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Mercuric ion inhibition of eukaryotic transcription factor binding to DNA

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Abstract

Mercury has harmful effects in both rodents and humans. In rodent tissue culture cells exposed to $HgCl_2$, the metal ions were observed to concentrate in cell nuclei and to associate with chromatin. Thus, transcription factors and other proteins associated with chromatin are possible targets of mercuric ion toxicity. In this study, mercuric ions were found to inhibit the DNA binding activity of the Cys_2His_2 zinc finger proteins transcription factor IIIA (TFIIIA) and Sp1. These factors are prototypes of the largest eukaryotic protein superfamily. Neither the presence of excess zinc ions nor β-mercaptoethanol prevented inhibition by mercuric ions. Mercuric ions also inhibited DNA binding by the non-zinc finger protein AP2. Zinc finger–DNA binding was inhibited when both TFIIIA/5S RNA complex and TFIIIA alone were preincubated with concentrations as low as 15 μM mercuric ion. Inhibition occurred in less than 1 min and was not readily reversible. Mercuric ions also inhibited the digestion of DNA by the restriction enzymes *Bam*HI or *Eco*RI. Inhibition of transcription factors as well as potentially other DNA binding proteins by micromolar concentrations of mercuric ion suggests additional biochemical mechanisms for mercury toxicity in promoting disease via alterations in gene transcription patterns.

Keywords: Mercury toxicity; Zinc fingers; Xenobiotics; DNA–protein interactions; Cys_2His_2 protein motifs; DNase I footprinting; Restriction enzymes

1. Introduction

Mercuric ions are known to have positive and negative effects on bacterial transcription factors. The ions bind to the MerR transcription factor in bacteria switching the protein to an active conformation that allows it to turn on transcription of mercury resistance genes [1]. Mercuric ions inhibit the ability of the methylated Ada protein to transcriptionally activate its own gene [2]. When animals are exposed to mercuric ions, the metal accumulates in cell nuclei where it preferentially binds to non-histone proteins of chromatin [3–5]. Mercuric ions also bind chromatin in rat fibroblasts cultured *in vitro* [6]; at mercuric concentrations between 10 and 15 μM, cell survival drops about 50% after 24 hr. Methylmercury concentrations of 10–15 μM in rodent brain tissue cause biochemical changes, reduced neuronal numbers, altered mitoses, and degenerative changes [7–10]. A 50 μM methylmercury concentration in human infant brain tissue causes cortical layers to become severely deranged [11]. The presence of mercury in cell nuclei could result in adverse effects on gene function if mercury ions, at low concentrations (10–15 μM), are capable of having deleterious effects on gene regulatory proteins. Mercury-induced alterations in gene expression could be manifested in developmental, reproductive, and carcinogenic effects, which are known to occur in animal systems. To help understand the biochemical mechanisms responsible for the adverse effects of metal ions on biological systems and human health, it is important to identify potential protein targets for their toxic action and elucidate the inhibitory mechanisms, including concentration dependence and kinetics. Cysteine and histidine amino acids in proteins are reactive to electrophilic metal ions, and such residues are proposed to be primary targets for metal ions, especially those in the heavy element category [12]. One class of cysteine-rich proteins are regulatory factors that contain

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Abbreviations: TFIIIA, transcription factor A for RNA polymerase III; Cys_2His_2, two cysteine and two histidine amino acids; ICR, internal control region; S, sedimentation constant; DNase I, deoxyribonuclease I; DEAE, diethylaminoethyl; RNase A, ribonuclease A; and SV40, simian virus 40.

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Cys_2His_2 zinc binding domains first identified in TFIIIA and referred to as "zinc fingers" [13,14]. TFIIIA binds to the ICR of the 5S ribosomal RNA gene and stimulates 5S RNA synthesis by RNA polymerase III [15]. Zinc finger proteins are proposed to be targets for xenobiotics and possibly responsible for the carcinogenic effects of metal ions [16].

Inhibitory effects of cadmium, lead, and arsenic on eukaryotic transcription factors were examined previously in two distinct types of zinc finger proteins, the estrogen hormone receptor and TFIIIA [17–20]. These proteins are prototypes of transcription factor superfamilies. Hormone receptor-type proteins contain two Cys_2Cys_2 zinc binding domains, whereas TFIIIA-type proteins contain different numbers of Cys_2His_2 zinc binding domains. In the estrogen receptor, micromolar amounts of arsenic inhibited hormone binding and millimolar amounts inhibited DNA binding, whereas micromolar concentrations of cadmium ions were not detrimental to the DNA binding function and could replace zinc in the receptor finger structure with no loss of function [17,18]. Micromolar amounts of cadmium and lead ions inhibited TFIIIA [19,20]. It is important to mechanistically determine if mercury ions can also have deleterious effects on gene expression in general and on eukaryotic gene regulatory proteins in particular and at what concentrations. In the present study, the mechanistic effects of mercuric ions on DNA binding proteins are extended by investigating mercuric ion inhibition of TFIIIA, transcription factor Sp1 (another Cys_2His_2 finger protein that binds GC-rich regions in RNA polymerase II promoters), and the non-finger transcription factor AP2 [21,22].

