Metal Response Element-binding Transcription Factor-1 Is Activated by Degradation of Metallothionein

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Cytosolic zinc-binding protein, metallothionein (MT), is normally saturated with Zn. It is thought that Zn-saturated MT (Zn-MT) acts as a major intracellular Zn pool. Metal-response element-binding transcription factor-1 (MTF-1) plays an important role in Zn-mediated MT transcription. Here, we showed that degradation of Zn-MT activates MTF-1. We measured activated MTF-1 using an electrophoretic mobility shift assay. Interleukin-6 induced MT expression and increased MTF-1 activity. MTF-1 activation was not observed in MT-overexpressing cells. MT-dependent MTF-1 activation was observed only after treating MT-overexpressing cells with cycloheximide (CHX), a protein synthesis inhibitor. CHX-treatment increased the degradation/synthesis ratio of protein. An increase in the degradation/synthesis ratio for the MT protein is expected to increase the level of labile Zn and activate MTF-1. Recombinant MTF-1 was activated by H2O2 only in the presence of Zn-MT. Oxidative stress activated MTF-1 DNA-binding activity in primary cultured hepatocytes but not in MT-deficient hepatocytes. These findings suggest that degradation of Zn-MT activates MTF-1, and that MT plays an important role in zinc-mediated signal transduction.

Key words —— metal response element-binding transcription factor-1, metallothionein, zinc, oxidative stress

INTRODUCTION

Zn is an essential micronutrient involved in structural and regulatory cell functions.1) Metallothionein (MT), the cytosolic Zn-binding protein, is normally saturated with Zn,2) and is thought to maintain Zn homeostasis.3,4) In mammals, four MT genes have been cloned, MT-I to IV. MT-I and -II are expressed in many cell types in various organs and tissues, and in most cultured cells. By comparison, MT-III and -IV show a very restricted cell-type-specific expression pattern.5) The present study focuses on MT-I and -II. Transcription of MT is induced by heavy metals, including Zn, and is mediated by metal response element (MRE), a cis-element.6–8) MRE-binding transcription factor-1 (MTF-1) is a highly conserved Zn-finger transcription factor that regulates the transcription of MT.9–11) The overloading of Zn in MT activates MTF-1, and then induces MT synthesis. Newly synthesized metal-free MT (apo-thionein) binds to Zn. Finally, a decrease in available Zn causes inactivation of MTF-1. In summary, MT synthesis induces MTF-1 inactivation, although, it is known that MT activates nuclear factor-κB (NF-κB), which requires zinc for activation.12,13) Independent findings suggest that Zn-MT acts as a Zn donor for some enzymes14,15) and transcription related factors, including Sp1 and the estrogen receptor.16–18) The synthesis and degradation of MT, especially the degradation of Zn-MT, have implications for the availability of Zn and Zn-mediated signal transduction. In this study, we examined whether degradation of MT activates MTF-1.
MATERIAL AND METHODS

Cells and Cell Culture —— Rat H4IIEC3 hepatoma cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (FBS) at 37°C in 5% CO₂/95% air. For MT stable expression, Chinese Hamster Lung (CHL) cells were transfected with pBPVGRPMT or pBPVGRPTM by the calcium phosphate precipitation method as previously described. After G418 selection and cloning, the presence of the transgene was detected by PCR. The concentration of MT was determined using the ¹⁰⁹Cd/hemoglobin affinity assay. Mouse hepatocytes were isolated by the in situ two step collagenase perfusion method as previously described. The hepatocytes were plated in Williams’ medium E containing 5% FBS, 1 µM dexamethasone (Dex) and 1 µM insulin.

Preparation of Nuclear Extracts and Electrophoretic Mobility Shift Assays —— Nuclear extracts were prepared by the method of Dalton et al. Binding reactions in the electrophoretic mobility shift assays (EMSA) were performed using binding buffer containing 12 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pH 7.9), 5 mM MgCl₂, 60 mM KCl, 0.5 mM dithiothreitol, 2 µg of poly(dI-dC), 2.5 pmol of mutant MRE oligonucleotide (mutMREs; 5’-GATCCAGGGAGCTAATTACTCCGCAGGAAGTA-3’), and 5–50 fmol of ³²P-labeled MRE oligonucleotide (MREs; 5’-GATCCAGGGAGCTCTGACACAGCCCGAAAAGTA-3’), with 0.2–0.5 µl of recombinant human MTF-1 protein (1.0 pmol) in a total volume of 20 µl. After incubation on ice for 30 min, protein-DNA complexes were separated electrophoretically at 4°C on 10% nondenaturing polyacrylamide gels. The gels were dried and the labeled complexes were detected by radioluminography.

