ROLE OF CHELATABLE METAL IONS IN ARYL HYDORCARBON RECEPTOR (AHR) FUNCTION

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Introduction

The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor. In the absence of ligand, the AhR exists as part of a cytosolic protein complex associated with two molecules of heat shock protein (hsp90). Exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin, TCDD) results in dissociation of the cytosolic complex and accumulation of the AhR within in the nucleus (receptor transformation). Dimerization of the AhR with the nuclear aryl hydrocarbon receptor translocator (Arnt) protein results in formation of a protein:DNA complex at sequence-specific enhancer elements (dioxin responsive elements, DRE) which drives TCDD-inducible expression of adjacent genes.

Unlike other ligand activated transcription factors (e.g., steroid hormone receptors) the role of chelatable metal ions (e.g., Zn^{++}, Ca^{++}, Mg^{++}, etc) in aryl hydrocarbon receptor (AhR) function is unclear. In the case of steroid hormone receptors, chelation of metal ions results in inhibition of receptor function via disruption of the zinc finger motifs identified within the DNA binding domains. While the AhR lacks a zinc finger motif, there is evidence to suggest that its function requires the presence of metal ions. Previous studies in our laboratory demonstrated that EDTA treatment of cytosolic extract prior to addition of ligand resulted in a significant decrease in AhR:DNA complex formation with no alteration of ligand binding activity[1,2]. However, addition of EDTA or the chelator 1,10-phenanthroline after ligand-induced transformation had no effect on formation of a DNA complex suggesting that AhR transformation, but not ligand binding or DNA binding of the transformed receptor, is sensitive to metal depletion. In contrast, another laboratory reported that inhibition of AhR:DNA complex formation occurred in the presence of the chelating agent 1,10-phenanthroline as well as its non-chelating isomers suggesting that metal ions are not required for AhR transformation[3]. In the present study, we provide additional evidence that chelatable metal ions are required AhR transformation. However, once the AhR has transformed, the formation of the AhR:DNA complex is resistant to metal ion depletion.

Materials and Methods

Preparation of Cytosol. Liver tissue was harvested from male Hartley guinea pigs and male Sprague-Dawley rats. Hepatic cytosol was prepared in HDG buffer (25 mM Hepes, pH 7.5, 1 mM DTT, and 10% (v/v) glycerol) as previously described and was stored at -80°C until use[5]. Chelex-100 resin was washed with HDG and 2ml was placed in a 3ml syringe. To the column 0.5ml of rat or guinea pig cytosol, either untreated or TCDD (20nM) treated, was added and spun at 2500g for 2min. This method of treatment allowed the Chelex treated cytosol to remain undiluted.

Sucrose Density Centrifugation. Cytosol (20 mg/ml) was incubated with either 10 nM [^{3}H]TCDD in the absence or presence of 1 mM TCDF for 1 h at 4^{o}C. After treatment with dextran-coated charcoal to remove unbound and loosely bound radioligand, samples were subjected to centrifugation in 10-30% sucrose (v/v) gradients and fractionated as we have previously described[4]. [^{14}C]-BSA and [^{14}C]-catalase were included in the gradients as internal sedimentation markers. The specific binding of [^{3}H]TCDD was determined by subtracting the radioactivity present in each fraction of a gradient containing [^{3}H]TCDD and TCDF (non-specific binding) from the radioactivity in the corresponding fractions from a gradient containing [^{3}H]TCDD alone (total binding).

Dialysis of Chelex-Treated Cytosol. Cytosol samples were dialyzed overnight at 4°C in HDG buffer in the absence or presence of 50μM of either $CdCl_2$, $ZnCl_2$, $MgCl_2$, or $MnCl_2$. Cytosol samples were then incubated with DMSO or 20nM TCDD for 2h at 20°C and subjected to gel retardation analysis as described below.

Gel Retardation Analysis. The oligonucleotide sequence used in this assay 5'-GATCTGGCTCTTCTCACGCAACTCCG-3' and the complementary strand 5'-GATCCGGAGTTGCGTGAGAAGAGCCA-3' were synthesized, purified, annealed and radiolabeled with γ-[^{32}P]-ATP. This sequence represents DRE3 of the mouse CYP1A1 gene. Guinea pig hepatic cytosol (16mg protein/ml) was incubated with DMSO (20 μl/ml) or TCDD (20nM) for 2h at 20°C. Cytosol samples were mixed with poly (dI·dC) and incubated for 15 min at 20°C followed by the addition of ^{32}P-labeled for an additional 15 min. AhR:Arnt:DRE complexes were resolved on a 4% non-denaturing polyacrylamide gel as described previously[5]. Protein-DNA complexes were visualized by autoradiography of the dried gels.

Results and Discussion

Previous studies have employed a variety of soluble chelating agents to examine the role of metal ions in AhR function with the results open to a variety of interpretations. Earlier work used 1,10-phenanthroline, but inhibition of AhR binding to the DRE was also observed in the presence of the non-chelating isomers of phenanthroline[3]. The classic chelators EDTA and EGTA also reduce formation of AhR:DRE complexes when applied prior to TCDD[1-3]. However, removal of EDTA by gel filtration restored AhR:DRE complex formation without replenishing ion levels[3]. While these results suggest that the effects of chelating agents are independent of their ability to bind metal ions, an alternative hypothesis is that these agents interact with metal ions integrated within the AhR complex. While this interaction is insufficient to extract the metal ion(s) from the complex, these chelating compounds are able to distort critical structural characteristics of the AhR such that the receptor fails to bind DNA. Once removed, AhR structure and function are restored. It is important to note that all data indicates that the pre transformed AhR is more sensitive to chelator effects than the post transformed receptor suggesting that a structural change in the AhR protects critical chelator targets.

