPAzf, k_{fast} values show a trend wherein the values for PARP-1zf-1 exceed those for XPAzf. Values of k_{fast} are shown in Table 2, while Table 3 shows values for k_{slow} . For the most robust analysis, the values of k_{fast} for XPAzf and PARP-1zf-1 are compared at 15 μ M peptide, the concentration where residual plots reveal the best fit of the 2 phase decay model, and 200 μ M As(III). For this concentration, the k_{fast} value for PARP-1zf-1 ($7.9 \pm 0.13 \text{ min}^{-1}$) shows approximately a four-fold increase over the value for XPAzf ($1.9 \pm 0.30 \text{ min}^{-1}$), demonstrating that while As(III)-XPAzf formation is thermodynamically comparable to As(III)-PARP-1zf-1 formation, the latter reaction may be kinetically favorable.

In order to probe the specific contribution of zinc finger structure to determining As(III)-binding rates, the As(III)-binding kinetic experiments were repeated with a mutant PARP-1zf-1 peptide containing a histidine to cysteine mutation conferring a C4 zinc finger motif (PARP-1zf-1-C4mut). Data from these experiments were fit with the same two-phase decay model as described above, and observed association rate constants compared with those for PARP-1zf-1 and XPA-1. Values of k_{fast} and k_{slow} for As(III) binding to PARP-1zf-1-C4mut are reported in Table 4. Observed values of k_{slow} for the association between the C4 mutant PARP-1zf-1 and As(III) are quite consistent with the values observed for both wild type PARP-1zf-1 and XPAzf. The values for k_{fast} for the C4 mutant PARP-1zf-1 are consistent with those observed for wild type PARP-1zf-1 and, as with the values for wild type PARP-1zf-1, are higher than those observed for XPAzf. Finally, at the 15 μ M concentration of peptide with 200 μ M As(III), k_{fast} for the C4 mutant of PARP-1zf-1 shows approximately a four-fold increase over k_{fast} for XPAzf, as does k_{fast} for wild type PARP-1zf-1. These results suggest that the kinetic advantage for As(III)-PARP-1zf-1 formation over As(III)-XPAzf formation is likely due to additional structural elements present within these peptides, rather than the additional thiolate residue in the XPAzf peptide.

5. DISCUSSION

5.1. Differences and similarities between As(III)-XPAzf and As(III)-PARP-1zf-1 binding thermodynamics and kinetics

Under the present reaction conditions, the fast phase of As(III)-PARP-1zf-1 peptide binding proceeds at a rate approximately four times faster than the fast phase of As(III)-XPAzf

binding, suggesting that despite the similarity in binding affinities between these two peptides, a kinetic advantage may still exist for As(III)-PARP-1zf-1 binding over As(III)-XPAzf binding. Additionally, the C4 mutant of PARP-1zf-1 demonstrated similar observed values of k_{fast} to those seen for wild-type PARP-1zf-1. Since As(III) may coordinate separately with three separate cysteine residues within XPAzf or PARP-1zf-1, it is likely that k_{fast} and k_{slow} may represent separate steps of the binding mechanism. It is not possible to determine which is the initial step. However, while it is generally accepted that the slow step of the reaction mechanism determines reaction rate, the coordination of As(III) with even one cysteine residue may disrupt zinc finger structure sufficiently to prevent Zn(II) binding and function in the context of the full-length protein. Thus, if k_{fast} reflects the first of two reaction steps between As(III) and the model peptides, As(III) may be said to show a kinetic advantage for binding PARP-1zf-1 over XPAzf.

These findings suggest that for these peptides, and in the present experimental system, the presence of a fourth cysteine residue in the zinc finger motif does not confer a significant kinetic advantage for As(III) binding. However, the findings from one set of C3H1 and C4 zinc finger peptides may not necessarily be extrapolated to all such motifs, given that the variable three-dimensional structures of zinc finger motifs may influence binding kinetics and thermodynamics independently of the number of cysteine residues. Despite previous evidence that certain C4 zinc finger proteins may exhibit enhanced affinity for As(III) [38], a significant difference in observed K_d for As(III) binding between PARP-1zf-1 and XPAzf peptides was not observed in the present study, suggesting that the As(III)-binding affinity of these two model peptides is not exclusively influenced by the number of thiolate residues. In comparing the kinetics of different As(III)-zf interactions, the similar As(III)-binding rates of wild type PARP-1zf-1 and the C4 mutant of PARP-1zf-1 suggest that the discrepancy in As(III) binding rates between PARP-1zf-1 and XPAzf may likewise be attributable to structural elements apart from their C3H1 vs. C4 motifs. Future kinetic studies utilizing an XPAzf mutant peptide with a cysteine to histidine substitution may be useful in further confirming this finding.