In Vitro Transcription/Translation —— Recombinant human MTF-1 proteins were synthesized in vitro with the TnT® T7 quick coupled transcription/translation system (Promega, Madison, WI, U.S.A.) using 1.0 µg of plasmid (pRc/CMV-MTF) per reaction as previously described.

Preparation of Zinc-saturated MT —— Zn-MT was prepared by adding zinc (10 µmol) to the apo-MT solution (0.1 µmol). The reaction mixture was subjected to dialysis to purify Zn-MT. The apo-MT was prepared from Cd, Zn-MT (Sigma, St. Louis, MO, U.S.A.) by adding 1 mM HCl. The reaction mixture was subjected to dialysis to purify the apo-

RESULTS AND DISCUSSION

To determine the effect of MT on MTF-1 activity, we compared the DNA-binding activity in MT-induced cells and in cells stably overexpressing MT. The MT concentration increased in Interleukin (IL)-6 treated cells (Fig. 1A). MTF-1 DNA-binding activity transiently increased after 12 hr of IL-6-treatment (Fig. 1B). By comparison, MTF-1 DNA-binding activity did not increase in MT-overexpressing cells (approximately 300-fold MT concentration, Fig. 2A and 2B). The IL-6-induced transient activation of MTF-1 was not observed in MT-deficient (MT-KO) cells (Fig. 4). This result indicates that the IL-6-induced activation of MTF-1 correlates positively with induction of MT. It is possible that activation of MTF-1 required a dramatic change in the concentration of MT, but not a high concentration of MT. A drastic change of the concentration of MT may bring a drastic change of labile intracellular zinc pool. The activation of MTF-1 after IL-6 treatment may be caused by an increase of labile zinc pool. To confirm this possibility, an increase of labile zinc pool was caused by cycloheximide (CHX)-treatment, which blocked protein synthesis (included MT synthesis). CHX-treatment causes a decrease in the concentration
of MT in IL-6 treated cells but not in IL-6-free cells (Fig. 3A). CHX-induced activation of MTF-1 was observed only in IL-6 treated cells (Fig. 3B). By comparison, activation of MTF-1 was not observed in MT-KO cells (Fig. 4A and 4B). The activation of MTF-1 was striking in cells overexpressing MT (Fig. 3C). IL-6-mediated MT induction occurred during the inflammation reaction. Once the MT synthesis/degradation balance is disrupted, the activity of MTF-1 will be altered. It is known that MT-KO mice are hypersensitive to lipopolysaccharide (LPS)-induced coagulatory disturbance and LPS/D-galactosamine-induced lethality. It is possible that this hypersensitivity is related to a lack of MT-mediated MTF-1 activation.

The accelerated degradation of MT is observed under oxidative stress conditions. For this reason, we analyzed the effect of the oxidative agents H2O2 and tert-butyl hydroperoxide (tBH), on the activity of MTF-1. Incubation with zinc induced the formation of MTF-1/MRE complexes (Fig. 5A). By comparison, incubation with H2O2 alone was not able to induce the formation of MTF-1/MRE complexes. Incubation with H2O2 and Zn-saturated MT induced the formation of MTF-1/MRE complexes in a dose-dependent manner. These results.
suggest that H$_2$O$_2$ induces the degradation of MT and increases the level of free Zn. This hypothesis is supported by the observation that tBH-induced MTF-1 activation does not occur in MT-KO cells (Fig. 5B). In summary, MT acts as a sensor for oxidative stress. Under oxidative stress conditions, Zn is released from Zn-MT and induces the activation of MTF-1. Oxidative stress-induced MT transcription is mediated by certain cis-elements, MRE, and the antioxidant response element (ARE). The identity of the transcriptional factors involved in oxidative stress-induced ARE-mediated MT transcription is unclear, although, activation by MTF-1 is reported to induce this process. Using an in vitro transcription system, Zhang et al. showed that Zn-saturated MT is required for oxidative stress-mediated activation of MT transcription. It is unclear which proteins are the main zinc sources for activation of MTF-1. As shown in Fig. 5B, tBH-induced MTF-1 activation was not observed in MT-KO cells. Our result suggests that MT is the main zinc source for MTF-1 activation in response to oxidative stress.

Here, we showed that MT induces MTF-1 activity. In IL-6 treated cells, it is possible that activation of MTF-1 is induced by a dramatic change in the concentration of MT. Oxidative stress induced the activation of MTF-1 via degradation of Zn-MT. IL-6-mediated MT induction is observed during endotoxemia. Oxidative stress mediated MT induction occurs in response to a change in environmental conditions. For this reason, there are many candidate target genes for MTF-1. A change in MTF-1 activity may be an adaptive response to changes in environmental conditions. Further studies are necessary to reveal the mechanisms controlling MT-mediated activation of MTF-1.

REFERENCES