

Published in final edited form as:

Mar Environ Res. 2000 ; 50(1-5): 89–92.

## Cadmium and lead interactions with transcription factor IIIA from *Xenopus laevis*: a model for zinc finger protein reactions with toxic metal ions and metallothionein

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### Abstract

Zinc finger proteins comprise the largest class of eukaryotic transcription factors. The metal binding sites in these proteins have been proposed as plausible targets for exchange reactions between zinc and toxic metal ions that lead to the alteration of function of the proteins in gene transcription. According to the present work, both  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$  displace  $\text{Zn}^{2+}$  from transcription factor IIIA (TFIIIA). Neither product binds to the internal control region (ICR) of the 5 S rRNA gene, the normal binding site for Zn-TFIIIA. Furthermore, the adduct of Zn-TFIIIA with ICR is also reactive with  $\text{Cd}^{2+}$  and  $\text{Pb}^{2+}$ , leading to the dissociation of the DNA–protein complex. Cd-TFIIIA reacts with apometallothionein (apoMT) to form Cd-MT and apoTFIIIA. Similarly,  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  can be exchanged in the reaction of Cd-TFIIIA with Zn-MT. Zn-finger 3 of TFIIIA has also been examined to compare the reactivity of a single finger motif with fingers in the holoprotein. Zn-finger 3 reacts with much faster kinetics than the holoprotein.

### Keywords

Zinc-finger; Cadmium; Lead TFIIIA; Metallothionein

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The most common protein DNA binding motif among transcription factors in eucaryotes is the Zn finger structure that is stabilized through binding of a  $\text{Zn}^{2+}$  ion to two imidazole nitrogen (N) and two cysteine sulfhydryl (S) ligands. In the absence of  $\text{Zn}^{2+}$  its conformational integrity is lost and the domain no longer associates with DNA. Because transcription factors play such a central role in cell regulation, Zn finger proteins have attracted much attention.

The prototypical Zn finger transcription factor is transcription factor IIIA (TFIIIA), isolated from the immature ovary of *Xenopus laevis*. It binds to the internal control region (ICR) of the 5 S ribosomal RNA gene and stimulates its transcription. The product 5 S rRNA competes with the ICR for binding Zn-TFIIIA to inhibit its own synthesis. TFIIIA largely comprises nine consecutive Zn finger domains which differentially interact with the ICR DNA or with 5 S rRNA.

Studies with TFIIIA and other Zn finger structures suggest that the  $\text{Zn}^{2+}$  is not bound as tightly in these molecules as in numerous other types of Zn-metalloproteins. Thus, when  $\text{N}_2\text{S}_2$  Zn finger proteins are prepared, buffers commonly contain  $\text{Zn}^{2+}$  to insure that the isolated proteins are saturated with metal ion (Del Rio & Setzer, 1991). Furthermore, competing small ligands such as EDTA rapidly inactivate TFIIIA and other Zn fingers,

whereas, such reactions involving other Zn-metalloproteins commonly occur very slowly (Hanas, Hazuda, Bogenhager, Wu & Wu, 1983; Petering, Krezoski, Chen, Pattanaik & Shaw, 1991). For these and other reasons, investigators have speculated that cellular control of gene regulation might include control by alteration in accessibility of Zn finger proteins to intracellular  $Zn^{2+}$ . Furthermore, these same properties make Zn finger sites attractive targets for toxic metal ions to bind in competition with  $Zn^{2+}$  (Makowski & Sunderman, 1992).

A protein where both of these possibilities may occur is in MTF-1, the transcriptional control protein for induction of metallothionein (MT) synthesis by  $Zn^{2+}$ . MTF-1 contains six Zn finger domains, is required for  $Zn^{2+}$  responsive MT induction *in vivo*, and binds to its DNA metal response element in a Zn dependent fashion *in vitro* (Radtke et. al., 1993). It has also been assumed that MTF-1 is the binding site for  $Cd^{2+}$  which results in MT induction (Andrews, 1990).

The present study examined interactions of  $Zn^{2+}$  and  $Cd^{2+}$  or  $Pb^{2+}$  with TFIIIA and reactions of these structures with MT to explore the chemical possibilities for reaction of Zn finger proteins with toxic metal ions and competing metal binding ligands.

TFIIIA was isolated from *E. coli* containing the cloned gene for *Xenopus laevis* TFIIIA (Del Rio & Setzer, 1991). The 106 base pair ICR was also obtained from a plasmid grown in *E. coli* and used as a  $^{32}P$ -labelled DNA binding site for TFIIIA in a standard electrophoretic mobility shift assay (Romaniuk, 1990). Finger 3 of TFIIIA (F3) was made by peptide synthesis and used for comparison with the native structure.

Isolation of Zn-TFIIIA in the absence or presence of  $Zn^{2+}$  resulted in protein samples that varied in Zn to protein stoichiometry from 2 to  $9\pm 2$ , confirming the qualitative view that  $Zn^{2+}$  is readily lost from the protein or difficult to acquire. One can reproducibly bind nine  $Zn^{2+}$ ,  $Cd^{2+}$ , or  $Pb^{2+}$  ions per mol of TFIIIA by titrating the apoprotein with metal ion at pH 7.4.

Titration data have been used to measure metal ion binding constants for F3. The results in Table 1 show that  $Zn^{2+}$  does not bind strongly to F3, and both  $Cd^{2+}$  and  $Pb^{2+}$  display larger formations constants, indicating that these metal ions bind preferentially to F3 in comparison with  $Zn^{2+}$ . Available information for two other finger peptides is also shown in Table 1. It is seen that there can be a large variation in binding affinity of  $Zn^{2+}$  for related finger structures. In the case of CP1, peptide association with  $Zn^{2+}$  is much more favorable than with  $Cd^{2+}$ . These results show first that individual Zn finger sites will be differentially sensitive to the concentration of cellular  $Zn^{2+}$  and second that certain Zn finger proteins will be preferentially susceptible to exchange of  $Zn^{2+}$  with  $Cd^{2+}$  and, presumably, also with  $Pb^{2+}$ .