5.2. The relative concentrations of Zn(II) and As(III) in a system may significantly influence zinc finger occupancy, suggesting a potential mechanism behind evidence for Zn(II)-rescue of As(III)-mediated adverse effects

While it is known that As(III) may replace Zn(II) in the zinc finger motifs of certain key DNA repair proteins, leading to adverse events, such as increased mutational frequency, DNA-damage retention, and PARP-1 activity reduction [12, 13-22, 26], it has also been demonstrated that Zn(II) supplementation may mitigate these effects [26, 27]. Both of these observations may be accounted for by the similar relative affinities of Zn(II) and As(III) for XPAzf peptide, and slightly higher affinity of Zn(II) for PARP-1zf-1 peptide over As(III). The K_d values for these binding reactions suggest that in a cellular context, relative levels of zinc finger occupancy may be dictated as a function of metal concentration. Given that free zinc may be present in as low as picomolar concentrations depending on cell type [39, 40], it is plausible that low-level As(III) exposure (at nanomolar level) could lead to As(III)-zinc finger binding, and that local fluctuations in relative Zn(II) and As(III) concentration could significantly contribute to this phenomenon.

The importance of relative metal concentrations is underscored by the hypothesized existence of significant levels of cellular PARP-1 wherein the first zinc finger remains unbound by zinc, a phenomenon suggested by Bossak et al. upon finding that PARP-1zf-1 displays an extraordinarily low affinity for Zn(II) in comparison with other measured Zn(II)-zf affinities [35]. Our observed K_d of $0.10 \pm 0.059 \mu\text{M}$ for Zn(II) binding to 15 μM PARP-1zf-1 is in reasonable agreement with Bossak et al.'s value of 26 nM. In another study by Bal et al., a tentative value of $K=8.5 \pm 1.5 \times 10^8 \text{ M}^{-1}$ is provided for the association constant between Zn(II) and XPAzf, corresponding to a dissociation constant of approximately 1.2 nM. The value of K_d determined from the present study is three orders of magnitude lower than this, possibly due to different experimental conditions and methods; however, is still consistent with the idea that XPAzf demonstrates a relatively low affinity for Zn(II) in comparison with many other zinc finger proteins [37].

The comparison between Zn(II) and As(III) in terms of relative peptide-binding affinity provides a possible explanation for the recent findings which have demonstrated a potential role for Zn(II) supplementation in reversing or helping to prevent adverse outcomes from As(III) exposure in experimental systems [26, 27]. The more favorable kinetics of Zn(II) binding to XPAzf and PARP-1zf-1, and the comparable stability of Zn(II) and As(III) interactions with each of these peptides, suggest that in a physiological context, elevated cellular zinc levels due to supplementation may cause reduced As(III) occupancy of these zinc finger motifs through Zn(II) competition with As(III). Since PARP-1 and XPA are involved in DNA repair, this evidence strongly suggests that dietary Zn(II) supplementation may have potential utility as a chemopreventive intervention in vulnerable populations, to mitigate adverse health effects of low level As(III) exposure. It is worth noting that dietary selenium supplementation has also been proposed as another such potentially protective intervention to alleviate As(III) exposure, however, distinct mechanisms likely underlie the chemopreventive effects of Zn(II) and selenium [41-43].

It must be acknowledged that there are multiple factors in a cellular environment that are not recapitulated in the present experimental system, which may influence binding kinetics and thermodynamics of Zn(II) and As(III) interactions with zinc fingers. On a fundamental level, co-factors present in the cellular environment may influence binding affinities, through alterations in the precise conformation of the zinc finger motif, or stabilization of protein-metal interactions. For example, a decrease in Zn(II)-zinc finger affinity under acidic conditions has been previously reported, with no concomitant effect on As(III)-zinc finger affinity [44]. The thermodynamic favorability of zinc finger binding to one metal over the other may therefore be subject to change based upon the specific conditions of the local cellular environment. Previous investigations into the role of glutathione in both As(III)-zinc finger and Zn(II)-zinc finger binding also highlight the complexity of the cellular environment in modulating metal-zinc finger binding. Reduced glutathione has been shown to enhance the rate of both Zn(II)-zinc finger [45], and As(III)-zinc finger formation [46], further underscoring the importance of local fluctuations in relative metal concentrations in determining zinc finger occupancy. However, in vivo, such effects are likely to be subject to local fluctuations in glutathione concentration and pH level. An extensive analysis of these topics is therefore somewhat beyond the scope of this paper, which focuses on generating a baseline understanding of the physical chemistry underlying interactions between As(III),

Zn(II), and the model peptides in the absence of any endogenous compounds that may mediate such interactions in vivo. Nonetheless, in future peptide-based studies, it would be interesting to see whether the inclusion of varying concentrations of glutathione would have differential effects on As(III) binding to zinc fingers, vs. Zn(II) binding to zinc fingers, or C3H1 binding vs. C4 zinc finger binding to either of these metals.