2. Materials and methods

2.1. TFIIIA isolation

Ovarian tissue was surgically removed from anesthetized 4- to 5-cm *Xenopus laevis* frogs (Nasco) and homogenized in buffer A [50 mM Tris–HCl (pH 7.6), 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride]. The homogenate was centrifuged for 20 min at 10,000 *g*, and aliquots were layered onto 15–30% (v/v) glycerol gradients in buffer A. Gradients were centrifuged for 24 hr at 34,000 rpm in a Beckman SW41 rotor; all manipulations were performed at 0–4°. The 7S particle complex containing TFIIIA and 5S ribosomal RNA sedimented faster than the 5S hemoglobin molecule and was identified by UV absorption. The ribonucleoprotein particles were purified to 90% homogeneity by DEAE ion exchange chromatography as described previously [13]. The 5S RNA was removed from TFIIIA by digestion of the 7S particle (20 μg/mL) with RNase A (10 μg/mL) in buffer B [20 mM Tris–HCl (pH 7.6), 320 mM KCl, 2 mM $MgCl_2$, 0.4 mM dithiothreitol, 0.1% (v/v) Nonidet P-40 detergent] for 30 min at room temperature and then placed on ice. Protein concentration was determined by the method of Bradford, using bovine serum albumin as a standard [23].

2.2. DNA binding reactions

A 303 bp DNA insert containing the 120 bp *Xenopus borealis* somatic 5S ribosomal RNA gene was ^{32}P end-labeled on the coding strand by first digesting a pT7–73 plasmid containing the insert with *Bam*HI followed by alkaline phosphatase removal of the 5′ phosphates. After removal of alkaline phosphatase by phenol-chloroform extraction, the 5′ ends were rephosphorylated with polynucleotide kinase and [γ-^{32}P]ATP. The end-labeled plasmid was digested with *Eco*RI to excise the insert, and the 303 bp end-labeled fragment was purified on a 6% (w/v) polyacrylamide gel. The specific activity of the 5S gene insert was determined by absorbancy at 260 nm and Cerenkov counting. To study the effects of the mercuric ion on TFIIIA function, TFIIIA in buffer B was diluted 5-fold in buffer C [20 mM Tris–HCl (pH 7.6), 70 mM NH_4Cl, 7 mM $MgCl_2$, 0.2 mM β-mercaptoethanol, 0.1% (v/v) non-ionic detergent Nonidet P-40] at room temperature with mercuric chloride (Aldrich Chemical Co.) at the concentrations and times indicated in the figure legends. TFIIIA was then diluted (20-fold) to 10 nM in the same buffer minus the mercury, end-labeled 5S gene was added to a final concentration of 1 nM (about 10^4 cpm), and the binding reaction (20 μL) took place for 15 min at room temperature. DNA binding reactions with transcription factors Sp1 and AP2 (obtained from Promega Life Sciences) were performed in similar fashion using, as a template, the SV40 promoter. DNA was end-labeled according to the instructions of the vendor.

2.3. DNase I protection analysis

DNase footprints of specific DNA–protein interactions were analyzed as previously described [24]. DNase I was added to the DNA binding reactions described above at a final concentration of 1–2 μg/mL and incubated for an additional minute at room temperature. The digestion was terminated by the addition of 100 μL stop buffer [20 mM Tris–HCl (pH 7.6), 1 mM EDTA, 0.1% SDS, 30 μg sonicated salmon sperm DNA/mL]. The digested DNA was ethanol-precipitated and suspended in 4 μL formamide solution [20 mM Tris–HCl (pH 7.6), 95% deionized formamide, 1 mM EDTA, 0.01% xylene cyanol, and bromphenol blue], heated at 95° for 5 min, and electrophoresed through a 7 M urea–7% (w/v) polyacrylamide gel until the xylene cyanol marker migrated two-thirds down the gel. The gel was then transferred to blotting paper, dried, and subjected to autoradiography overnight at −70° using Kodak XAR-5 film.

