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Interaction of arsenite with a zinc finger CCHC peptide: Evidence for formation of an As–Zn-peptide mixed complex

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ABSTRACT

The interaction of arsenite with a Cys_3His (CCHC) zinc finger model (34–51) HIV-1 nucleocapsid protein p7 (NCp7) peptide in the absence and presence of Zn^{II} was studied using fluorescence spectroscopy, CD (circular dichroism) and ESI-MS (Electrospray Ionization Mass Spectrometry). We found that arsenic forms different complexes with the free peptide and the zinc finger peptide. In the former case the peptide conformation differed greatly from that of the zinc finger, whereas in the second case a mixed As–Zn-peptide complex was formed with partial preservation of zinc finger conformation. An apparent stability constant was estimated for the mixed As–Zn-peptide complex ($K = 2083\ M^{-1}$ and $442\ M^{-1}$ at 25 °C and pHs 6 and 7, respectively). Our study also shows that the interaction of arsenic with the CCHC motif is facilitated by glutathione (GSH), through formation of a GS–As-peptide conjugate.

1. Introduction

Arsenic is a most toxic element in the environment introduced from both geochemical and anthropogenic sources. This metalloid is a classical example of dichotomy: on the one hand arsenicals are known carcinogens while on the other hand they have important antitumoral properties [1]. Arsenic is an ancient drug used in traditional Chinese medicine and in actuality, arsenic trioxide (ATO), is used in the treatment of relapsed acute promyelocytic leukemia (APL) [1–4].

The biological consequences of arsenic exposure are multiple but a critical event is elevation of reactive oxygen species which is then linked to induction of apoptosis through caspase activation [5, 6]. In this context, arsenic-containing compounds are potent modulators of the thioredoxin system, including thioredoxin, thioredoxin reductase, and NADPH [7], which controls to a large extent intracellular redox reactions, regulates apoptosis, and protects cells from oxidative stress damage. The ability of ATO to target and block thioredoxin reductase has been proposed to be responsible for its pro-apoptotic activity [6–8]. A presumed molecular target of ATO in APL patients is PML-RARα, a fusion protein containing sequences from the promyelocytic leukemia protein (PML) zinc finger protein and retinoic acid receptor alpha. It was recently demonstrated that arsenic binds directly to cysteine residues in Cys_3His (CCHC) and Cys_4 (CCCC) zinc finger motifs located within the RBCC domain of PML-RAR and PML, inducing degradation of PMLRARα and leading to apoptosis by promoting a specific postranslational modification (SUMoylation) of the PML moiety [9–13].

ATO was also found to decrease the activity of poly(ADP-ribose) polymerase (PARP), a zinc finger protein sharing the CCHC motif, involved in DNA repair [14]. Thus, zinc finger proteins have emerged as potential target for therapeutic metal compounds such as arsenicals and antimonials [4]. Interestingly, zinc finger proteins have also been found in micro-organisms, such as trypanosomatids and viruses. One subtype of the zinc finger domain is the CX2CX4HX4C (CCHC) structure that occurs twice in the nucleocapsid protein p7 (NCp7) of human immunodeficiency virus type 1 (HIV-1) and is absolutely conserved among all known strains of retroviruses, except human foamy viruses [15]. NCp7 protein contributes to the selection and packaging of the viral genome, as well as exercising additional functions critical to viral replication, through interactions with single-strand nucleic acids and viral proteins [16–17]. Recently we have reported the interaction of Sb^{III} with the C-terminal CCHC zinc finger (34–51) of HIV-1 NCp7, and confirmed Sb^{III} binding and Zn^{II} ejection [18].

Even though some studies have already evidenced the ability of As^{III} to interact with and modulate the function of zinc finger proteins, the binding events of the metal to the zinc finger motif and its impact on the protein structure are still poorly understood. In this work, the

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interaction of As^{III} with the C-terminal CCHC zinc finger (34–51) NCp7 peptide in the absence and presence of Zn^{II} was studied. Surprisingly, we found that arsenic forms different complexes with the free peptide and the zinc finger peptide. In the former case the peptide conformation differed greatly from that of the zinc finger, whereas in the second case a mixed As–Zn-peptide complex was formed with partial preservation of zinc finger conformation. Our study also shows that the interaction of As^{III} with the CCHC motif is facilitated by reduced glutathione (GSH), through formation of a GS–As–Zn-peptide conjugate.

2. Experimental

2.1. Chemicals

KGCWKCGKEGHQMKDCTE, CCHC-type zinc finger (ZF) peptide derived from the HIV nucleocapsid NCp7 protein [18,22] was obtained from Gen Script Corporation (Piscataway, NJ, USA) at a purity higher than 98%. GSH, Sodium arsenite ($NaAsO_2$) and ethylenediaminetetraacetic acid (EDTA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The $As(GS)_3$ conjugate was prepared by mixing GSH and sodium arsenite at 0.1 M and 0.033 M final concentrations, respectively, at pH 8.2 under nitrogen atmosphere [19].

2.2. Preparation of zinc finger peptide complex

The Zn^{II}-associated peptide (ZF) was obtained as described previously [18] by incubating 2mM peptide with $Zn(acetate)_2$ at 1:1.2 M ratio in aqueous solution for 2 h at pH 6 and 37 °C under nitrogen atmosphere.

