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Cys redox reactions and metal binding of a Cys_2His_2 zinc finger

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Abstract

The elucidation of mechanisms by which cysteine (Cys) redox reactions influence metal binding to zinc finger domains is important for understanding the structure and function of zinc fingers. The present studies utilize electrospray ionization mass spectrometry (ESI-MS) to analyze Cys redox reactions and their influence on metal ion binding to a synthetic polypeptide similar in motif to the third zinc finger of the RNA polymerase II transcription factor, Sp1 (Sp1-3). The differential specificity of metal binding events to this zinc finger domain is demonstrated over a range of redox-altering dithiothreitol, hydrogen peroxide, and hydrogen ion concentrations. By analyzing this Cys_2His_2 zinc finger domain at single Da resolution with ESI-MS, shifts in the natural isotope cluster demonstrate that a Cys thiol and thiolate can contribute to Zn^{2+} and other metal ion coordination. These experiments provide insight into the basic redox chemistry and metal binding mechanisms of Cys_2His_2 zinc finger domains.
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Zinc finger proteins comprise a large class of eukaryotic proteins that have diverse roles in many cellular processes including transcription and translation, DNA replication and repair, metabolism, cell signaling and proliferation, and apoptosis [1]. The term "zinc finger" describes a variety of compact protein domains stabilized by structural zinc (Zn^{2+}) ions that interact minimally with Cys residues [2]. The TFIIIA-type zinc finger domain binds Zn^{2+} via two Cys and two His residues, and folds into a ββ-hairpin containing the Cys residues followed by a loop, and then an α-helix containing the His residues; the α-helix also forms the DNA binding region of this type of zinc finger [3,4]. Zinc fingers are key targets for small molecules known to alter cellular physiology. Reactive oxygen species (ROS) are proposed to interact with zinc finger domains and eject Zn^{2+} by a mechanism involving oxidizing coordinating Cys residues to disulfides [5,6]. The Cys_2His_2 zinc finger transcription factor, Sp1, is under redox control both in vitro and in vivo [7]. The redox regulation of zinc fingers is one mode of gene regulation that affects a variety of redox sensitive physiological states including proliferation [8], differentiation [9], apoptosis [10], and senescence [11]. In primary breast tumors, DNA binding of ER (estrogen receptor) zinc fingers is inhibited by oxidation mechanisms presumably involving Cys residues [12]. Zn^{2+} homeostasis is a process linked to redox conditions in the cell [13] and studies have indicated that reactive small molecules could control Zn^{2+} transfer from metallothionein to zinc-dependent proteins [14,15]. Additionally, some cell signaling properties of nitric oxide (NO) are proposed to occur through reactions with Cys residues and Zn^{2+} coordination sites [15–17].

Xenobiotic metals with carcinogenic and/or toxic potential are another class of small molecules that can disrupt zinc finger domains and induce alterations in gene expression [18]. Recent studies in a yeast model have indicated that the chronic exposure to environmentally relevant doses of cadmium (Cd^{2+}) ions can disrupt

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DNA repair mechanisms possibly through interacts with zinc finger domains [19]. These doses are similar to levels of Cd^{2+} that can accumulate in the human body [19]. Lead (Pb^{2+}) ions were also reported to inhibit the DNA repair process conceivably through interactions with zinc finger domains [20], and studies have indicated that Pb^{2+} ions can cross the cell membrane and associate with mostly nuclear proteins [21]. Because metals such as Cd^{2+} and Pb^{2+} specifically interact with Cys residues and zinc finger proteins are critical components of the DNA repair machinery, zinc finger proteins are predicted to be in vivo targets for these toxic metals. In vitro studies with TFIIIA demonstrated that micromolar concentrations of Cd^{2+} and Pb^{2+} ions can inhibit specific DNA binding through interactions with the zinc finger structure [22,23]. Gel shift analyses revealed that Pb^{2+} ions can alter the specific DNA binding ability of Sp1 in vivo and in vitro [24,25]. Potentially these metals could alter the zinc finger conformation by replacing Zn^{2+} or by binding the finger structure in addition to Zn^{2+}. This first hypothesis is supported by electrospray ionization mass spectrometry (ESI-MS)[1] experiments that revealed Zn^{2+} can be replaced by copper (Cu^{2+}) ions within the estrogen receptor DNA binding domain (ER-DBD) [26].

