Mimosine Attenuates Serine Hydroxymethyltransferase Transcription by Chelating Zinc

IMPLICATIONS FOR INHIBITION OF DNA REPLICATION*

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Mimosine is a naturally occurring plant amino acid and iron chelator that arrests the cell cycle in the late G1 phase, although its mechanism of action is not known. Some studies indicate that mimosine prevents the initiation of DNA replication, whereas other studies indicate that mimosine disrupts elongation of the replication fork by impairing deoxyribonucleotide synthesis by inhibiting the activity of the iron-dependent enzyme ribonucleotide reductase and the transcription of the cytoplasmic serine hydroxymethyltransferase gene (SHMT1). In this study, the mechanism for mimosine-induced inhibition of SHMT1 transcription was elucidated. A mimosine-responsive transcriptional element was localized within the first 50 base pairs of the human SHMT1 promoter by deletion analyses and gel mobility shift assays. The 50-base-pair sequence contains a consensus zinc-sensing metal regulatory element (MRE) at position −44 to −38, and mutation of the MRE attenuated mimosine-induced transcription repression. Mimosine treatment eliminated MRE- and Sp1-binding activity in nuclear extracts from MCF-7 cells but not in nuclear extracts from a mimosine-resistant cell line, MCF-7/2a. MCF-7 cells cultured in zinc-depleted medium for more than 16 days were viable and lacked cytoplasmic serine hydroxymethyltransferase protein, confirming that mimosine inhibits SHMT1 transcription by chelating zinc. The disruption of DNA-protein interactions by zinc chelation provides a general mechanism for the inhibitory effects of mimosine on nuclear processes, including replication and transcription. Furthermore, this study establishes that SHMT1 is a zinc-inducible gene, which provides the first mechanism for the regulation of folate-mediated one-carbon metabolism by zinc.

Mimosine is an amino acid found in plants of the *Mimosa* and *Leucaena* genera and a tyrosine analog (1). It is a cell-specific inhibitor of DNA replication in *vivo*; sensitive cell lines include human MCF-7 (2), HL60 (3) and Chinese hamster ovary (4), whereas human neuroblastoma (2), K562 (3), and *Xenopus* and mouse embryonic cells are resistant (5, 6). Mimosine is an effective iron chelator and a structural analog of deferiprone, a compound that is used clinically to lower systemic iron in patients who suffer from iron overload (7, 8) (Fig. 1).

Mimosine arrests cell cycle progression in late G1 and although it elicits a plethora of biological effects, its mechanism of action has not been established. Mimosine has been proposed to arrest DNA synthesis at the replication fork by repressing deoxyribonucleotide synthesis (9–12). Mimosine and deferoxamine inhibit the activity of the iron-dependent enzyme ribonucleotide reductase in *vivo* by chelating iron (9, 11, 12) and deplete cellular deoxyribonucleotide pools in some but not all studies (2, 12). Furthermore, mimosine and deferoxamine have been shown to inhibit the transcription of the cytoplasmic serine hydroxymethyltransferase gene (SHMT1) in MCF-7 cells but not in mimosine-resistant SH-SY5Y neuroblastoma (2). The cytoplasmic serine hydroxymethyltransferase enzyme (CSHMT) catalyzes the tetrahydrofolate-dependent conversion of serine to glycine, a reaction that generates folate-activated one-carbon units that are required for purine, thymidine, and methionine biosynthesis (13). Therefore, it has been proposed that mimosine may impair deoxyribonucleotide biosynthesis by inhibiting CSHMT and ribonucleotide reductase (5, 14). Other studies demonstrate that mimosine disrupts the initiation phase of DNA replication at the origins of replication in Chinese hamster ovary cells. Mimosine is novel in this regard; it prevents formation of replication intermediates (4, 10). Mimosine also affects gene expression at the level of transcription and translation. Mimosine increases the expression of p21WAF1 (5, 15) and p27kip1 (16) while decreasing the transcription of the *SHMT1* (2), *Mcp-1*, and *Mip-2* (17) genes and depleting cyclin D levels (11). Mimosine also inhibits the activity of several enzymes in *vivo* including tyrosinase, dopamin β-hydroxylase, deoxyhypusyl hydroxylase, and H1 kinase (3). However, none of these effects provide a definitive mechanism for cell cycle arrest, nor do they account for the cell-specific effects that mimosine exerts. In this study, we elucidated the molecular mechanism for mimosine-induced transcriptional repression of the human SHMT1 gene to provide insight into its inhibitory effect on cell cycle progression.