$Cd^{2+}$  and  $Pb^{2+}$  stoichiometrically and rapidly displace  $Zn^{2+}$  from Zn-F3, consistent with the results shown in Table 1. Similarly, using an ultrafiltration method to separate TFIIIA from unbound metal ions, it was determined that these metal ions react rapidly with Zn-TFIIIA to form Cd- or Pb-TFIIIA. It had previously been shown that binding of  $Cd^{2+}$  inhibited subsequent association of TFIIIA with its ICR DNA binding site (Hanas & Gun, 1996). It was important to find out whether the Zn-TFIIIA-ICR adduct was also reactive with these metal ions. An electrophoretic mobility shift assay was done on reaction mixtures of  $Cd^{2+}$  or  $Pb^{2+}$  with the preformed Zn-TFIIIA-ICR adduct. With  $13 \mu M$   $Zn^{2+}$  in the buffer to help maintain Zn-saturated TFIIIA, four- and seven-fold excesses of  $Pb^{2+}$  and  $Cd^{2+}$ , respectively, were sufficient to completely dissociate the preformed adduct of Zn-TFIIIA-ICR. Thus, binding of Zn-TFIIIA to the ICR does not protect it from metal ion exchange reactions, which destroy its ability to specifically associate with DNA.

Previous experiments showed that apoMT can remove  $Zn^{2+}$  from TFIIIA, either in the absence or presence of the ICR (Petering et al., 1999; Zeng, Vallee & Kägi, 1991). In Fig. 1, a reaction of apoMT with Cd-TFIIIA is shown, indicating that Cd-MT and apoTFIIIA form efficiently with a modest rate constant of  $40 \text{ s}^{-1} \text{ M}^{-1}$ . In the cell this reaction might rescue the Cd-TFIIIA molecule. The metal ion exchange reaction between Cd-TFIIIA and Zn-MT, which produces Zn-TFIIIA and Cd-MT, was also observed by ultrafiltration but took place at a slower rate. The same reaction of Cd-F3 with Zn-MT occurred rapidly, indicative of the greater kinetic reactivity of the peptide than the holoprotein.

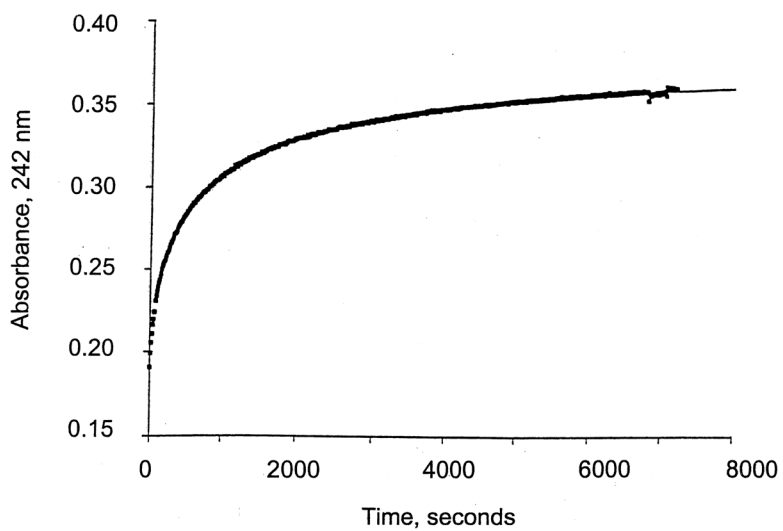
In summary, these results demonstrate how differential binding of toxic metal ions to Zn finger transcription factors can occur and show that even when bound to their DNA binding sites, Zn finger structures can be readily reactive with  $Cd^{2+}$  or  $Pb^{2+}$ . The fact that both metal ions cause dissociation of TFIIIA from the 5 S rDNA ICR indicates that these metal ions alter the finger domain conformation needed to specifically interact with DNA. In particular, this result brings into question the hypothesis that  $Cd^{2+}$  binds to MTF-1 and activates its binding to DNA. Finally, the much larger reactivity of the individual Zn finger, Zn-F3, than the holoprotein Zn-TFIIIA, suggests that tertiary structural features play a significant role in its reactions.

## Acknowledgments

This research was supported by NIH grants ES-04026 and ES-04184.

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**Fig. 1.** Kinetics of  $\text{Cd}^{2+}$  transfer between Cd-TFIIA ( $42 \mu\text{M}$  Cd) and apoMT ( $6 \mu\text{M}$ ). Formation of Cd-MT was followed at 242 nm. Buffer: 20 mM HEPES, pH 7,  $25^\circ\text{C}$ .

**Table 1**Log formation constants for  $M^{2+}$ -finger complexes at pH 7.4 and 25°C of TFIIIA and other finger peptides<sup>a</sup>

	<b>F3</b>	<b>Sp1 (F3)</b>	<b>CP1</b>
Co <sup>2+</sup>	5.1	6.5	7.2
Zn <sup>2+</sup>	7.4	9.2	11.2
Cd <sup>2+</sup>	8.3		8.7
Pb <sup>2+</sup>	8.7		

<sup>a</sup>Abbreviations and references: Sp1 (F3), finger 3 of Sp1 transcription factor (Posewitz & Wilcox, 1995), CP1, consensus peptide derived from available sequences of N<sub>2</sub>S<sub>2</sub> domains (Krizek, Merkle & Berg, 1993).