Metal Replacement in "Zinc Finger" and Its Effect on DNA Binding

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Metal replacement studies were used to investigate the metal requirement of a bacterially expressed polypeptide encoding the zinc finger DNA binding domain of the estrogen receptor. Apoloppeptide was generated by dialysis of native polypeptide against low-pH buffer under reducing conditions. Specific DNA binding can be restored by refolding the apoloppeptide in the presence of ionic zinc, cadmium, or cobalt. However, refolding in the presence of copper or nickel fails to regenerate DNA binding activity. While cobalt-reconstituted polypeptide has a reduced affinity for its AGGTCA-binding site compared to zinc- or cadmium-polypeptide, it has the surprising property of increased cooperative DNA binding. Our work indicates that metal substitution results in a range of effects upon DNA binding in vitro. The potential biological significance of metal substitution in vivo is discussed. — Environ Health Perspect 102(Suppl 3):196–198 (1994)

Key words: zinc fingers, copper, nickel, cobalt, cadmium, metal toxicity, DNA binding, nuclear hormone receptor, estrogen receptor

Introduction

Although originally identified in the Xenopus transcription factor TFIIEA less than 10 years ago (1), zinc fingers now form the largest known class of DNA-binding proteins. Folding of individual zinc finger domains is achieved in part through coordination, by cysteine or histidine side-chains, of one zinc atom. However, although zinc is generally presumed to be the endogenous metal ion within zinc fingers, few studies have actually demonstrated this to be the case. In fact, it is known from in vitro studies that, in some instances, metals other than zinc are capable of functioning in zinc fingers (2–5). Even metals which fail to function in zinc fingers may be of physiologic significance if they can compete with zinc for the metal-coordinating ligands of the fingers. Thus, it is important to characterize the interaction of metals other than zinc with zinc finger domains.

For these studies we have chosen a member of the nuclear hormone receptor subclass of zinc fingers (6). These proteins, which include receptors for a wide variety of hormones including estrogen, proges-
terone, glucocorticoid, retinoid, and thyroid hormones, act as ligand-induced transcription factors. They interact with their DNA-binding sites via a zinc finger motif DNA-binding domain. The structures of the DNA binding domains for both the glucocorticoid and estrogen receptors have been determined by NMR spectroscopy (7,8). As well, a glucocorticoid receptor DNA-binding domain/ DNA cocrystal structure has recently been solved (9). Both the estrogen and glucocorticoid receptor DNA-binding domains form zinc finger structures in which each of two zinc atoms is coordinated by four cysteine residues. However, unlike many other zinc fingers in which each finger exists as a structurally independent domain, the fingers of these receptors fold in on one another, forming one domain.

Like most other nuclear hormone receptors, the estrogen receptor binds, as a dimer, to a DNA sequence which contains two "half-sites." This estrogen response element, or ERE, consists of two AGGTCA half-sites spaced by three basepairs and arranged as inverted repeats (10,11). While an estrogen receptor DNA-binding domain polypeptide binds only as a monomer to an isolated half-site, it can interact cooperatively to form dimers on an actual ERE. Thus, the effect of metal replacement on both DNA binding affinity and cooperativity can be measured for the estrogen receptor zinc finger domain.

A purified, bacterially expressed polypeptide encompassing the DNA-binding domain of the estrogen receptor was employed in all of our studies (2). Apoloppeptide was generated by exposing the native polypeptide to low pH. Dialysis of this apoloppeptide against a neutral pH buffer fails to regenerate specific binding to an ERE half-site (Figure 1). Nor is specific binding observed when the apoloppeptide is similarly dialyzed against the identical buffer containing copper or

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nickel (Figure 2), although these metals do not appear to interact directly with the polypeptide (2,3). However, when apopolypeptide is dialysed against zinc-, cadmium-, or cobalt-containing buffer, specific binding is reconstituted (Figure 2). Methylation interference experiments demonstrate that native zinc-, cadmium-, and cobalt-reconstituted forms of the polypeptide interact with the AGGTCA (read TGACCT on the other strand) half-site in a qualitatively similar manner (Figure 3). The reduced intensity of the guanine band within the TGACCT half-site indicates that in each case, the polypeptide is interacting specifically with the half-site sequence. Quantification of DNA binding, however, has revealed that cobalt-reconstituted polypeptide has an affinity for theERE half-site some 10-fold lower than that of the native zinc- or cadmium-reconstituted form (Table 1).

The ability of zinc, cadmium, and cobalt to reconstitute the DNA-binding properties of the native polypeptide are consistent with the expected structural contribution of these metals, as they can all bind to sulfhydryl ligands with tetrahedral geometries. The inability of apoceptor DNA-binding to be restored by copper or nickel is also not unexpected. Square-planar geometries are more common for nickel. Nickel binding may result in fingers distorted from the normally tetrahedral metal site, resulting in a polypeptide incapable of specifically interacting with the DNA. Copper, on the other hand, in the form of copper(I), has a high affinity for sulfhydryl ligands but has less stringent geometric requirements than zinc. Therefore, it is not likely to demand proper folding of the DNA-binding domain.