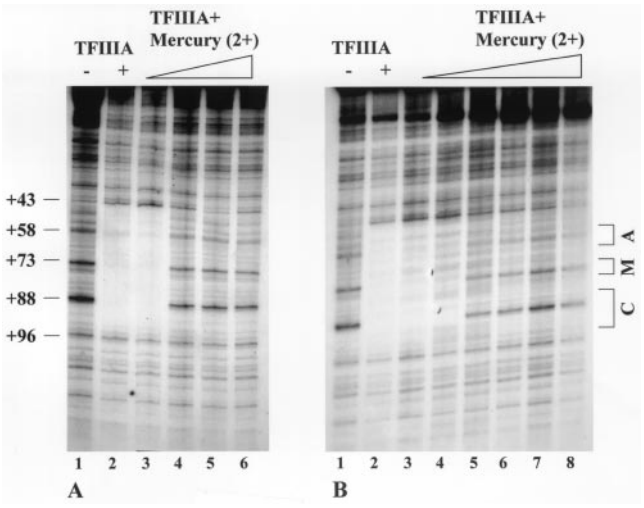

Fig. 1. Mercuric ion inhibition of TFIIIA binding to the 5S RNA gene. The nucleotide positions marked on the left margin of panel A (+43, +58, +73, +88, and +96) are on the coding strand of the 5S RNA gene and are relative to the +1 transcription start site [15]. The locations of the transcriptional promoters C box, intermediate element (M), and A box on the 5S RNA gene ICR are indicated on the right margin of panel B. TFIIIA-DNA binding and DNase protection reactions electrophoresed in lanes 1 and 2 were performed with 1 nM end-labeled 5S RNA gene in the absence or presence of 10 nM TFIIIA. TFIIIA (200 nM) used in the reactions electrophoresed in lanes 3–6 (panel A) was first preincubated with 10, 25, 50, or 100 μM mercuric ions (Hg^{2+}) followed by a 20-fold dilution into the DNA binding reaction. TFIIIA (200 nM) used in the reactions electrophoresed in lanes 3–8 (panel B) was first preincubated with 5, 10, 15, 20, 25, or 30 μM mercuric ions (Hg^{2+}) followed by a 20-fold dilution into the DNA binding reaction. DNA binding, DNase I protection reactions, TFIIIA isolation, polyacrylamide gel electrophoresis, 5S RNA gene end-labeling, autoradiography, and metal incubations are described in "Materials and methods."

3. Results

3.1. Mercuric ion inhibition of TFIIIA binding to the 5S RNA gene

To examine whether mercuric ions inhibit zinc finger transcription factors, the DNase I protection assay was employed. TFIIIA protection of a specific DNA sequence from DNase I digestion indicates that this region of DNA is "covered" by TFIIIA and, thus, is not available to DNase I. In this assay, TFIIIA binds to and protects from DNase digestion a large surface on the 5S RNA gene, from nucleotides +43 to +96, relative to the +1 start site of transcription (Fig. 1A, lane 2). If no functional TFIIIA is present, DNase I nicks this area of the gene as shown by the large number of ^{32}P DNA fragments in this region (lane 1); there are obvious DNase I cuts at nucleotides +88, +73, and +58 [7]. TFIIIA incubated with concentrations of mercuric ions ranging from 10 to 100 μM resulted in considerable loss of DNase I protection at 25 μM but not at 10 μM (Fig. 1A, lanes 4 and 3). A more definitive concentration–response DNase I protection assay was executed (Fig. 1B) to obtain a clearer picture of the inhibitory mercuric ion concentrations for TFIIIA. In this autoradiogram, strong inhibition of TFIIIA binding first occurred at 15 μM (lane 5). Inhibition of TFIIIA-dependent DNase I protection occurred over the entire TFIIIA binding region, from +43 to +96 on the 5S RNA gene where all nine zinc fingers of the factor interact.

The DNase I protection reactions contained 0.2 mM β-mercaptoethanol, a reagent necessary for TFIIIA activity. Excess thiol groups may be binding mercury ions, thereby increasing the overall mercuric ion concentration necessary for TFIIIA inhibition. In this regard, we previously reported that 20 μM mercuric ions are not inhibitory in a TFIIIA-dependent DNase I footprinting assay [19]. However, we believe that this apparent discrepancy is explained by the presence of 0.4 mM dithiothreitol in the previous DNA binding assay, which represents about a 4-fold molar excess of free thiol groups compared with the present assay. In the assays exhibited in Fig. 1, TFIIIA was exposed to mercuric ions and then diluted 20-fold into the DNA binding reaction. TFIIIA binding was not inhibited when these diluted mercury ion concentrations were added directly to the DNase I protection assay (not shown). In addition, inhibition by mercury occurred in the presence of excess thiol reagent. These data indicate that the inhibition of TFIIIA seen in the presence of mercuric ions is not easily reversible.

3.2. Inhibition of TFIIIA zinc fingers by mercuric ions in the presence of excess zinc

Because mercuric ions are able to inhibit TFIIIA binding to the 5S RNA gene and because it is known that the TFIIIA zinc fingers are responsible for this binding, results presented indicate that mercuric ions are disrupting the TFIIIA zinc finger structure. One plausible inhibitory mechanism would be that mercuric ions are displacing zinc ions in a competitive fashion from the cysteine-rich metal coordination sphere in the finger structure, leading to an alteration in DNA binding. Such a mechanism might predict that a large excess of zinc ions could compete with mercury binding and protect activity. To determine if zinc in solution is able to competitively block this finger inhibition, increasing amounts of zinc ions (up to 100 μM) were added to the TFIIIA incubation reaction along with a constant amount of mercuric ion (15 μM). The results of this DNA binding assay are shown in Fig. 2. Mercuric ions inhibited TFIIIA zinc fingers in the presence of all the zinc ion concentrations evaluated, as shown by the loss of TFIIIA-dependent DNase I protection on the 5S RNA gene ICR (lanes 4–8). Mercuric ions, as indicated by the data, did not appear to compete with and replace the zinc ions in the metal coordination sphere of the finger but rather inhibited finger function and structure by another mechanism.

