Dietary Manganese Modulates Expression of the Manganese-Containing Superoxide Dismutase Gene in Chickens<sup>1–3</sup>

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Abstract

To investigate the possible mechanism(s) by which dietary manganese (Mn) levels and sources modulate the expression of the manganese-containing superoxide dismutase (MnSOD) gene at both the transcriptional and translational levels, we used 432 8-d-old male broiler chicks in a 1 plus 4 × 2 design. Chicks were given either a diet without Mn supplementation [control (C)] or diets supplemented with 100 (optimal) or 200 (high) mg Mn/kg diet from inorganic Mn sulfate (I) or 3 organic complexes of Mn and amino acids with weak (W), moderate (M), or strong (S) chelation strength up to 21 d of age. Compared with C chicks, chicks fed Mn-supplemented diets had higher (<i>P</i> < 0.01) Mn concentrations, specificity protein 1 (Sp1) DNA-binding activities, MnSOD mRNA levels, MnSOD mRNA-binding protein (MnSOD-BP) RNA-binding activities, MnSOD protein concentrations, and MnSOD activities within heart tissue, but lower (<i>P</i> < 0.01) heart activating protein-2 (AP-2) DNA-binding activities compared with those fed the I and W diets and lower (<i>P</i> < 0.05) heart activating protein-2 (AP-2) DNA-binding activities. Chicks fed M diets had higher (<i>P</i> < 0.05) heart Mn concentrations, MnSOD mRNA levels, and MnSOD-BP RNA-binding activities compared with those fed the I and W diets and lower (<i>P</i> < 0.01) AP-2 DNA-binding activities than those fed other treatment diets. These results suggest that dietary Mn could modulate the expression of the MnSOD gene in broilers by altering Sp1 and AP-2 DNA-binding activities at the transcriptional level and enhancing MnSOD-BP RNA-binding activity at the translational level. Additionally, an organic Mn source with moderate chelation strength could be more effective than other Mn sources in activating MnSOD gene expression at both the transcriptional and translational levels. J. Nutr. 141: 189–194, 2011.

Introduction

Manganese (Mn) is an essential diet component for animals and humans, because it is a critical component of manganese-containing superoxide dismutase (MnSOD).<sup>7</sup> MnSOD is the primary antioxidant enzyme in mitochondria that plays a key role in the detoxification of superoxide free radicals and protects cells from oxidative stress. Previous research has focused on the effect of a Mn-deficient diet on MnSOD activity and mitochondrial ultrastructure in mice (1), rats (2), and chickens (3,4). MnSOD is a nuclear-encoded mitochondrial protein synthesized in the cytosol and post-translationally modified for transport into the mitochondrion (5). The biosynthesis of MnSOD might be controlled at transcriptional, post-transcriptional, and/or post-translational levels (6).

MnSOD is highly expressed in differentiated organs that contain a large number of mitochondria such as the heart, liver, and kidneys. Of all the tissues that express MnSOD in humans (7), mice (8), and chickens (9), the heart has the highest steady-state mRNA expression level. A series of studies in broiler chicks from our laboratory has indicated that, although Mn concentrations were significantly lower in the heart than in other soft tissues, such as the liver and pancreas, MnSOD activity was significantly higher and very sensitive to supplemental Mn levels in corn-soybean meal diets (3,4). Furthermore, MnSOD activities in the liver and pancreas of broilers were not affected by dietary supplemental Mn levels (3,4) and heart MnSOD mRNA
levels were consistently sensitive to supplemental Mn levels in corn-soybean meal diets (10–14). However, no information is available on the molecular mechanisms underlying the transcriptional and translational regulations of MnSOD gene expression in chickens or other animals by dietary Mn.

Several commercial organic Mn sources, including amino acid (AA) complexes, chelates, and proteinates, have been developed as supplements to animal feeds. Our previous studies have demonstrated that when Mn sources were supplied through either diet or i.v. injection, the organic Mn source with moderate chelation strength was more effective than inorganic Mn sulfate or organic Mn sources with weak or strong chelation strength in terms of enhancing heart MnSOD mRNA level of broilers (10–13). However, the mode of action by which organic Mn sources with different chelation strengths mediated the expression of MnSOD gene in broilers remains unclear.