We have used a solid chelating matrix, Chelex 100, to examine the role of chelatable metal ions in AhR function. Using a solid matrix allowed us to eliminate direct interactions between the chelator and the AhR as a mechanism of inhibition. Our approach was to subject guinea pig cytosol to either standard gel or Chelex column filtration both before and after TCDD-induced transformation. We were then able to use gel retardation analyses to compare the ability of the AhR in these samples to form DNA complexes. The process of gel filtration, in itself, did not

alter AhR:DNA complex formation [Figure 1A]. Chelex treatment of guinea pig cytosol prior to TCDD incubation, virtually eliminated AhR:DRE complex formation. In contrast, AhR:DRE complex formation was only moderately inhibited in cytosol samples subjected to Chelex treatment after TCDD incubation [Figure 1B]. These observations were not due to loss of ligand binding activity as evidenced by determination of specific binding activity in non-Chelex and Chelex-treated samples [Figure 2]. Finally, dialysis did not restore AhR:DRE complex forming activity to chelex-treated cytosol [Figure 3, lanes 3,4] suggesting that the observed inhibition was not due to a soluble contaminant of the Chelex preparation. Adding 50μM of either $CdCl_2$ or $ZnCl_2$ during dialysis, however, restored the ability of AhR to bind DRE in Chelex treated samples [Figure 3, lanes 5-8]. Metal ion specificity was observed in that neither $MgCl_2$ nor $MnCl_2$ were able to restore AhR:DRE complex formation [Figure 3, lanes 9-12]. These results suggest that chelatable metal ions are required for AhR function. The fact that receptor DNA binding activity is more resistant to Chelex treatment after TCDD-induced transformation than before TCDD exposure suggests that metal ions are required for AhR transformation, but not for DNA binding.

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References

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Figure 1: Analysis of AhR:DRE complex formation following gel filtration [A] and Chelex treatment [B] of guinea pig cytosol.

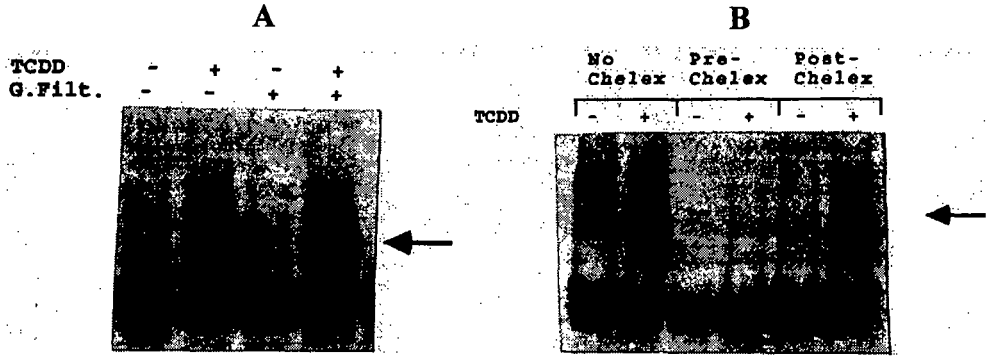


Figure 2: Ligand binding activity in guinea pig cytosol is not altered by Chelex treatment.

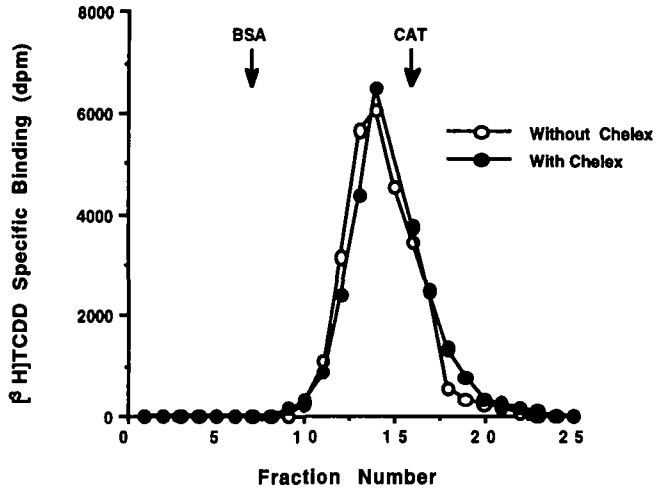
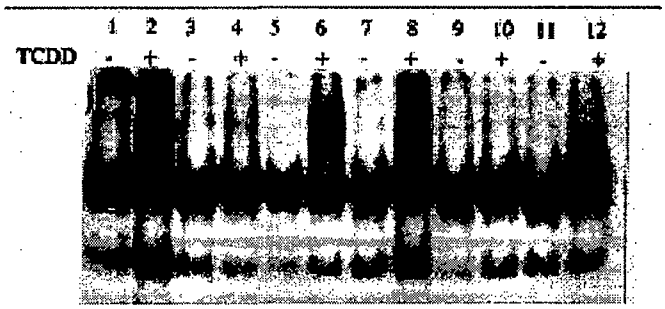


Figure 3. Reconstitution of AhR:DRE complex formation in Chelex-treated samples by select divalent cations.



Lane	Treatment
1-2	control (no Chelex, no metal)
3,4	Chelex
5,6	Chelex, $CdCl_2$
7,8	Chelex, $ZnCl_2$
9,10	Chelex, $MgCl_2$
11,12	Chelex, $MnCl_2$