EXPERIMENTAL PROCEDURES

Materials—Fetal bovine serum and α-minimal essential medium (αMEM) were obtained from Hyclone Laboratories. Zinc-depleted fetal bovine serum was prepared as described elsewhere (18). Mammary adenocarcinoma (MCF-7) cells and the mimosine-resistant cell line MCF-7/2a (2) were maintained in αMEM with 11% fetal calf serum at 37 °C in a 5% CO2 atmosphere. Mimosine was purchased from Sigma. Oligonucleotides were purchased from Sigma Genosys; pfu *Taq* polymerase and dNTPs were from Stratagene, and [γ-32P]dATP was purchased from PerkinElmer Life Sciences.

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‡ The abbreviations used are: SHMT1, cytoplasmic serine hydroxymethyltransferase gene; MEM, minimal essential medium; MRE, metal response element; NF, nuclear factor.
Generation of SHMT1 Promoter Deletion Fragments—The human SHMT1 proximal promoter from −409 to −1, and all deletion and mutated fragments thereof were cloned 5′ of the firefly luciferase gene in the basic (promoterless) pGL3 reporter plasmid (Promega). Fragments of the promoter were generated by PCR and sequence verified. The following primer pairs were used to generate promoter fragments: for the −298 to −109 fragment, the forward primer was 5′-ATGCAGATCTTCCTTCCTCACTTAAGC-3′, and the reverse primer was 5′-ATGCAGATCTCTTTGGGCTTGGCCCCG-3′. The following serial promoter deletion fragments were generated using the underlined sequences on the forward and reverse primers indicate NheI and BglII restriction endonuclease sequences, respectively, that facilitated the cloning into the pGL3 vector. For all primer sets, the cycling conditions were 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 7 min for 30 cycles. The concatenated vector ((−219 to −1)(−50 to −1)) was constructed by cloning a double-stranded oligonucleotide identical to the sequence −50 to −1 at the 3′ end of the −219 to −1 fragment in the basic pGL3 vector. Mutations were generated using the QuikChange site-directed mutagenesis kit (Stratagene), and the sequence was verified. To mutate the nuclear factor 1 (NF1) site, the forward oligonucleotide primer was 5′-GGTGCGGCCCATGCGGTCGCTTCCTGTATGGGCCG-3′, and the reverse primer was 5′-CGCCCAATCAGAGAGCGCAGC-3′. To mutate the metal response element (MRE) site, the forward oligonucleotide primer was 5′-GCTGGCGGCCCATGCGGTCGCTTCCTGTATGGGCCG-3′, and the reverse primer was 5′-CGCCCAATCAGAGAGCGCAGC-3′. Bold letters indicate base pair changes in the NF1/MRE element. The cycling conditions were 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min for 30 cycles.

Gene Reporter Assays—MCF-7 cells were seeded in 96-well plates and upon attachment were treated with equal volumes of phosphate-buffered saline or mimosine (final concentration of 100 μM) for 24 h prior to transfection. Cells were transfected with one of various SHMT1 promoter constructs using FuGENE transfectamine (Roche Applied Science) following the manufacturer’s protocol and cultured for an additional 24 h in culture medium that contained (experimental) or lacked

![Mimosine and Deferiprone](http://www.jbc.org/content/374/11/397/F2)