6. CONCLUSION

While both model peptides show similar relative binding affinities for As(III), PARP-1zf-1 peptide shows a possible kinetic advantage over XPAzf peptide for binding As(III) which is likely due to elements of three-dimensional peptide structure apart from the number of cysteine residues present. The relative similar affinities that As(III) and Zn(II) show for each peptide suggest that zinc finger occupancy in a cellular system may be strongly influenced by the relative concentrations of each metal.

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Highlights

- Poly (ADP-Ribose) Polymerase 1 is abbreviated to PARP-1.
- Xeroderma Pigmentosum Complementation Group A is abbreviated to XPA.
- Similar K_d values are shown for As(III) binding to XPA and PARP-1 zinc finger peptides.
- k_{fast} for As(III) binding to PARP-1 peptide is approx. 4× faster than to XPA peptide.
- Zn(II) binds faster than As(III) to both XPA and PARP-1 zinc finger peptides.

A.

Figure 1. Zn(II)- and As(III)- dependent changes in fluorescence spectra of XPAzf and PARP-1zf-1

Intrinsic fluorescence emission spectra of 30 μ M XPAzf (A) and 30 μ M PARP-1zf-1 (B) with added Zn(II) or As(III). Panels C and D show plots of fluorescence at 303 nm and 350 nm of 15 μ M XPAzf and PARP-1zf-1, respectively, vs. concentration of As(III) or Zn(II). Maximum fluorescence is assumed to represent maximum peptide binding by Zn(II), while minimum fluorescence is assumed to represent maximum peptide binding by As(III). In

panels **C** and **D**, the data point closest to the X axis represents baseline fluorescence of the apo-peptide.

Figure 2. Fluorescence change over time at 303 or 350 nm for 30 μ M XPAzf (A) and PARP-1zf-1 (B) peptides respectively, with added As(III) or Zn(II)

Panels show decreasing or increasing peptide intrinsic fluorescence intensity after the addition at time=0 of As(III) or Zn(II) respectively. 90 μ L of peptide solution was introduced to a cuvette containing 10 μ L metal solution to produce the final concentrations listed in the legend. A SpectraMax M2 Multi-Mode Microplate Reader was used in kinetics mode to monitor real-time changes in peptide conformation after metal addition, using an average of 6 readings at 5 second intervals for 5-10 minutes. The appearance of a plateau in the measured fluorescent intensity is correlated with reaction equilibrium.

Figure 3. Fluorescence decay over time for 30 μM XPAzf (A) and PARP-1zf-1 (B) peptides with added As(III)

Decreasing peptide intrinsic fluorescence intensity over time is seen after the addition of As(III) at time=0. 90 μL of peptide solution was introduced to a cuvette containing 10 μL As(III) solution to produce the final concentrations listed in the legend. A SpectraMax M2 Multi-Mode Microplate Reader was used in kinetics mode to monitor real-time changes in peptide conformation after As(III) addition, using an average of 6 readings at 5 second intervals for 5-10 minutes. The appearance of a plateau in the measured fluorescent intensity is correlated with reaction equilibrium.

Table 1

Observed dissociation constants for Zn(II) and As(III) binding to XPAzf and PARP-1zf-1. Values for XPAzf at 15 μ M are the average of the two best out of three data sets, $\pm$ *SEM*. Values for all other peptide concentration are the average of 3 fits, $\pm$ *SEM*. R squared values are listed below values of observed K_d .

Reaction	Observed K_d at 15 μ M peptide (μ M)	Observed K_d at 30 μ M peptide (μ M)
Zn(II)-XPAzf binding	1.4 ± 2.4 $R^2=.9910$	6.2 ± 4.5 $R^2=.9945$
As(III)-XPAzf binding	2.3 ± 1.7 $R^2=.9592$	5.2 ± 2.6 $R^2=.9773$
Zn(II)-PARP-1zf-1 binding	0.10 ± 0.059 $R^2=.9975$	0.38 ± 0.28 $R^2=.9923$
As(III)-PARP-1zf-1 binding	5.4 ± 0.77 $R^2=.9975$	8.0 ± 3.2 $R^2=.9823$

Table 2

Values of k_{fast} at 15, 30, 45, and 60 μ M peptide for the binding of XPAzf (**A**) and PARP-1zf-1 (**B**) by As(III). Values are the average of at least 2 data sets $\pm$ average SEM.

