# The Prospective Role of Abnormal Methyl Metabolism in Cadmium Toxicity

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**Several lines of evidence point to the probable role of abnormal methylation processes in the toxicology of metals and other xenobiotics. The spectrum of toxic effects exhibited by such metals as Ni, As, and Cd, as well as by Zn deficiency, often resemble those seen in animals chronically fed methyl-deficient diets. These metal-associated pathologies include cancer, atherosclerosis, birth defects, neurological disturbances, and pancreatic lesions. In addition, each of the above agents has been shown to alter normal methyl group metabolism *in vivo* or *in vitro*. In the present studies, we compared the effects on the enzyme DNA methyltransferase (MTase) of two metal ions: the essential metal Zn and the carcinogen Cd. MTase extracts were obtained from the hepatic nuclei of rats fed a methyl-deficient diet (lacking choline and folate) for 7 and 24 weeks. Control animals were fed the same diet supplemented with each of these vitamins. Zn and Cd both inhibited MTase in the nuclear extracts from both the control and the methyl-deficient rats. The inhibitory activity of Cd was greater than that of Zn regardless of whether the nuclear extracts were from the control or the deficient animals. In addition, the kinetics of Cd inhibition of MTase activity were different in the nuclear extracts from the control and methyl-deficient rats. The results provide evidence that the carcinogenic effects of Cd may be mediated in part through abnormal DNA methylation. *Key words:* Cd, Zn, carcinogenesis, DNA methyltransferase, methyl deficiency. *Environ Health Perspect* 110(suppl 5):793–795 (2002).**
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Methyl-deficient (MD) diets cause liver cancer in rodents (*1*). In addition, physiological methyl insufficiency has been associated with increased risk of cancer and other diseases such as pancreatic damage, atherosclerosis, birth defects, and neurological disturbances, both in humans and in experimental animals (*2*). Abnormal biological methylation appears to be implicated in the development of such diseases, and the chief physiological methyl donor *S*-adenosylmethionine (SAM) appears to be a key intermediate involved in these pathologies. One major effect of the chronic feeding of MD diets is the alteration of DNA methylation (*3*). This reaction is catalyzed by the enzyme DNA methyltransferase (MTase), which transfers methyl groups from SAM to the C5 position of cytosine residue in DNA (*4*). Alterations in MTase activity are seen in both the preneoplastic and the neoplastic livers of MD rats (*3*). Abnormalities in DNA methylation are associated with many diseases (*5*) and appear to be implicated in inappropriate gene expression (*6*).

Several toxic metals, including Cd, are known to produce toxic effects resembling those seen with physiological methyl insufficiency (*7,8*). Cd exerts a synergistic effect with the DNA-hypomethylating agent azacytidine in the induction of metallothionein in liver cells (*9*). Cd also reacts with the cysteine and histidine residues in proteins (*10,11*). DNA MTase is a Zn finger–containing protein that possesses both a cysteine residue in the active center and $Cys_2/His_2$ Zn finger motifs in the N-terminal region (*4*) and thus provides a reasonable target for mediating the adverse effects of Cd in mammalian cells. We studied the inhibitory effects of Cd on DNA MTase in the livers of rats chronically fed a normal methyl-sufficient (MS) diet and the corresponding carcinogenic MD diet, both with and without a brief pretreatment of the animals with the carcinogen aflatoxin $B_1$ ($AFB_1$).

## Materials and Methods

The dietary regimes and the design of $AFB_1$ dosing have been described previously (*12*). At 5 weeks of age, the rats were randomly assigned to receive either $AFB_1$ (25 µg/rat/day, 5 days/week) or control dimethylsulfoxide (100 µL/rat/day). After 3 weeks of dosing (8 weeks of age), the rats were either maintained on the MS diet or placed on the MD diet for 7 or 24 weeks. Four treatment/diet groups were thus examined: control/MS (C/MS), control/MD (C/MD), $AFB_1$/MS, and $AFB_1$/MD. Unless otherwise specified all chemicals were supplied by Sigma-Aldrich (St. Louis, MO, USA). The nuclear extracts were isolated from the rat livers either at 7 or at 24 weeks of the different dietary regimes (*13*), and DNA MTase activity was determined in the nuclear extracts as described previously (*14*). Unless otherwise indicated, the reaction mixture contained 1–2 µg hepatic DNA, 1 µL (0.55 µCi) [methyl-$^3$H]SAM (Perkin-Elmer Life Sciences, Inc., Boston, MA, USA) (specific activity, 13 Ci/mmol), 10 µL nuclear extract MTase (containing up to 50 µg of protein), and the corresponding concentrations of Zn or Cd acetate (Alfa Aesar, Ward Hill, MA, USA) in 20 µL of standard buffer solution (20 mM Tris–HCl buffer, pH 7.5; 1 mM EDTA, 0.2 mM dithiothreitol; and protease inhibitors: 0.1 mM phenylmethylsulfonyl fluoride, 1 µg/mL leupeptin, and 10 µg/mL each of TLCK, TPCK, and E-64). The enzyme activity was expressed as counts per minute (cpm) of radioactivity incorporated per milligram of protein from [methyl-$^3$H]SAM into DNA during the incubation of the reaction mixture at 37°C for 1 hr under the above conditions. In one study, the effects of Cd and Zn on prokaryotic methylase Sss I (New England Biolabs, Beverly, MA, USA) activity were examined (*14*).