Our objectives in this experiment were to identity the possible molecular mechanism(s) by which dietary Mn regulates MnSOD gene expression in the heart tissue of broilers at both the transcriptional and translational levels, as well as the underlying relationship between the regulation of MnSOD gene expression and supplemental Mn source, to provide a more effective nutritional strategy for protecting tissues from oxidative damages.

Materials and Methods

Animals and experimental design. All experimental procedures were approved by the Office of the Beijing Veterinarians. Male broiler chicks (Arbor Acres, Huadu Broiler Breeding) were handled in accordance with the guidelines for raising broilers (15). The broilers were housed in electrically heated, thermostatically controlled stainless steel cages with fiberglass food and water containers and were allowed ad libitum access to experimental diets and tap water containing no detectable Mn. During the first week, the birds were fed a corn-soybean meal basal diet with no Mn supplementation (Table 1, containing 16.13 mg Mn/kg diet by analysis), which was formulated to meet or exceed the requirements of the broilers for all nutrients except for Mn. This diet was used to deplete Mn stores in the body. At 8 d of age, a total of 432 chicks were randomly divided into 9 treatment groups with 6 replicate cages (8 chicks/cage) for each treatment in a completely randomized design involving a 4 × 2 (source × level) factorial arrangement of treatments plus 1 Mn-unsupplemented basal diet control (C). The 4 supplemental Mn sources tested were inorganic Mn sulfate (I; reagent grade MnSO₄·H₂O) and 3 organic complexes of Mn and AA with weak (W; Availa Mn, formation quotient (Qf) = 2.35) or moderate (M; Mn-AA B, Qf = 16.85) or organic Mn sources with weak or strong chelation strength in terms of enhancing heart MnSOD mRNA level of broilers (10–13). However, the mode of action by which organic Mn sources with different chelation strengths mediated the expression of MnSOD gene in broilers remains unclear.

Our objectives in this experiment were to identity the possible molecular mechanism(s) by which dietary Mn regulates MnSOD gene expression in the heart tissue of broilers at both the transcriptional and translational levels, as well as the underlying relationship between the regulation of MnSOD gene expression and supplemental Mn source, to provide a more effective nutritional strategy for protecting tissues from oxidative damages.

were selected based on body weight mean and killed by cervical dislocation. The heart was excised and quickly frozen in liquid nitrogen until analysis. The samples of 3 individual chicks from each cage were pooled prior to analysis; thus, 6 replicates were produced from each treatment.

Measurements of Mn, total RNA, total protein concentrations, and MnSOD activity. Concentrations of Mn in diets and heart tissue were determined by inductively coupled plasma emission spectroscopy (Thermal Jarrell Ash) as described by Li et al. (10). Total RNA was measured on the basis of the UV absorbance at 260 nm. The total protein concentration in sample supernatant was measured using the bicinonic acid protein assay kit (Pierce). MnSOD activity was measured by the nitrite method described by Li et al. (10).

RNA extraction and quantitative RT-PCR. Total RNA in heart tissue was extracted using Trizol (Invitrogen) according to the manufacturer’s instructions. RT was performed using the SuperScript III first-strand synthesis system for RT-PCR kit (Invitrogen) and oligo (dT)₂₀ as a primer. Quantitative PCR was performed in triplicate on an ABI Prism 7000 apparatus (Applied Biosystems) according to optimized PCR protocols (17). The primers for MnSOD (forward, 5'–GAGAAATTGTGCGTGACATCA-3'; reverse, 5'–CCAGGCGCCTCTTGTATTTCT-3'; PCR product length 138 bp) and reference gene β-actin (forward, 5'–GAGGAAATTGTGCGTGACATCA-3'; reverse, 5'–CCTGAAACTCTCATTGCCA-3'; PCR product length 152 bp) were used for the amplification reactions, respectively. We used the relative standard curve method to quantify gene expression, as previously described (17). The result was expressed as the ratio of MnSOD mRNA abundance/β-actin mRNA.

