

Inhibitory Effects of Heavy Metals on Transcription Factor Sp1

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Abstract: Heavy metals are expected to affect the biological activity of various metal-containing proteins, including transcriptional regulators. We studied the effects of several heavy metal ions on the DNA-binding activity of a Zn-finger transcription factor, Sp1. With respect to both DNA elements through which Sp1 acts positively and negatively, Cd²⁺ inhibited DNA-binding of Sp1 at 20 μM or higher, while the toxic effect of Zn²⁺ was obvious only at more than 200 μM. Inhibition was also apparent for Cu²⁺ but less remarkable for Hg²⁺. The inhibition by Cd²⁺ was relieved by the addition of Zn²⁺ at much lower concentrations than that of Cd²⁺. These results suggest that the toxic potential of heavy metals could be largely influenced by the intracellular Zn²⁺ concentration.

Key words: Heavy metal, Cadmium, Zinc, Transcription factor, Sp1

Metal-containing proteins could be a major target of toxic heavy metals. One of the mechanisms for such toxicity has been assumed to be the disturbance of proper metal-protein interactions that are essential for maintaining correct protein structures¹. Zn-finger transcription factors, a category of transcriptional regulators, have Zn-containing structures that form the DNA-binding domains². It is easily expected that the activity of these proteins might also be influenced by co-existing heavy metals. In fact, it has been reported that heavy metal ions such as Cd²⁺ could exert detrimental effects on Zn-finger factors including TFIIIA and Sp1^{3,4}.

Sp1 was first identified as a transcriptional activator that recognizes a GC-rich motif widely present in various viral and mammalian gene promoters and enhancers⁵⁻⁸. However, later it was reported that Sp1 could act also as a negative regulator of transcription in a number of cases⁹⁻¹¹. During the course of a study about human metallothionein-IIA (*hMT-IIA*) gene regulation, we found that Sp1 acts negatively via

a particular metal responsive element (MRE)¹². In the present work, we studied the effects of several heavy metals on the DNA-binding activity of Sp1, both in cases where it acts positively and negatively.

DNA-binding activity of Sp1 was estimated by an electrophoretic mobility shift assay (EMSA). As the source of Sp1, HeLa cell crude nuclear extracts (NEs) were prepared as described previously¹³. Double-stranded oligonucleotides were end-labeled with T4 polynucleotide kinase and [γ -³²P]ATP, to be served as probes. NE and a ³²P-probe (15 fmoles) were incubated in reaction mixtures (12.5 μl each, containing 10 mM Hepes-K (pH 7.9), 2 mM MgCl₂, 50 mM KCl, 16 mM NaCl, 10 mM DTT, 10% glycerol, 0.2 mg/ml bovine serum albumin and 80 μg/ml poly (dI-dC)) with or without heavy metal ions at 25°C for 20 min. The protein-DNA complexes formed were electrophoresed for 1.5 h in 5% polyacrylamide gel in a buffer containing 22 mM Tris and 22 mM boric acid, and were detected by autoradiography. All experiments were repeated at least three times to confirm reproducibility, and typical results are shown.

Sp1 binds to MREb of the *hMT-IIA* gene, and is likely to negatively regulate transcription probably by competing with an MRE-binding transcriptional activator MTF-1¹². The

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effects of various divalent heavy metal ions (including Zn^{2+} , Cd^{2+} , Cu^{2+} and Hg^{2+}) on the Sp1/MREb interaction were examined by EMSA with a ^{32}P -labeled MREb probe¹³. The results are shown in Fig. 1. Inhibition of the complex formation by Cd^{2+} was obvious above $10 \mu M$ (lanes 5 to 7). Cu^{2+} also strongly blocked Sp1/MREb binding above $20 \mu M$ (lanes 11 to 13). By contrast, inhibition by Zn^{2+} was observed only at much higher concentrations (lanes 25 and 26). Hg^{2+} (lanes 15 to 20) moderately inhibited complex formation above $50 \mu M$, but complete inhibition was not observed even up to $1,000 \mu M$. These results demonstrate that Cd^{2+} and Cu^{2+} have relatively higher potentials in terms of the inhibitory effect on the DNA-binding activity of Sp1. Such an effect might possibly affect *hMT-IIA* gene regulation *in vivo*; those metals could weaken the binding of Sp1 to MREb at moderate concentrations, allowing the activator MTF-1 to be accessible to MREb.