## Results

We compared the effects of Cd and Zn on DNA methylation by examining homologous methylation of hepatic DNA by hepatic MTase in nuclear extracts isolated from the livers of rats treated with $AFB_1$ and subsequently fed the MS and MD diets for 7 and 24 weeks. Figure 1 shows the reaction velocities of the DNA methylation in the four treatment groups after 7 experimental weeks as a function of the log of metal concentration in the MTase assay. The increase of metal concentration from 1 to 500 µM causes a marked inhibition of hepatic DNA methylation regardless of whether the extracts had been obtained from the MS or the MD livers or whether the animals had been pretreated with $AFB_1$.

With either the MS or MD dietary groups, the inhibition by Zn of hepatic DNA methylation displays simple kinetics (Figure 1); accordingly, the graph of an inverse of the reaction velocity versus Zn concentration shows no deviation from linearity (Figure 2). These results suggest that only one type of Zn-binding site is implicated in inhibiting DNA methylation.

With the MS groups, the inhibition by Cd of hepatic DNA methylation is also described by a simple kinetic curve (Figure 1), and the plot of an inverse of the reaction velocity as a function of Cd concentration is linear (Figure 2). However, in each group, the concentration of Cd causing a 50% inhibition

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of hepatic DNA methylation is 3- to 4-fold lower than the corresponding concentration of Zn (Figure 3). With the MD groups, the kinetics of Cd inhibition of hepatic DNA methylation are described by S-shape curves (Figure 1), and the dependence of the reciprocal of the reaction velocity on Cd concentration show marked deviations from linearity (Figure 2). These results suggest that the impairment of DNA methylation by Cd can involve more than one process. With the MD groups, the concentrations of Cd causing a 50% inhibition of hepatic DNA methylation are also much lower than the corresponding concentrations of Zn (Figure 3).

The experiments described in Figures 1–3 were repeated with the same four experimental groups at 24 weeks. The kinetics of Cd and Zn inhibition of DNA methylation in the 24-week hepatic nuclear extracts were similar to those observed in the corresponding extracts isolated at 7 weeks. Similarly, both Cd and Zn inhibit the activity of the bacterial Sss I MTase (Figure 4).

## Discussion

The present results on the inhibition by Cd and Zn of mammalian and bacterial MTases suggest a possible mechanism to explain the carcinogenicity of Cd. The kinetics of inhibition by Cd and Zn of the methylation of hepatic DNA from MS rats is qualitatively similar. This inhibition may be caused by binding of these metals to the cysteine residue in the active center of MTase (*4*). Both Cd and Zn also inhibit the activity of prokaryotic Sss I MTase (Figure 4), which,

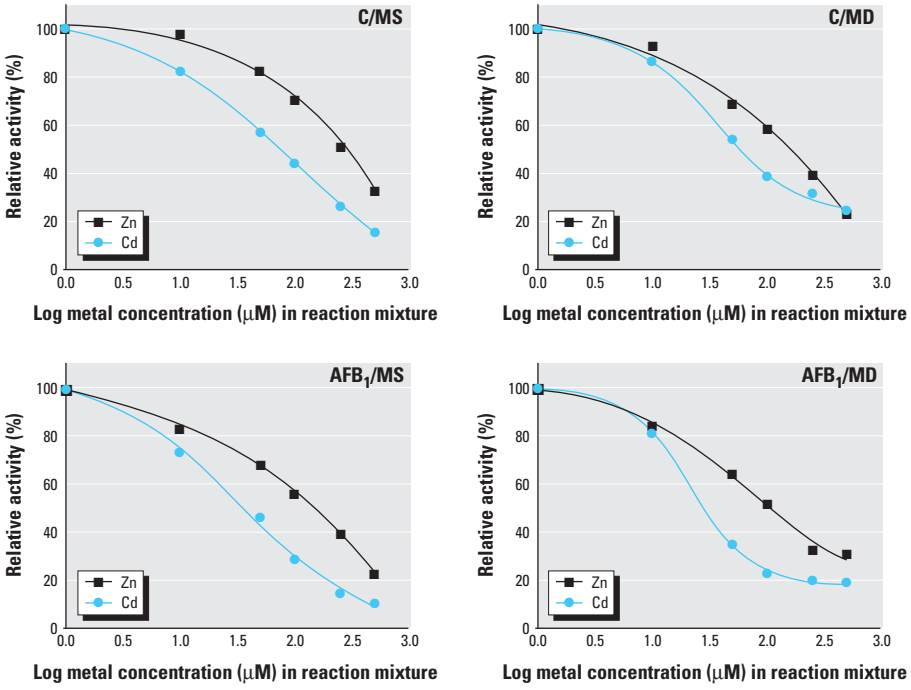


**Figure 1.** The effect of MD diet on the kinetics of the inhibition by Cd and Zn of hepatic DNA methylation by hepatic DNA MTase. In each group, 100% activity corresponds to the activity obtained without the presence of a metal in MTase assay.