**Table 2. Transcriptional repression of the SHMT1 proximal promoter by mimosine.** MCF-7 cells were cultured in the presence and absence of 100 μM mimosine for 24 h and then transiently transfected with plasmids containing either the control SV40 promoter or SHMT1 5′-proximal promoter sequences cloned 5′ to the firefly luciferase cDNA in the promoterless vector pGL3-basic. Firefly luciferase activity serves as an indicator of promoter activity and was assayed 24 h following transfection. Firefly luciferase activity was normalized to Renilla luciferase activity, which results from the co-transfection of a control plasmid as described under “Experimental Procedures.” Data are presented as relative luciferase activity (the ratio of firefly/Renilla luciferase activity). Black bars indicate normalized firefly luciferase activity in untreated cells; white bars indicate normalized firefly luciferase activity in mimosine-treated cells. All values are the average of three measurements, and variance is displayed as the standard error of the mean. Inhibition of promoter activity by mimosine is indicated by the percentages that appear at the top of the black bars. Site-specific mutagenesis was used to alter three base pairs in the NF1 element of the construct −219 to −1 from 5′-TGGCT-3′ to 5′-GGTGC-3′. Site-specific mutagenesis was used to alter three base pairs in the MRE element of the construct −219 to −1 from 5′-TGCGCT-3′ to 5′-TAGTC-3′.
(control) 100 μM mimosine. Firefly luciferase activity was measured using Dual Glo luciferase kit (Promega) and normalized to Renilla luciferase activity that was generated from the co-transfected control pRL-cytomegalovirus vector. In all cases, luciferase activity measurements were corrected for background; all luciferase activity values were a minimum of 20-fold greater than background. All luciferase data is presented as a ratio of firefly luciferase to Renilla luciferase activity.

**Results**

**Identification of Mimosine-responsive Transcriptional Elements**—A mimosine-responsive cis element was identified in the SHMT1 5′-promoter by comparing SHMT1 promoter activity in transiently transfected MCF-7 cells that were cultured in the presence and absence of 100 μM mimosine (Fig. 2). Mimosine inhibited the activity of the SV40 promoter by 27%, whereas the activity of the SHMT1 proximal promoter (409 to −1) was inhibited by 96%. A mimosine-responsive element was localized to the first 50 base pairs of the SHMT1 proximal promoter by gel mobility shift assays. Mimosine exposure did not affect qualitatively the SDS-PAGE profile of MCF-7 nuclear protein (Fig. 4) but did inhibit MRE and Sp1 binding activity. Nuclear extracts from MCF-7 cells bound a double-stranded oligonucleotide probe that contains the sequence of the overlapping NF1/MRE element −50 to −31 as is evident from the gel shift (Fig. 5), whereas nuclear extracts from mimosine-treated cells displayed no or very weak binding activity. Mutation of the NF1/MRE element resulted in a 2-fold decrease in the transcriptional activity of the −219 to −1 promoter fragment compared with the −219 to −1 fragment of the non-mutated probe. Mimosine exposure eliminated the binding activity of nuclear extracts for all probes. Collectively, these data demonstrate that factors present in MCF-7 nuclear extracts bind to the MRE consensus sequence located within the mimosine-responsive promoter fragment −50 to −1 (Fig. 2), but this activity is lost in MCF-7 cells that were exposed to mimosine.

**Analysis of nuclear extracts by SDS-PAGE**. Nuclear extracts were prepared from MCF-7 cells and MCF-7/2a cells (a mimosine-resistant cell line) grown for 48 h in αMEM or αMEM containing 300 μM mimosine. Nuclear extracts (10 μg) were run on an 8% polyacrylamide gel, and protein bands were visualized by silver stain. Mimosine and Serine Hydroxymethyltransferase Transcription

![Fig. 4. Analysis of nuclear extracts by SDS-PAGE](image-url)
induced inhibition of cell cycle or SHMT1 transcription (2). Nuclear extracts isolated from mimosine-treated MCF-7/2a cells retained the ability to bind the probe containing the NF1/MRE consensus sequence (−50 to −31) of the SHMT1 promoter, which contains an overlapping NF1/MRE element. The mutated MRE probe contained the nucleotide sequence −50 to −31 of the SHMT1 promoter, except the MRE element was changed from 5'-TGGCTC-3' to 5'-TACAATC-3'. The mutated NF1 probe contained the nucleotide sequence −50 to −31 of the SHMT1 promoter, except the NF1 element was changed from 5'-TTGGCTC-3' to 5'-GAGACT-3'. In all cases, the addition of unlabeled probe refers to the nonmutated probe that represents nucleotide sequence −50 to −31 of the SHMT1 promoter. The arrow designates the mimosine sensitive band shift.