Western blotting. Heart tissue was homogenized in ice-cold lysis buffer (50 mmol/L Tris, pH 7.4, containing 150 mmol/L NaCl, 1 mmol/L PMSF, 1 mmol/L EDTA, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS) and then sonicated at 4°C for 2 min (2 s with 10-s intervals). After incubating on ice for 30 min, the homogenate was centrifuged at 12,000 × g for 5 min at 4°C. The supernatant was
subjected to Western-blot analysis (18) by using specific antibodies for MnSOD (Abcam, diluted 1:3000) and β-actin (Sigma, diluted 1:5000). Data were presented as the ratio of MnSOD protein band intensity: β-actin protein.

**Nuclear extract and electrophoretic mobility shift assay.** Mined tissue was homogenized in HEPES buffer (10 mM/L HEPES, pH 7.9, containing 1.5 mM/L MgCl₂, 10 mM/L KCl, 0.5 mM/L DTT, 0.2 mM/L PMSF, and 5 mg/L each of aprotinin, pepstatin, leupeptin, and chymostatin) and incubated on ice for 30 min. After the addition of Nonidet P-40 to a final concentration of 5 g/L, the homogenate was vortexed vigorously for 15 s and centrifuged at 14,000 × g for 1 min at 4°C. The pellet was incubated with HEPES buffer containing 420 mM/L NaCl for 30 min on ice. Nuclear extract supernatant was obtained by centrifugation at 12,000 × g for 5 min at 4°C.

Electrophoretic mobility shift assay (EMSA) was performed using consensus sequences for transcriptional factor specificity protein 1 (Sp1), 5′-CGAGAGGGGCCTTCCGGCTCT-3′, and activator-protein 2 (AP-2), 5′-GAGTGGGCCTTCCGGCTCT-3′. The complementary single-stranded oligonucleotide was end-labeled separately using biotin-11-dUTP and terminal deoxynucleotidyl transferase according to instructions of the biotin 3′ end DNA labeling kit (Pierce) and then annealed before being used in the binding reactions. The binding specificity between Sp1 or AP-2 protein and its respective consensus sequences was verified by competition and supershift assay. For the competition experiments, synthetic wild-type and mutant oligonucleotides were used in 100-fold molar excess and incubated with nuclear extract for 20 min before the biotin-labeled probe was loaded. For the supershift assay, the nuclear extract was preincubated with Sp1 (Abcam) or AP-2 (α-β, Abcam) antibody for 60 min at room temperature prior to adding oligonucleotide probe. Samples were then subjected to a 6% nondenaturing PAGE in Tris borate-glycine buffer (45 mmol/L Tris borate, 1 mmol/L EDTA, pH 8.3) and transferred to a nitrocellulose membrane. The biotin-labeled DNA-protein complex was detected using streptavidin-HRP conjugate and chemiluminescent substrate according to instructions of the Lightshift chemiluminescent EMSA kit (Pierce). The data were expressed as relative densitometry units of the DNA-protein complex in each experimental group compared with those of the C group.

**RNA EMSA.** Heart tissue was homogenized in lysis buffer (25 mM/L Tris, pH 7.0, containing 40 mM/L KCl, 0.1 mM/L EDTA, 1% Triton X-100, 0.1 mM/L PMSF, 10 mg/L leupeptin, and 200 U/L aprotinin). The supernatant fluid was obtained by centrifugation at 12,000 × g for 15 min at 4°C. A 41-base MnSOD cRNA probe corresponding to the MnSOD mRNA-binding protein (MnSOD-BP) binding region (19) was synthesized from a template containing a T7 polymerase promoter sequence. The sequence for the template was 5′-TATTITGTATAGG TAACACAAATGTTGCGTCCATCTTGGTCTGATGTCATGTA-3′. The labeled sense-strand transcript was prepared following the procedure for incorporation of biotin-14-CTP into RNA by T7 RNA polymerase (Invitrogen). The competitor-iron-responsive element cRNA probe was prepared from the template 5′-GGTTCCGTTCACAAA CACTGTGAACCGGAAAACCATATGATGTCATGTA-3′. The binding specificity between MnSOD-BP and MnSOD mRNA was confirmed by competition assay. For the competition assay, 100-fold of unlabeled MnSOD-BP cRNA probe or competitor-iron-responsive element cRNA sequence was mixed with the cellular proteins for 20 min at room temperature before addition of the specific labeled probe. Samples were subjected to a 4% native polyacrylamide gel and detected according to instructions of the Lightshift chemiluminescent EMSA kit (Pierce). The data were expressed as relative densitometry units of the RNA-protein complex in each experimental group compared with those of the C group.