Sp1 is known to activate transcription via the GC-box sequence located between MREa and MREb of the *hMT-IIA* gene^{14,15}. We then examined how Cd^{2+} and Zn^{2+} affect the binding of Sp1 to this positive element, in comparison with MREb. EMSA was performed with either a 20-base pair MREb or GC box probe (Fig. 2a) in the presence of various concentrations of $ZnSO_4$ (Fig. 2b) or $CdSO_4$ (Fig. 2c). Zn^{2+} reduced the complex formation above $200 \mu M$, for both the MREb and GC box probes (Fig. 2b). Cd^{2+} also inhibited Sp1 binding to both motifs in an essentially identical manner (Fig. 2c), so that the effects of heavy metals are similar irrespective of the DNA sequence through which Sp1 acts positively or negatively. These observations further imply that heavy metals could modulate the expression of a number of genes, by suppressing both positive and negative functions of Zn-finger transcription factors.

Next we examined the effect of Zn^{2+} , the essential structural component of Zn-finger transcription factors, on the inhibition of Sp1 by Cd^{2+} (Fig. 3). In both binding reactions with MREb (lanes 1 to 6) and the GC box (lanes 7 to 12) probes, Cd^{2+} almost completely inhibited the complex formation at $100 \mu M$ (lanes 3 and 9; compare with controls in lanes 1 and 7, respectively). Zn^{2+} suppressed the inhibitory effect of Cd^{2+} at concentrations much lower than that of Cd^{2+} , irrespective of the target sequence (lanes 4 to 6 for MREb; lanes 10 to 12 for GC box). Zn^{2+} alone did not raise the levels of complex formation (lanes 2 and 8). These results demonstrate that the toxic effect of Cd^{2+} on Sp1 greatly depends on the concentration of co-existing Zn^{2+} . At low Zn^{2+} levels, an excess of Cd^{2+} appears to destroy Zn-finger structures, thereby abolishing DNA-binding activity. At sufficiently high Zn^{2+} levels, however, the finger structures