Expression of the SHMT1 Gene Is Zinc-dependent—The effect of zinc availability on CSHMT protein levels was determined in MCF-7 cells by Western blot analyses (Fig. 7). MCF-7 cells passaged in zinc depleted culture medium (18) displayed a 50% decrease in CSHMT protein levels after 8 days of culture and greater than a 95% decrease from day 16 through day 40. MCF-7 cells passaged in zinc deficient medium that was supplemented with 4 μM zinc exhibited similar CSHMT protein levels as those passaged in αMEM. Similar effects on CSHMT

![Fig. 5. Effect of mimosine on NF1/MRE binding activity in MCF-7 cells.](image)

![Fig. 6. Effect of mimosine on NF1/MRE and Sp1 binding activity in MCF-7 and MCF-7/2a cells.](image)

![Fig. 7. Effect of zinc depletion on CSHMT protein levels in MCF-7 and MCF-7/2a cells.](image)
protein levels were observed for the mimosine-resistant MCF-7/2a cell line (Fig. 7).

**DISCUSSION**

The results of this study establish *cis* elements that bind zinc finger transcription factors, including MREs and Sp1, as mimosine-responsive transcriptional elements and thereby provide a definitive mechanism for mimosine-induced repression of *SHMT1* gene transcription. Iron chelators, including deferiprone and deferoxamine, exhibit appreciable affinity for zinc and can deplete the labile intracellular zinc pool in cultured murine thymocytes (7, 8). In patients with iron overload, chronic deferiprone treatment is associated with zinc deficiency (29) and impairments in folate metabolism are strongly associated with human pathologies including cancers, cardiovascular disease, and developmental anomalies, further investigation into the role of zinc in regulating folate metabolism is clearly warranted.

Mimosine impairs the binding activity of the zinc finger proteins MTF-1 and Sp1, an observation that suggests a general mechanism for the effect of mimosine on nuclear processes including replication and transcription. Biological responses elicited by mimosine may result from inhibition of zinc-dependent DNA binding metalloproteins including those involved in DNA replication initiation (25, 26) and translation (27). Whereas the *in vivo* zinc chelating activity of mimosine and other iron chelators would be expected to be invariant among cell types, mimosine is a cell-specific inhibitor of both cell cycle and *SHMT1* transcription. Previously, we reported the generation of the mimosine-resistant MCF-7/2a cell line, which displayed normal to elevated *SHMT1* transcriptional activity in the presence of either mimosine or deferoxamine (2), indicating that the inhibitory effect of mimosine on cell cycle progression and *SHMT1* transcription results from a common mechanism (2). In support of this suggestion, both MFT-1 and Sp1 binding activity is unaffected by mimosine in MCF-7/2a cells, indicating that mimosine cannot effectively prevent the incorporation of zinc into zinc finger DNA binding proteins in this resistant cell line, although *SHMT1* expression remains sensitive to zinc depletion of the culture medium (Fig. 7). Therefore, cellular mimosine resistance is most likely conferred by limiting the cellular accumulation of metal chelators, either by enhancing their metabolism or efflux or by preventing their import, which thereby prevents the sequestration of intracellular zinc.

The regulation of *SHMT1* transcription by MTF-1 indicates that zinc status may regulate folate-mediated one-carbon metabolism. The CSHMT enzyme plays a key role in the regulation of folate-mediated one-carbon metabolism; it serves as a metabolic switch that preferentially directs folate-activated one-carbon units into the thymidylate synthesis pathway and impairs the homocysteine remethylation pathway by sequestering 5-methyltetrahydrofolate rendering it unavailable for the remethylation of homocysteine to methionine (13). Zinc status is known to affect folate-mediated one-carbon metabolism, but the underlying molecular mechanisms have not been established. Zinc deficiency in rats lowers plasma homocysteine concentrations by 65% and reduces hepatic 5-methyltetrahydrofolate concentrations (28). Although the effects of zinc deficiency on lowering plasma homocysteine and hepatic 5-methyltetrahydrofolate concentrations have been attributed to very modest increases in hepatic methionine synthase activity (28), they are also consistent with an attenuation of *SHMT1* expression and subsequent derepression of the homocysteine remethylation cycle. Because human zinc deficiency is common (29) and impairments in folate metabolism are strongly associated with human pathologies including cancers, cardiovascular disease, and developmental anomalies, further investigation into the role of zinc in regulating folate metabolism is clearly warranted.

REFERENCES