To facilitate the determination of DNA-binding constants for the various metal-substituted forms of the polypeptide, DNA containing a single half-site was used for the studies described above. Since both the monomeric and dimeric bound forms of the polypeptide can be resolved upon binding to an actual ERE (Figure 4), it was possible to quantitate the extent of cooperativity. A general model for ERE binding is presented in Figure 5. The polypeptide binds to either of the two hexameric half sites with identical affinity, “K”. Binding of the second polypeptide occurs with an affinity equal to $\tilde{\omega}K$, where $K$ is the polypeptide association constant for a half site and $\tilde{\omega}$ is the cooperativity parameter. Thus, the binding equation can be derived from the equilibria:

$$P + f_{AF_B} = b_{A^*F_B}$$

$$(1)$$

$$P + f_{AF_B} = f_{AB}$$

$$(2)$$

$$P + b_{AB} = b_{A^*B^*}$$

$$(3)$$

$$P + b_{AB} = b_{A^*B^*}$$

$$(4)$$

where $P$=free protein, $f_{AF_B}$=free DNA, $b_{A^*F_B}$=protein bound to site A only, $f_{AB}$=protein bound to site B only, $b_{A^*B^*}$=protein bound to both sites A and B.

Cooperativity values were determined by mathematical best fit of data over a range of $\tilde{\omega}$ values. Data fitting was accomplished with a computer program designed by PF Predki.* A cooperativity parameter

*The program was written for an Apple Macintosh computer and runs under HyperCard. It is available on request from the author.

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Table 1. Dissociation constants of polypeptide binding to ERE hexamer sequence as determined by double-reciprocal plot analysis of mobility shift assay results.

| Polypeptide form | $K_D$ nM |
|------------------|----------|
| Native           | 48       |
| Zinc             | 66       |
| Cadmium          | 48       |
| Cobalt           | 720      |

*Reproduced from Predki and Sarkar (2).
of $>1$ indicates positive cooperativity while a value of 1 indicates no cooperativity and a value $<1$ indicates negative cooperativity.

Previously, we determined a value of 109 for $\omega$ upon binding of native polypeptide to the ERE (Table 2). Here, we observe that cadmium substitution has only a minor effect on the cooperativity of binding to an ERE (Figure 6A). However, cobalt-substituted polypeptide has an approximately 5-fold increased cooperativity (Figure 6B). Thus, cobalt substitution has the effect of decreasing the affinity of the receptor with respect to copper. To understand the cooperativity of the polypeptide, we observed that the polypeptide appears to undergo upon DNA binding by analogy to the glucocorticoid receptor DNA binding domain (9). This distortion may be more energetically favorable with cobalt than with zinc or cadmium polypeptides.

While our studies demonstrate a variety of effects of metal substitution on the DNA-binding properties of the estrogen receptor DNA-binding domain, the physiologic significance of these observations is not entirely clear. However, our results do provide a useful starting point for further investigation.

One function likely to be effected by metal substitution is transcriptional regulation itself. Our results suggest, for instance, that misincorporation of copper would lead to a transcriptionally inactive receptor. In fact, if copper binding causes misfolding, the receptor may be rapidly degraded within the cell. Presumably, under normal physiologic conditions, the intracellular concentration of copper is low enough that such events are insignificant (unless copper is directly involved in regulating receptor activity). However, in certain circumstances the intracellular concentration of copper may become significant. For instance, this could occur in Wilson and Menkes diseases, in which certain cells and tissues retain elevated levels of copper. In addition, in a zinc deficient state, copper may be able to competively interfere with the decreased cellular concentration of zinc. In fact, some symptoms of zinc deficiency may be directly attributable to misfolding of steroid receptors (12).

Another potential effect of metal substitution in zinc fingers was proposed recently by Sunderland and Barber (13). It is known that a number of redox metals are capable, even when bound to protein ligands, of catalyzing the production of high energy radicals (14–16). If these radicals are of high enough energy that they can cause damage to DNA.

![Figure 6](image_url)  
**Figure 6.** Species distributions of cadmium (A) and cobalt (B) reconstituted polypeptide with the ERE. In each case the points correspond to data points determined experimentally from mobility shift autoradiographs. The fraction of monomer or dimer present is plotted as a function of % bound. The curves are mathematically determined from best fit of the data and the $w$ values corresponding to these curves are indicated in Table II. Mobility shift assays were performed as previously described (2). For quantitative purposes, multiple exposures of varying lengths of time were taken in order to ensure a linear response. For determination of cooperativity parameters, free (f) and bound (complex a and b) forms were quantitated by scanning laser densitometry of the X-ray film on a LKB Ultrascan XL.

| Table 2. Cooperativity values for interaction with an ERE for various metal substituted forms of the estrogen receptor DNA-binding domain polypeptide. |
|-------------------------------|-----------------|
| Polypeptide form | $\omega$ |
| Native | 109 |
| Cadmium | 85 |
| Cobalt | 516 |

Substitution of zinc for such a metal, for example cobalt, would effectively place this metal in close proximity to DNA. It would, in effect, target it to a region of DNA important for genetic regulation, damage of which could have serious consequences for the cell. Of course, the metal need not be within the zinc finger to generate radicals. Metals may also bind to suitable ligands on the exterior of the zinc finger domain. This mode of metal binding likely explains the existence of other apparent low affinity metal-binding sites on such domains (17).

In summary, the experiments demonstrated that metal replacement can result in a range of effects upon DNA binding in vitro. Both DNA binding affinity and the DNA-dependent protein–protein interactions mediating cooperative binding were affected to various extents by metal substitution. Perhaps even more critical is the potential significance of these observations in vivo. Clearly, further investigations into the potential biological ramifications of zinc finger metal substitution are warranted.
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