2.3. Circular dichroism spectroscopy

Circular dichroism (CD) spectra were recorded at 25 °C on a Chirascan spectropolarimeter (Applied Photophysics, UK) in the wavelength range of 190–280 nm, in 10-mm cuvet with the peptide at 20 μM final concentration in phosphate buffer at pH 6. All spectra represent the average of 5 scans. Spectra were subtracted from that of the buffer registered in the same experimental conditions.

2.4. Electrospray ionization mass spectrometry (ESI-MS)

The ZF + As sample was prepared by adding aqueous sodium arsenite (1 mM) to 0.5 mM ZF aqueous solution and adjusting pH to 6.5. The ZF + $As(GS)_3$ was prepared by adding to an aqueous solution of ZF (1 mM) an equimolar amount of the $As(GS)_3$. Samples were diluted 10-fold in 0.1% trifluoroacetic acid (TFA) just before injection.

Positive electrospray ionization (ESI) was performed on a Waters/Micromass QTOF-2 mass spectrometer and equipped with an electrospray ionization source operated in positive ion mode [20]. Sample was introduced into the inlet at 4.0 uL/min in water. ESI capillary voltage was held constant at 2.85 kV and a cone voltage of 38 V was used. The ESI source was maintained at 105 °C and N_2 desolvation gas at 100 °C was used throughout the experiment. All spectra were internally calibrated using polyalanine over the entire mass range. Data were processed using Masslynx 4.0 software.

2.5. Fluorescence measurements and titration

Fluorescence measurements were carried out on a Cary Eclypse spectrofluorometer (Varian, Inc., Australia) at 25 °C in 1-cm cuvet, exploiting the intrinsic fluorescence of the peptide. Kinetics were recorded with excitation at 280 nm and emission at 360 nm in 50 mM phosphate buffered (pH 6) or 200 mM phosphate buffered (pH 7) solution. The fluorescence emission spectra were recorded with excitation at 280 nm. Preformed ZF peptide (20 μM) was titrated fluorimetrically with sodium arsenite in 50 mM phosphate pH 6 or 200 mM phosphate pH 7 at 25 °C.

Assuming that the predominant peptide species in this assay are Zn-peptide and $(As)_n$–Zn-peptide, the fraction of $(As)_n$–Zn-peptide species was calculated from $[(I_{Zn} - I_m)/(I_{Zn} - I_{As})]$, where I_{Zn} represents the fluorescence intensity of the peptide bound to Zn^{II}, IAs is the fluorescence intensity of ZF peptide bound to As^{III} and I_m is the measured fluorescence intensity at the specific As/Zn ratio.

From the fluorimetric titration data, $[(As)_n$–Zn-peptide] was calculated as follows:

$$\left[(As)_n - Zn - peptide\right] = [peptide]_{tot}\left[(I_{Zn} - I_m)/(I_{Zn} - I_{As})\right] \quad (1)$$

where $[peptide]_{tot}$ represents the total concentration of peptide (free and bound to Zn and As).

The concentrations of Zn-peptide and As were also deduced as follows:

$$[Zn - peptide] = [peptide]_{tot} - \left[(As)_n - Zn - peptide\right] \quad (2)$$

$$[As] = [As]_{tot} - n\left[(As)_n - Zn - peptide\right] \quad (3)$$

where $[As]_{tot}$ is the total concentration of As added to the solution.

Considering that arsenite is added in large excess compared to the peptide concentration, Eq. (3) can be simplified as:

$$[As] \approx [As]_{tot}. \quad (4)$$

To estimate the stoichiometry of arsenite binding to the zinc form of the peptide, the following reaction and apparent stability constant (K) were considered:

$$n\ As + ZF - peptide \leftrightarrow (As)_n - ZF - peptide \quad (5)$$

$$K = \left[(As)_n - ZF - peptide\right]/[ZF - peptide][As]^n$$

From Eqs. (1), (2), (4) and (5), the following equation can be deduced:

$$Ln[(I_{Zn} - I_m)/(I_m - I_{As})] = n\ Ln\left([As]_{tot}\right) + Ln(K) \quad (6)$$

n was determined from the linear regression of Eq. (6).

In this assay, the following reaction and equilibrium constant were finally considered:

$$As + Zn - peptide \leftrightarrow As - Zn - peptide \quad (7)$$

$$K = [As - Zn - peptide]/[As][Zn - peptide].$$

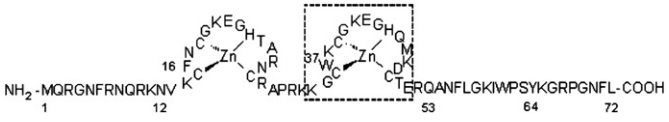
From Eqs. (1), (2), (4) and (7), the following equation can be deduced:

$$1/(I_{Zn} - I_m) = 1/K[As]_{tot}(I_{Zn} - I_{As}) + 1/(I_{Zn} - I_{As}) \quad (8)$$

K was determined from the linear regression of Eq. (8).

3. Results and discussion

The zinc-finger peptide model used in this study is (34–51)NCp7, a peptide corresponding to the distal (C-terminal) finger motif of NCp7 (boxed in structure below):



The choice of this isolated CCHC motif is justified because: (i) the binding information is essentially encoded by the CCHC motifs