In Figs. 1 and 2, TFIIIA was incubated in different concentrations of metal ions for 20 min at 23° before assaying for DNA binding, which occurred in an additional 15-min incubation. To help elucidate the type of TFIIIA inhibition observed, a kinetic DNase I protection experi-

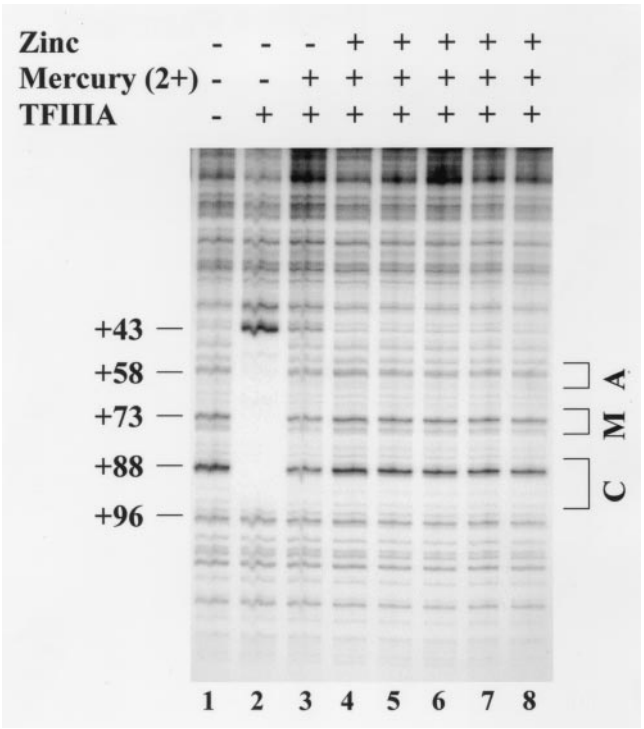

Fig. 2. Mercuric ion inhibition of TFIIIA binding in the presence of excess zinc ions. DNase I digestions electrophoresed in lanes 1 and 2 were of TFIIIA–DNA binding reactions containing 1 nM end-labeled 5S RNA gene in the absence or presence of 10 nM TFIIIA. TFIIIA (200 nM) used in the reactions electrophoresed in lanes 3–8 was preincubated for 20 min with 15 μM mercuric ions prior to a 20-fold dilution into the DNA binding reactions; TFIIIA reactions electrophoresed in lanes 4–8 were also preincubated at the same time with 20, 30, 50, 75, or 100 μM zinc chloride, respectively. DNA binding reactions, ^{32}P end-labeling of the 5S RNA gene, DNase I digestions, autoradiography, 5S RNA gene structure designations, gel electrophoresis, and TFIIIA isolation are described in "Materials and methods" and the legend of Fig. 1.

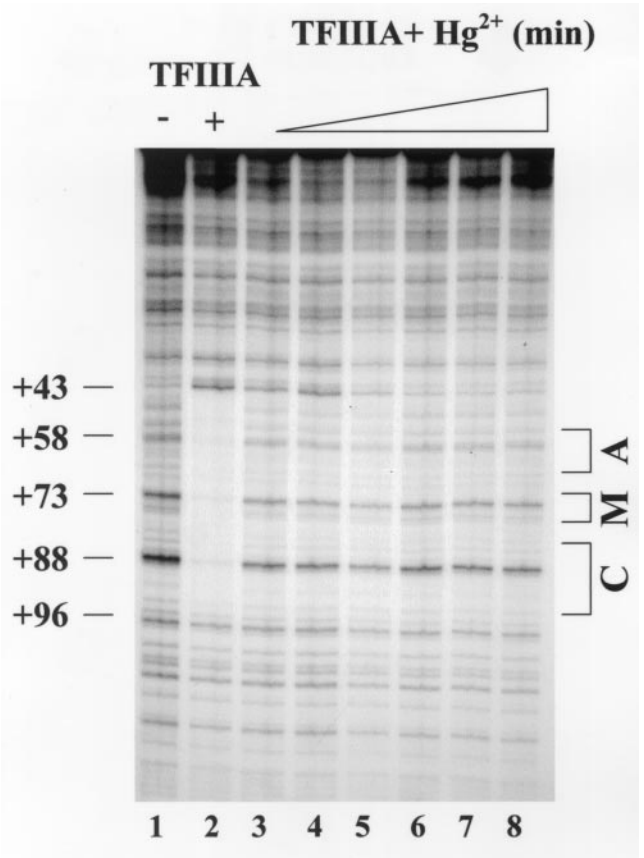

Fig. 3. Kinetics of mercuric ion inhibition of TFIIIA interaction with the 5S RNA gene. All of the TFIIIA–DNA binding reactions took place for 1 min prior to DNase I addition. The reactions electrophoresed in lanes 1 and 2 were performed with 1 nM end-labeled 5S RNA gene in the absence or presence of 10 nM TFIIIA; the TFIIIA used in the reaction for lane 2 was preincubated for 20 min in the absence of mercuric ions. The DNase I protection reactions electrophoresed in lanes 3–8 took place with 1 nM end-labeled 5S RNA gene and TFIIIA treated with 10 nM mercury. TFIIIA (200 nM) used in reactions electrophoresed in lanes 3–8 was preincubated with 30 μM mercuric ions (Hg^{2+}) for 0.5, 1, 2.5, 5, 10, or 20 min, respectively. TFIIIA reactions, analyses, and 5S RNA gene structural designations are described in "Materials and methods" and the legend of Fig. 1.