A number of metal binding studies with individual zinc fingers were previously performed under anaerobic conditions to avoid oxidation of the zinc finger Cys residues [27–31]. In the present study, metal coordination is examined under reducing and oxidizing environments that may resemble cell redox conditions more than studies performed in the absence of oxygen and reducing agents. Because of their compact and structurally defined motif, zinc fingers from consensus sequences as well as from native or altered fingers such as from the RNA polymerase II activator protein, Sp1, are used as models for Zn^{2+} coordination domains [27–34]. In the present studies, ESI-MS is used to analyze metal–ligand and redox reactions with a synthetic peptide similar in motif to the third zinc finger of Sp1 (Sp1-3). The use of ESI-MS with redox active molecules such as dithiothreitol (DTT) and hydrogen peroxide (H_2O_2) demonstrate differential specificity of Zn^{2+}, Cd^{2+}, and Pb^{2+} ions to this model Cys_2His_2 zinc finger domain. In addition, the natural carbon and Zn^{2+} isotopes can be discerned for the apo-Sp1-3 peptide (reduced and disulfide) and the metal-bound Sp1-3 peptide. This isotope cluster analysis allows for the protonation state of the Zn^{2+} coordinating Cys residues to be investigated under reducing and/or oxidizing conditions.

[1] *Abbreviations used:* M/Z, mass to charge ratio; Sp1, specificity protein 1 transcription factor; Sp1-3, the third zinc finger motif from the amino terminal end of Sp1 transcription factor; apo-Sp1-3 peptide, the reduced non-metal bound form of Sp1-3 finger motif; DTT, dithiothreitol; ESI-MS, electrospray ionization mass spectrometry; H_2O_2, hydrogen peroxide; TOF, time-of-flight; TFIIIA, transcription factor IIIA.

Materials and methods

Handling of Sp1-3 model peptide

The peptide (KKFACPECPKRFMSDHLSKHIK-THQNKK, monoisotopic mass 3365.7 Da), similar to zinc finger three of the RNA polymerase II activator, Sp1, was commercially synthesized by Genemed Synthesis, San Francisco, CA. This peptide was generated by Fmoc-solid phase peptide synthesis technology and purified by HPLC utilizing a C-18 column. The identity of the peptide was confirmed through MS and HPLC analysis at Genemed Synthesis. The purified Sp1-3 peptide was lyophilized and shipped in 1 mg aliquots which were then stored at −20 °C. The exact monoisotopic mass for this peptide structure was also confirmed in the present ESI-MS study. For experimental analysis, a Sp1-3 peptide aliquot was resuspended in 1–3 mM DTT in deionized water (Sigma Chemical) to a final peptide concentration of 1 mg/ml. This solution was then realiquoted and stored at −20 °C. Storage of the peptide in these DTT concentrations allowed the Cys residues to remain in the reduced state.

Electrospray ionization mass spectrometry

Sp1-3 peptide interactions with metal ions and redox agents were analyzed by ESI time-of-flight (TOF) mass spectrometry using an Applied Biosystems Mariner System (Foster City, CA). Typically, the diluted peptide and metal ions as acetate salts were reacted for 10 min at 25 °C and applied to the mass spectrometer utilizing the following buffers in deionized water: 30 μM to 1 mM DTT, 10–20 μM peptide, 5 mM ammonium acetate, pH 6.8, and 5% methanol. The pH of the buffer was lowered with acetic acid or raised with ammonium hydroxide. Experiments examining H_2O_2-mediated oxidation were performed by first binding metal ions to the peptide followed by 30–60 min treatment with 25–250 μM H_2O_2. These H_2O_2 additions did not alter the buffer pHs. The reactions were infused directly into the electrospray ionization source at a flow rate of 1 μl/min. Using the positive ion mode, spectra were obtained with isotopic resolution. The positive ion spectra were an average of 10 s per scan acquired over 5 min. The spectra were analyzed and deconvoluted using the software package, Data Explorer Version 4.0.0.1, supplied by Applied Biosystems. This software package was used to generate theoretical isotope clusters from the formula weight of potential Sp1-3 complexes. The instrument settings for these ESI-MS studies were as follows: spray tip potential, 1788.87; nozzle potential, 100.10; skimmer 1 potential, 11.01; quad DC potential, 6.23; deflection voltage, 0.39; einzel lens potential, −27.00; quad RF voltage, 700.2; nozzle temperature, 135.01 °C; push pulse potential, 675–700; pull pulse potential, 249.07; pull bias

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