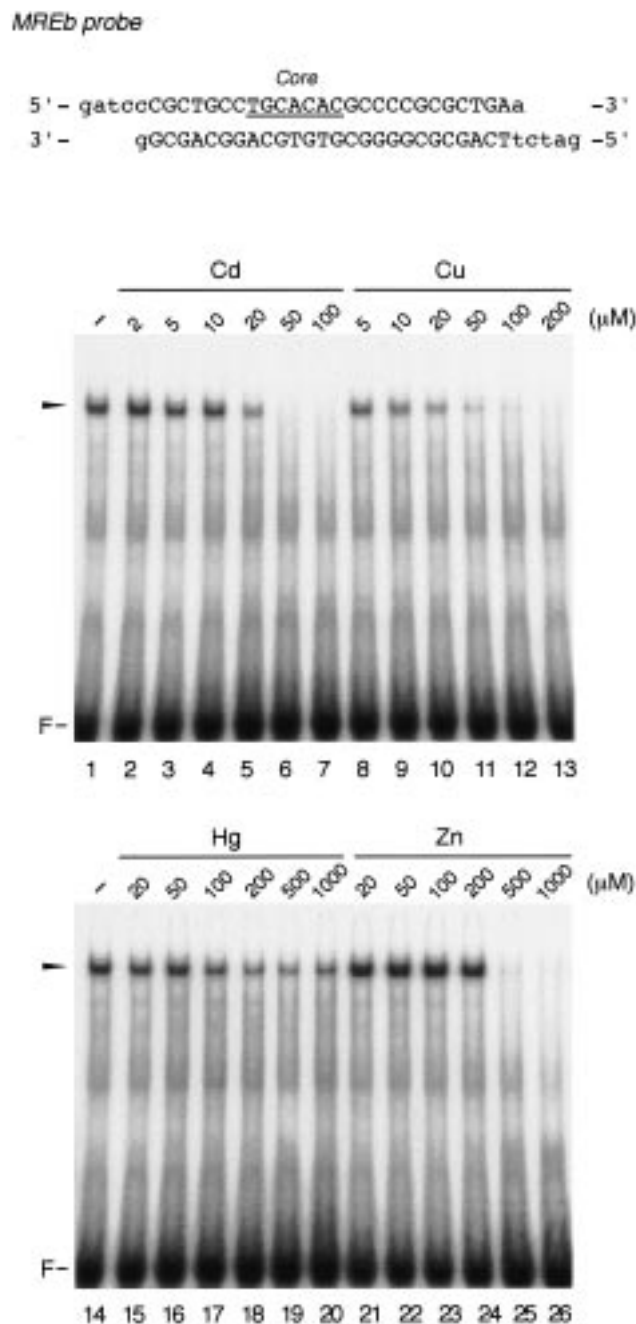


Fig. 1. Effects of heavy metals on the interaction between Sp1 and MREb.

DNA-binding of Sp1 was assayed by EMSA with a 28-base pair ^{32}P -MREb probe. The nucleotide sequence of the probe is shown at the top. Reactions contained $2 \mu g$ of HeLa cell NE and 15 fmoles of the probe. $CdSO_4$ (lanes 2 to 7), $CuSO_4$ (lanes 8 to 13), $HgCl_2$ (lanes 15 to 20) or $ZnSO_4$ (lanes 21 to 26) were added at the concentrations indicated. For the control reactions in lanes 1 and 14, no metals were added. After incubation at $25^\circ C$ for 20 min, protein-DNA complexes were resolved in 5% polyacrylamide gel and detected by autoradiography. Arrowheads indicate the specific Sp1/MREb complex. F indicates the free probe.