ment was performed. If this inhibition is rapid, a direct mercuric ion inhibition of the TFIIIA–DNA binding finger domains may be occurring. In these assays, TFIIIA was incubated with mercuric ions (30 μM) for the indicated times and then added to a DNA binding assay for 1 min. The autoradiogram in Fig. 3 demonstrates the kinetics of DNA binding inhibition as assayed by DNase I protection. Strong mercuric inhibition of TFIIIA-dependent DNase I protection was observed at the earliest 0.5-min metal exposure time point (lane 3). This rapid inhibition is consistent with a direct binding mechanism between mercury ions and TFIIIA DNA binding fingers.

3.3. Mercuric ion inhibition of TFIIIA zinc fingers bound to 5S RNA

TFIIIA zinc fingers bind 5S ribosomal RNA in a mechanism that is competitive with 5S gene DNA [25]; the fingers bind both DNA and RNA but not at the same time and not with the same mechanism. The TFIIIA–5S RNA complex is referred to as the 7S ribonucleoprotein particle. Previously, cadmium and lead ions were shown to inhibit TFIIIA only upon direct exposure to the free protein but not when the protein was bound to 5S RNA in the 7S particle [19,20]. These observations suggested that the metal ions were interacting and/or affecting finger structures that were in close contact with 5S RNA contact points such that when the RNA was removed, these finger regions became accessible to the cadmium and lead ions. Thus, the 5S RNA was able to protect portions of the zinc fingers from interacting with the cadmium and lead ions [19,20]. A similar experiment, shown in the autoradiogram in Fig. 4, was performed with mercuric ion inhibition of TFIIIA. Lanes 4 and 5 are control TFIIIA-dependent DNase I protection patterns in the presence and absence of 7S particle-specific 5S RNA, respectively; note that no TFIIIA binding was observed in the

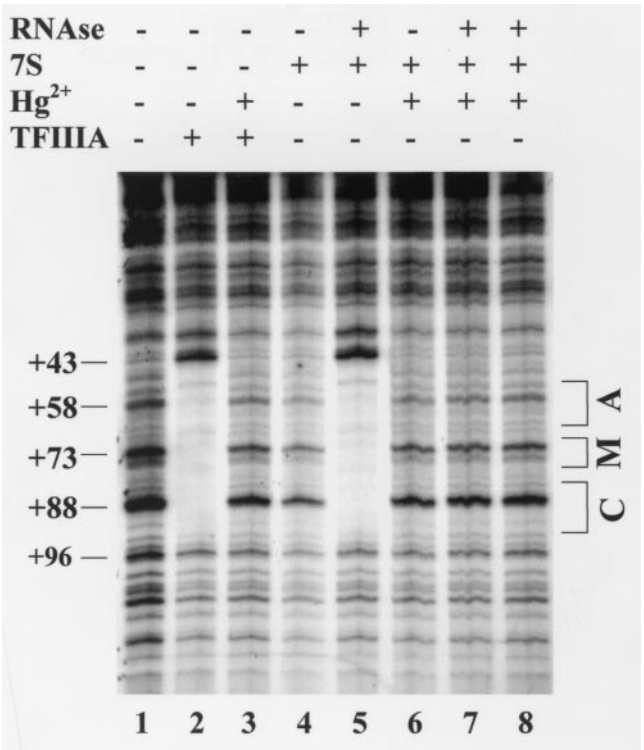

Fig. 4. Inhibition of the TFIIIA/5S RNA ribonucleoprotein complex by mercuric ions. Lane 1 is the DNase I digestion pattern of the end-labeled 5S gene; lanes 2 and 3 exhibit DNase I digestion protection patterns for TFIIIA (200 nM) incubated in the absence or presence of 30 μM mercuric ions (Hg^{2+}) for 20 min prior to a 20-fold dilution into the DNA binding reactions. DNase I protection patterns electrophoresed in lanes 4 and 5 are of the TFIIIA (10 nM) interaction with the 5S RNA gene (1 nM) when the factor is bound (lane 4) or unbound (lane 5) to 5S RNA; the RNA is removed in the DNA binding reaction in lane 5 by the addition of RNase A (10 μg/mL). DNase I patterns in lanes 6 and 7 are from binding reactions where the TFIIIA/5S RNA complex (200 nM) was initially incubated in 30 μM mercuric ions (Hg^{2+}) for 20 min prior to 20-fold dilution into the DNA binding reaction; 5S RNA was removed in the DNA binding reaction electrophoresed in lane 7 but not lane 6. The DNase I pattern in lane 8 is from a binding reaction where the TFIIIA/5S RNA complex (200 nM) was incubated initially in 15 μM mercuric ions (Hg^{2+}) for 20 min prior to a 20-fold dilution into the DNA binding reaction and RNA removal. DNase I digestion, ^{32}P end-labeling of the 5S RNA gene, gel electrophoresis, 5S RNA gene structure designations, autoradiography, and isolation of TFIIIA are described in "Materials and methods" and the legend of Fig. 1.