A.				
As(III)-XPAzf binding	50 μM As(III)	100 μM As(III)	150 μM As(III)	200 μM As(III)
k_{fast} at 15 μ M peptide (min ⁻¹)	1.8 $\pm$ 0.67	5.0 $\pm$ 2.4	1.7 $\pm$ 0.26	1.9 $\pm$ 0.30
k_{fast} at 30 μ M peptide (min ⁻¹)	1.4 $\pm$ 0.46	2.1 $\pm$ 0.27	1.4 $\pm$ 0.081	1.7 $\pm$ 0.092
k_{fast} at 45 μ M peptide (min ⁻¹)	1.5 $\pm$ 0.28	2.1 $\pm$ 0.11	1.7 $\pm$ 0.077	1.7 $\pm$ 0.049
k_{fast} at 60 μ M peptide (min ⁻¹)	2.8 $\pm$ 0.62	1.2 $\pm$ 0.06	2.2 $\pm$ 0.21	2.1 $\pm$ 0.11
B.				
As(III)-PARP-1zf-1 binding	50 μM As(III)	100 μM As(III)	150 μM As(III)	200 μM As(III)
k_{fast} at 15 μ M peptide (min ⁻¹)	3.0 $\pm$ 0.078	4.3 $\pm$ 0.71	6.3 $\pm$ 0.090	7.9 $\pm$ 0.13
k_{fast} at 30 μ M peptide (min ⁻¹)	2.4 $\pm$ 0.55	3.0 $\pm$ 0.26	5.6 $\pm$ 0.37	5.4 $\pm$ 0.27
k_{fast} at 45 μ M peptide (min ⁻¹)	3.9 $\pm$ 0.54	5.6 $\pm$ 1.4	7.8 $\pm$ 0.53	8.9 $\pm$ 0.93
k_{fast} at 60 μ M peptide (min ⁻¹)	9.8 $\pm$ 1.6	8.3 $\pm$ 1.6	5.4 $\pm$ 0.38	9.2 $\pm$ 0.70

Table 3

Values of k_{slow} at 15, 30, 45, and 60 μM peptide for the binding of XPAzf (**A**) and PARP-1zf-1 (**B**) by As(III). Values are the average of at least 2 data sets $\pm$ average SEM.

A.

As(III)-XPAzf binding	50 μM As(III)	100 μM As(III)	150 μM As(III)	200 μM As(III)
k_{slow} at 15 μM peptide (min^{-1})	0.28 ± 0.055	0.46 ± 0.074	0.46 ± 0.090	0.48 ± 0.21
k_{slow} at 30 μM peptide (min^{-1})	0.33 ± 0.051	0.33 ± 0.058	0.33 ± 0.066	0.34 ± 0.067
k_{slow} at 45 μM peptide (min^{-1})	0.26 ± 0.015	0.36 ± 0.014	0.43 ± 0.041	0.32 ± 0.048
k_{slow} at 60 μM peptide (min^{-1})	0.31 ± 0.031	0.30 ± 0.057	0.42 ± 0.055	0.34 ± 0.042

B.

As(III)-PARP-1zf-1 binding	50 μM As(III)	100 μM As(III)	150 μM As(III)	200 μM As(III)
k_{slow} at 15 μM peptide (min^{-1})	0.20 ± 0.039	0.48 ± 0.090	0.21 ± 0.030	0.36 ± 0.030
k_{slow} at 30 μM peptide (min^{-1})	0.54 ± 0.054	0.49 ± 0.025	0.29 ± 0.017	0.25 ± 0.042
k_{slow} at 45 μM peptide (min^{-1})	0.24 ± 0.030	0.28 ± 0.016	0.34 ± 0.014	0.35 ± 0.019
k_{slow} at 60 μM peptide (min^{-1})	0.57 ± 0.051	0.39 ± 0.030	0.20 ± 0.02	0.29 ± 0.031

Table 4

Values of k_{fast} (**A**) and k_{slow} (**B**) at 15, 30, and 60 μ M peptide for As(III) binding to the C4 mutant of PARP-1-zf-1. Reported values have been determined from the average of at least 2 data sets $\pm$ average SEM.

A.

As(III)- PARP-1zf-1-C4mut binding	50 μ M As(III)	100 μ M As(III)	200 μ M As(III)
k_{fast} at 15 μ M peptide (min^{-1})	2.9 ± 0.036	5.9 ± 0.062	8.4 ± 0.12
k_{fast} at 30 μ M peptide (min^{-1})	3.2 ± 0.11	6.8 ± 0.40	8.8 ± 0.19
k_{fast} at 60 μ M peptide (min^{-1})	4.1 ± 0.48	6.5 ± 0.23	11 ± 0.91

B.

As(III)- PARP-1zf-1-C4mut binding	50 μ M As(III)	100 μ M As(III)	200 μ M As(III)
k_{slow} at 15 μ M peptide (min^{-1})	0.35 ± 0.034	0.33 ± 0.030	0.46 ± 0.027
k_{slow} at 30 μ M peptide (min^{-1})	0.30 ± 0.024	0.33 ± 0.015	0.32 ± 0.014
k_{slow} at 60 μ M peptide (min^{-1})	0.30 ± 0.023	0.30 ± 0.017	0.31 ± 0.018