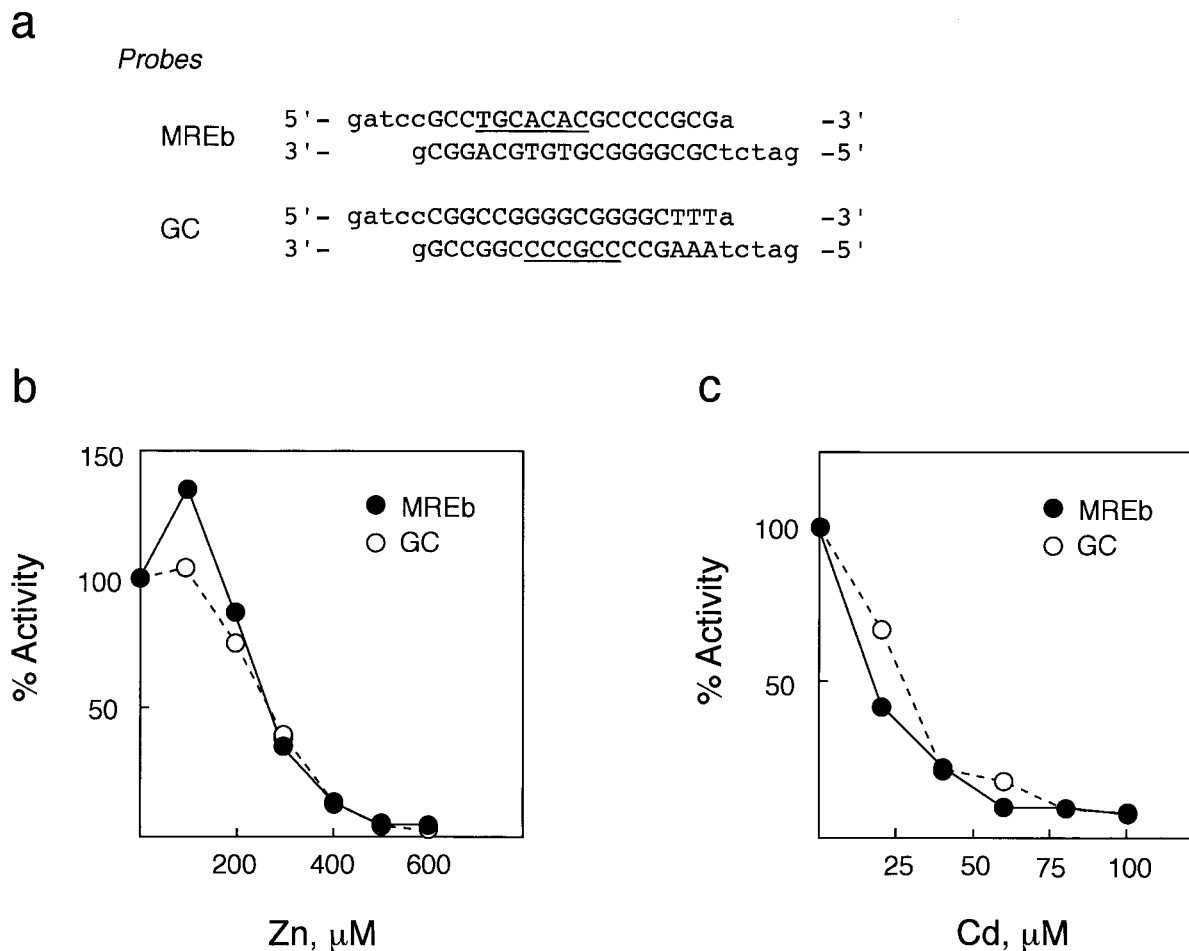


Fig. 2. Effects of Zn^{2+} and Cd^{2+} on Sp1 binding to different regulatory DNA elements through which Sp1 acts negatively or positively. EMSA was performed as in Fig. 1, except for using ^{32}P -labeled 20-bp oligonucleotide probes containing the MREb or GC box sequence of the *hMT-IIA* gene, the nucleotide sequences of which are shown in (a). The core motifs are underlined. Reactions were incubated with the concentrations of $ZnSO_4$ (b) or $CdSO_4$ (c) indicated. The Sp1 complex bands were quantified by densitometry with an imaging analyzer model TIAS 2300S (ACI Japan). Closed and open circles indicate reactions with MREb and the GC box, respectively. The value for the control without heavy metals is indicated as 100.

are likely to be fully occupied by Zn^{2+} even in the presence of Cd^{2+} , remaining functional. Since the protective effect of Zn^{2+} was observed at concentrations much lower than that of Cd^{2+} , Zn^{2+} appears to have a higher affinity to Sp1 than Cd^{2+} . Zn^{2+} could possibly have such a protective effect also against other toxic heavy metals and for other Zn-finger transcription factors, although this remains to be demonstrated. If such an observation *in vitro* could be extended to *in vivo*, fluctuation in the intracellular Zn^{2+} level, as a result of food intake or other physiological changes, might greatly influence the expression of heavy metal toxicity in various gene regulation processes.

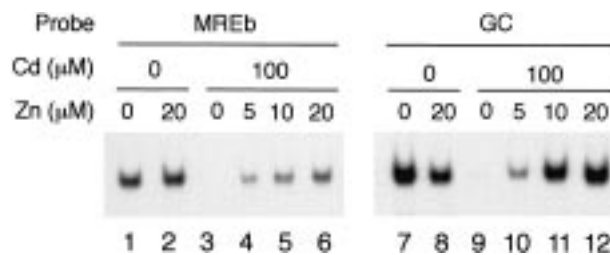


Fig. 3. Effect of Zn^{2+} on the inhibition of Sp1 by Cd^{2+} . EMSA reactions contained ^{32}P -labeled 20-bp oligonucleotide probes containing the *hMT-IIA* MREb (lanes 1 to 6) or GC box (lanes 7 to 12), and $CdSO_4$ and/or $ZnSO_4$ as indicated. Protein-DNA complexes formed were analyzed as in Fig. 1. Only a portion of the gel containing the Sp1 complex bands is shown.

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