presence of 5S RNA (lane 4, no DNase I protection from nucleotides +43 to +96). Importantly, TFIIIA binding was observed in the absence of 5S RNA (lane 5). This result indicates that the 5S RNA is preventing the TFIIIA zinc fingers from binding the 5S RNA gene. Lanes 6 and 7 exhibit the same experiment although with initial exposure of the 7S particle to 30 μM mercuric ions; the result in lane 7 (inhibition of TFIIIA binding after 5S RNA removal) was different from that in lane 5 (TFIIIA binding after 5S RNA removal). This demonstrates that the 7S particle-specific 5S RNA was not able to protect the TFIIIA zinc fingers from mercuric ion inhibition, as evidenced by the lack of DNA binding after removal of the 5S RNA by RNase digestion (lane 7). Mercuric ions may be binding the zinc fingers with

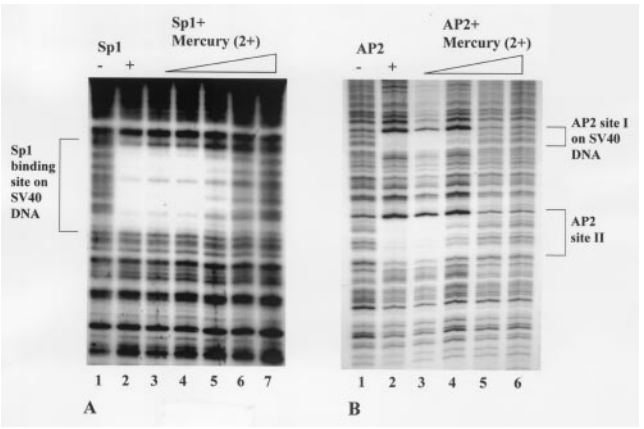

Fig. 5. Inhibition of transcription factors Sp1 and AP2 by mercuric ions. Electrophoresed in lane 1 is the DNase I digestion pattern of the SV40 ^{32}P end-labeled promoter DNA. Electrophoresed in lanes 2–7 (panel A) are the DNase I digestion patterns from Sp1–DNA binding reactions in which two footprinting units of Sp1 (Promega Life Sciences) were incubated for 20 min with 0, 5, 10, 15, 20, or 30 μM mercuric ions (Hg^{2+}), respectively, prior to DNA addition (10,000 cpm); binding reactions with SV40 DNA were continued for 15 min followed by DNase I digestion. Lanes 2–6 in panel B are identical reactions to lanes 2–6 in panel A except for the use of the AP2 transcription factor. Binding sites for Sp1 and AP2 on the SV40 early promoter are designated in the figure margins. DNase I protection experiments with transcription factors Sp1, AP2, and the SV40 viral DNA promoter were performed as described in "Materials and methods."

a different mechanism than that of cadmium and lead and at some distance from the finger-nucleic acid contact points. Lane 8 exhibits the same reaction electrophoresed in lane 7 except that the 7S particle was exposed to 15 μM mercuric ions. The positive controls for TFIIIA binding and mercuric ion inhibition of binding are in lanes 2 and 3, respectively.

3.4. Inhibition of DNA binding mechanisms of transcription factors Sp1 and AP2 by mercuric ions

Lead ions, up to 100 μM, do not inhibit the binding of the non-zinc finger transcription factor AP2 to DNA [20]. However, the Cys_2His_2 zinc finger transcription factor Sp1 is inhibited by micromolar concentrations of lead [20]. Therefore, it was of interest to determine if mercuric ions could inhibit these proteins and at what concentration ranges. In Fig. 5A, the ability of zinc finger protein Sp1 to bind to the SV40 viral DNA promoter region was evaluated in the presence of various mercuric ion concentrations. Strong inhibition of Sp1 DNA binding (loss of DNase I protection) was observed at the 20 μM mercuric ion concentration (lane 6) and mild inhibition was observed at the 15 μM concentration (lane 5). The ability of mercuric ions to inhibit the DNA binding ability of AP2 is exhibited in Fig. 5B. AP2 is an RNA polymerase II enhancer binding protein that regulates differential gene expression [22]. Mercuric ions (15 μM) were also found to inhibit AP2 binding to the two demarcated binding sites on the SV40 promoter region (lane 5, panel B).