**Statistical analysis.** To test the effect of supplemental Mn, data were analyzed using single df contrast to compare all supplemental Mn treatments with the control. Data excluding the control were further analyzed as a 4 × 2 (source × level) factorial arrangement of treatments by 2-way ANOVA with a model that included the main effects of supplemental Mn level and source, as well as their interaction. When an effect was significant (P < 0.05), means were compared by Duncan’s multiple comparison tests to determine specific differences between means. Data were presented as means ± SE. The CORR procedure of SAS was used to determine simple Pearson correlation coefficients.

**Results.**

**Performance.** Daily weight gain, feed intake, and gain:feed ratio of broiler chicks were not affected by dietary supplemental Mn level, source, or their interaction (Supplemental Table 1).

**Heart Mn concentration, MnSOD mRNA level, MnSOD protein, and MnSOD activity.** Compared with C chicks, chicks fed Mn-supplemented diets had higher (P < 0.01) Mn concentrations, MnSOD mRNA levels, MnSOD protein concentrations, and MnSOD activities in their heart tissue (Table 2). All 4 measured indices were higher (P < 0.05) in chicks fed high-Mn diets compared with those fed optimal-Mn diets. Heart Mn concentrations were higher (P < 0.05) in M chicks compared with I and W chicks but did not differ among other Mn source chicks. Heart MnSOD mRNA levels were higher (P < 0.01) in M chicks compared with the other Mn source chicks and also higher (P < 0.01) in S chicks compared with I chicks. Heart MnSOD activities were higher (P < 0.01) in M and S chicks than in I chicks. Supplemental Mn source did not affect heart MnSOD protein concentration. All 4 measured indices were correlated with dietary supplemental Mn level (r > 0.56; P < 0.01) (Supplemental Table 2) and also one another (r > 0.38; P < 0.01).

**Heart Sp1 and AP-2 DNA-binding activities.** Compared with C chicks, chicks fed Mn-supplemented diets had higher (P < 0.01) heart Sp1 DNA-binding activities and lower (P < 0.01) AP-2 DNA-binding activities (Table 3). Chicks fed high-Mn diets had higher (P < 0.01) heart Sp1 DNA-binding activities but lower (P < 0.05) heart AP-2 DNA-binding activities compared with those fed optimal-Mn diets. Heart AP-2 DNA-binding activities were lower (P < 0.01) in M chicks compared with the other Mn source chicks and also lower (P < 0.01) in W chicks compared with I and S chicks. Heart Sp1 DNA-binding activity was positively correlated with dietary supplemental Mn level (r = 0.91; P < 0.01), heart Mn concentration (r = 0.41; P < 0.01), and MnSOD mRNA level (r = 0.55; P < 0.01), whereas heart AP-2 DNA-binding activity was negatively correlated with dietary supplemental Mn level (r = −0.46; P < 0.01), heart Mn concentration (r = −0.53; P < 0.01), and MnSOD mRNA level (r = −0.76; P < 0.01).

**Heart MnSOD-BP RNA-binding activity.** Compared with C chicks, chicks fed Mn-supplemented diets had higher (P < 0.01) MnSOD-BP RNA-binding activities (Table 3). Chicks fed high-Mn diets had higher (P < 0.01) heart MnSOD-BP RNA-binding activities compared with those fed optimal-Mn diets. Heart MnSOD-BP RNA-binding activities were higher (P < 0.01) in M chicks compared with the other Mn source chicks and also higher (P < 0.01) in S chicks compared with I and W chicks fed either an optimal or high-Mn diet; however, heart MnSOD-BP RNA binding activities were higher (P < 0.05) in W chicks compared with I chicks fed a high-Mn diet, whereas I and W chicks fed optimal Mn diets did not differ. Heart MnSOD-BP RNA-binding activity was correlated with dietary supplemental Mn level (r = 0.66; P < 0.01), heart MnSOD mRNA level (r = 0.84; P < 0.01), MnSOD protein concentration (r = 0.53; P < 0.01), and MnSOD activity (r = 0.66; P < 0.01).
### Table 2: Effects of supplemental Mn level and source on heart Mn concentration, MnSOD mRNA level, MnSOD protein concentration, and MnSOD activity of broilers