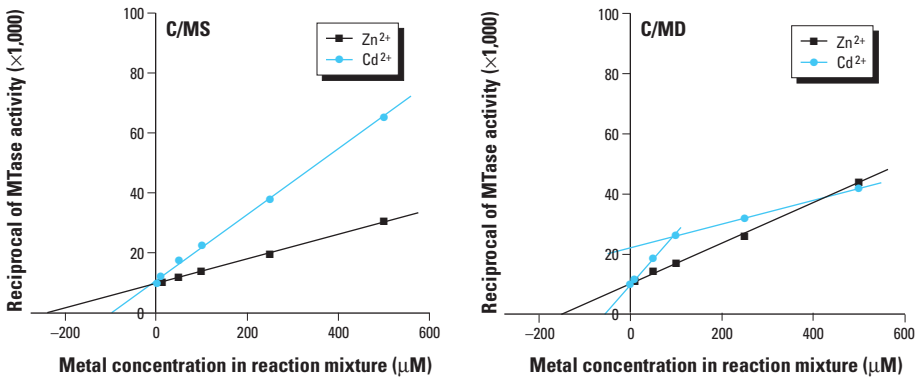


**Figure 2.** The inverse of the velocity of hepatic DNA methylation by hepatic DNA MTase as a function of metal concentration in MTase assay. The equation for the reaction velocity ($v$) in the presence of substrate $S$ and noncompetitive inhibitor $I$ may be rearranged (*14*) as $1/v = [(1 + K_s/S)/V_m] \times (1 + I/K_i)$, which gives the linear dependence of an inverse of the velocity on the inhibitor concentration at the constant substrate concentration.

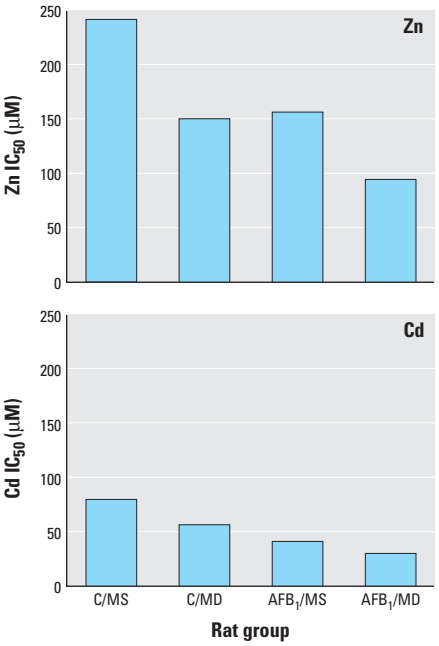


**Figure 3.** The effect of MD diet on Cd and Zn concentrations that caused 50% activity inhibition ($IC_{50}$) of hepatic DNA methylation by hepatic DNA MTase.

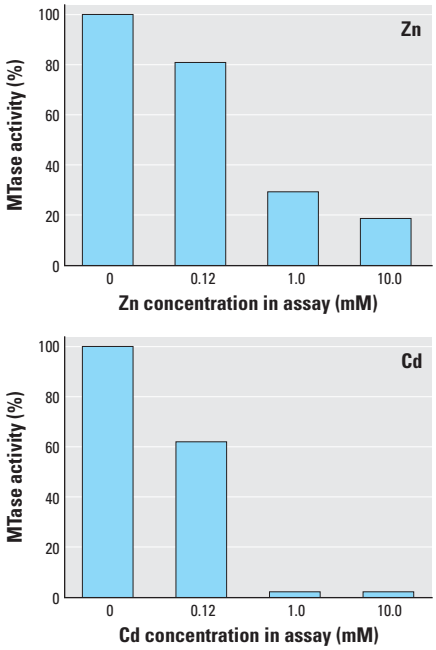


**Figure 4.** The inhibition by Cd and Zn of DNA methylation by Sss I MTase. The MTase assays contained 3 U of Sss I MTase and 1.5 μg of calf thymus DNA.

like mammalian MTase, possesses a cysteine residue in its active center (*4*).

The kinetics of inhibition by Cd and by Zn of the methylation by homologous MTase of hepatic DNA from MD rats, however, were quite dissimilar: such inhibition by Zn was similar to that seen with the MS extracts; the inhibition by Cd was not. This difference may be ascribed to the different effects of Cd and Zn on the binding of the Zn finger domain of mammalian MTase to DNA. Cd may prevent the binding of the MTase Zn finger to DNA. Such effects by Cd have been shown with other Zn finger proteins (*11,15*). The Zn finger domain in mammalian MTase is not essential for catalytic activity, but its absence alters site specificity and increases the *de novo* methylation activity of the enzyme (*16*). A weakening by Cd of the binding of the Zn finger MTase domain to DNA would thus be expected to be especially manifested when hypomethylated DNA is used as a substrate. Such was the case in these studies (*13*). In conclusion, the inhibition by Cd of mammalian MTase provides a biochemical mechanism by which the toxicity of this metal may be linked to that of other agents altering DNA methylation.

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