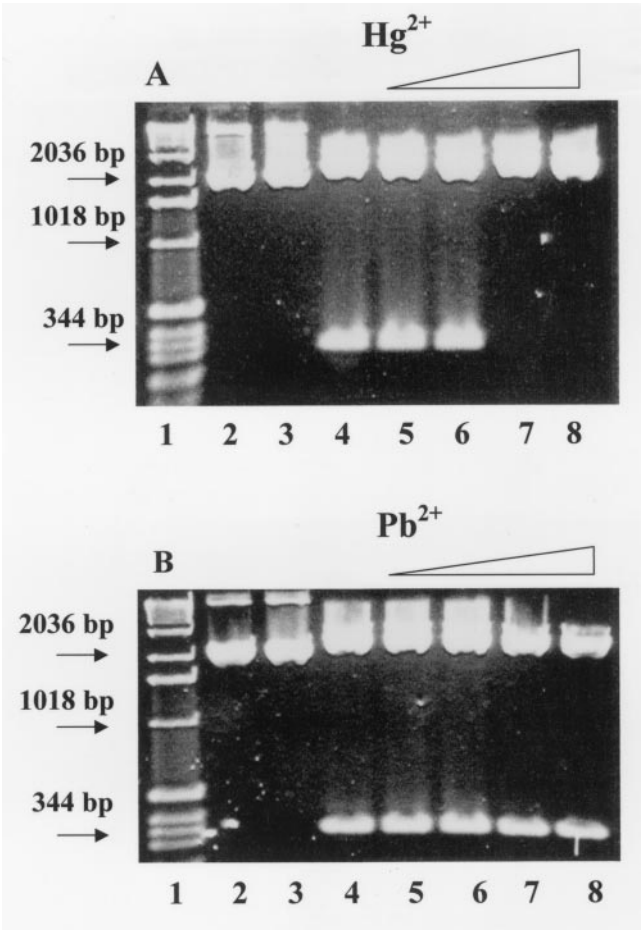

Fig. 6. Agarose gel electrophoresis of inhibition of restriction enzyme digestion by mercuric ions. *Bam*HI and *Eco*RI digestion of the pT7–73 plasmid containing the *X. borealis* somatic 5S gene was performed according to the directions of the manufacturer (Promega). Prior to DNA addition, the enzyme reactions were incubated with 5, 10, 25, or 50 μM (lanes 5–8) mercuric ions (Hg^{2+}, panel A) or lead ions (Pb^{2+}, panel B) for 30 min at 37°. The electrophoretic migration distance of the 303 bp 5S gene insert is demarcated in the right margins. Lane 1 contains electrophoretic DNA markers, and lanes 2–4 contain non-incubated, incubated, and incubated/digested plasmid DNA, respectively; band sizes (bp) are indicated in the left margins.

3.5. Mercuric ion inhibition of DNA digestion by restriction enzymes

Since mercuric ions were able to inhibit the non-zinc finger transcription factor AP2 at concentrations similar to those inhibitory for Cys_2His_2 zinc finger proteins (Fig. 5B), another non-finger protein–DNA binding system was investigated for inhibition by mercuric ions. This system involved the use of the restriction enzymes *Eco*RI and *Bam*HI to excise the 303 bp *X. borealis* somatic 5S ribosomal gene from the plasmid pT7–7. Fig. 6 exhibits the agarose gel electrophoretic profile of the digestion products of this reaction in the presence of enzymes incubated with increasing concentrations of mercuric ions (panel A) or lead ions (panel B). The enzyme reaction generating the 303 bp 5S RNA gene fragment was inhibited by mercuric ion concentrations of 25 and 50 μM (lanes 7 and 8, panel A) but not by 5 and 10 μM (lanes 5 and 6). This is the same concentration range in which mercuric ions inhibited TFIIIA (Fig. 1) and Sp1 and AP2 (Fig. 5). Mercuric ion inhibition was also observed for *Bam*HI or *Eco*RI digestion in another plasmid system (not shown). This result indicates that both *Bam*HI and *Eco*RI are being affected by mercuric ions (Fig. 6), and that the loss of the 303 bp band (panel A) is dependent upon inhibition of both enzymes. The effect of lead (Pb^{2+}) ions was also examined in this *Bam*HI-*Eco*RI dependent system, and they were found not to be inhibitory in the 5–50 μM range (lanes 5–8, panel B).

4. Discussion

The transcription factor TFIIIA is a member of the superfamily of transcriptional control proteins that contain repetitive domains of about 30 amino acids with two cysteines and two histidines that are involved in zinc binding; these domains are called “zinc fingers” [14]. Human TFIIIA and frog TFIIIA have very similar zinc finger structure and function even though there is only about 50% amino acid similarity [26,27]. Mammalian genomes contain about 1000 genes coding for distinct TFIIIA-type Cys_2His_2 finger proteins of differing size and binding specificity [28]. The zinc ions have important roles in finger structure and function. The functional conformation of the zinc fingers is controlled by the presence of zinc ions. Removal of these ions by chelation results in loss of specific DNA binding as well as conformational change [29]. Alteration of the zinc coordination sphere of the TFIIIA fingers due to single amino changes causes the loss of specific DNA binding [30]. These studies show that changes in the zinc coordination sphere also change the ability of the TFIIIA-type zinc fingers to specifically bind DNA. Electrophilic xenobiotics that interact with cysteine and histidine amino acids may also affect zinc finger structure and function. Studies have shown that micromolar amounts of cadmium and lead ions disrupt DNA binding function in TFIIIA and Sp1 Cys_2His_2 zinc fingers [19,20]. Importantly, lead ions have been shown to alter Sp1 DNA binding function in the developing rat brain [31]. Results in the present study indicate that micromolar concentrations of mercuric ions also disrupt zinc finger binding by these proteins *in vitro.* In a manner analogous to that observed with lead ions and the developing brain, it is possible that mercuric ions are affecting Cys_2His_2 zinc finger proteins *in vivo.* Certainly, in rats and in rat tissue culture cells treated with $HgCl_2$, the metal ions are found in the cell nucleus in association with chromatin where zinc finger transcription factors are performing their function [4,6]. However, at this point in the studies it is not known which Cys_2His_2 zinc finger proteins are affected by mercury *in vivo.*

The mechanism of action of mercuric ions may involve

binding of thiol groups. A single cadmium ion has been shown to bind two closely spaced thiol groups [17]. Crystallographic studies have demonstrated the presence of this orientation of thiol groups in TFIIIA-type zinc fingers [32]. Fig. 1 shows that a mercuric ion concentration of 15 μM was able to rapidly inhibit zinc finger structure. The speed and specificity of this reaction (completion in about 1 min) may be due to the association of mercuric ion with zinc finger thiols in a vicinal orientation. However, it is likely that this is not simply due to competition between zinc and mercuric ions since the presence of excess zinc ions did not assuage the mercuric ion inhibition (Fig. 2). TFIIIA binding to the 5S RNA gene involves three groups of three fingers each: the C-terminal finger group binds the A box at the 5′ end of the ICR; the middle group binds the M box or intermediate element in the middle of the ICR; the N-terminal group binds the C box at the 3′ end of the ICR [33]. If the C-terminal or the middle finger groups are mutated, the N-terminal group is still able to prevent digestion by DNase I. However, if the N-terminal group is altered, there is a total loss of DNase I protection over the ICR (+43 to +96) by not only the N-terminal group but the middle and C-terminal groups [30,33]. In Fig. 1, inhibition of TFIIIA-dependent DNase I protection along the entire ICR indicates that the mercuric ions, at the least, are altering the structure of the N-terminal group of fingers. DNase I protection of the C-box region should have been observed if only the middle or C-terminal fingers were affected. Fig. 5A shows another zinc finger protein, Sp1, in which there was complete inhibition of DNase I protection in the presence of mercuric ions.

Mercuric ion inhibition of Cys_2His_2 zinc finger proteins differs from that of cadmium and lead ion inhibition. 5S ribosomal RNA bound to TFIIIA is able to protect the TFIIIA zinc fingers from cadmium and lead inhibition [19, 20], but this was not the case with mercuric ions (Fig. 4). This result implies a different inhibitory mechanism for mercuric ions. Micromolar concentrations of lead and cadmium ions were also able to inhibit the Cys_2His_2 zinc finger protein Sp1 but were not able to inhibit the non-zinc finger protein AP2 [20]. Significantly, the same concentrations of mercuric ions were inhibitory to both Sp1 and AP2 (Fig. 5). In addition, mercuric ions were found to be inhibitory for restriction enzyme digestion, but lead ions were not (Fig. 6). These results indicate that mercuric ions may be more general inhibitors of DNA binding proteins, whereas lead and cadmium ions appear more specific for Cys_2His_2 zinc finger proteins.

The Cys_2His_2 zinc finger superfamily of proteins comprise the largest protein category in living systems [34]. The number and complexity of these proteins have increased over time. These proteins have the ability to bind nucleic acids, which implies that they could be involved in cell functions such as control of gene expression, cell growth, cell differentiation, and signal transduction. Data presented in this study indicate that the Cys_2His_2 family of zinc finger proteins may be the target of mercuric ions. In addition, mercuric inhibition of the transcription factor AP2 and restriction enzymes indicates that other types of DNA binding proteins may be targets as well. Mammals have cysteine-rich metallothionein proteins to act as xenobiotic cellular "mops," possibly to protect critical cysteines and histidines in Cys_2His_2 regulatory factors as well as in other cysteine- and histidine-rich proteins. As increasing amounts of heavy metals and other xenobiotics accumulate in the environment, animal cell defense mechanisms may become overwhelmed as the amount of environmental pollution, especially by heavy metals and other xenobiotics, increases. This could result in increased incidents of mercury- and other xenobiotic-related disorders. With respect to mercury toxicity, methylmercury is the chemical form of the metal that is generated in bacteria and that most readily enters animal cells. Once in the animal cell, this organic form can be converted to the inorganic mercuric form [35,36]. Therefore, these studies on mercuric ion inhibition of eukaryotic transcription factors are relevant to the overall cycle of methylmercury and mercuric ion toxicity. Mercuric ions also can enter animal cells and gain access to the nucleus and chromatin and induce toxic effects [4,6]. Although not yet demonstrated because of the added dangers associated with doing experiments with methylmercury, the organic form of the metal could interact with zinc finger proteins as well, since it also has specificity for thiol groups. Mercuric ion inhibition of transcription-regulating proteins responsible for normal cell function could result in abnormal cell growth. Suppression of transcription has been shown to be a factor in the development of tumors [37]. Mercury is a suspected carcinogen in rodent models and humans [38,39]. In mammals, mercuric ions may be acting as a tumor promoter causing cell proliferation. This study demonstrates the vulnerability of zinc finger as well as non-zinc finger transcriptional control proteins to mercuric ions and thus to the disruption of normal cell function.

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