<table>
<thead>
<tr>
<th>Item</th>
<th>C</th>
<th>I-100</th>
<th>W-100</th>
<th>M-100</th>
<th>S-100</th>
<th>I-200</th>
<th>W-200</th>
<th>M-200</th>
<th>S-200</th>
<th>( P )-value Level</th>
<th>Source</th>
<th>Level × Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Mn concentration, ( \mu \text{mol/kg fresh tissue} )</td>
<td>7.6 ± 0.4*</td>
<td>9.9 ± 0.5b</td>
<td>9.8 ± 0.5b</td>
<td>11.7 ± 0.7b</td>
<td>11.1 ± 0.5ab</td>
<td>10.9 ± 0.8b</td>
<td>10.8 ± 0.4b</td>
<td>12.2 ± 0.2b</td>
<td>12.0 ± 0.5b</td>
<td>0.05</td>
<td>0.02</td>
<td>0.97</td>
</tr>
<tr>
<td>MnSOD mRNA level,2</td>
<td>1.03 ± 0.03*</td>
<td>1.16 ± 0.01b</td>
<td>1.18 ± 0.02c</td>
<td>1.36 ± 0.02c</td>
<td>1.22 ± 0.03b</td>
<td>1.23 ± 0.02c</td>
<td>1.29 ± 0.02c</td>
<td>1.43 ± 0.03b</td>
<td>1.31 ± 0.02b</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.65</td>
</tr>
<tr>
<td>MnSOD protein concentration,2</td>
<td>0.41 ± 0.01*</td>
<td>0.46 ± 0.02</td>
<td>0.48 ± 0.01</td>
<td>0.48 ± 0.02</td>
<td>0.47 ± 0.02</td>
<td>0.48 ± 0.02</td>
<td>0.90 ± 0.02b</td>
<td>0.52 ± 0.02</td>
<td>0.52 ± 0.01</td>
<td>0.02</td>
<td>0.37</td>
<td>0.76</td>
</tr>
<tr>
<td>MnSOD activity,3</td>
<td>1278 ± 17</td>
<td>1334 ± 14</td>
<td>1383 ± 16</td>
<td>1386 ± 19</td>
<td>1398 ± 14</td>
<td>1398 ± 7</td>
<td>1390 ± 27</td>
<td>1465 ± 29</td>
<td>1436 ± 3</td>
<td>0.01</td>
<td>&lt;0.01</td>
<td>0.35</td>
</tr>
</tbody>
</table>

1 Data are means ± SE, \( n = 6 \). *Different from all Mn-supplemental groups, \( P < 0.05 \). Labeled means for a dose without a common letter differ, \( P < 0.05 \); \( y \) different from the corresponding 100 mg Mn/kg group, \( P < 0.05 \).
2 Expressed as the ratio of MnSOD mRNA abundance or MnSOD protein band intensity to \( \beta \)-actin mRNA or \( \beta \)-actin protein.
3 One nitrite unit (NU) was defined as the amount of enzyme needed to obtain 50% inhibition of nitrite formation.

### Table 3: Effects of supplemental Mn level and source on Sp1 DNA-binding activity, AP-2 DNA-binding activity, and MnSOD-BP RNA-binding activity of broilers

<table>
<thead>
<tr>
<th>Item</th>
<th>C</th>
<th>I-100</th>
<th>W-100</th>
<th>M-100</th>
<th>S-100</th>
<th>I-200</th>
<th>W-200</th>
<th>M-200</th>
<th>S-200</th>
<th>( P )-value Level</th>
<th>Source</th>
<th>Level × Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sp1 DNA-binding activity</td>
<td>1.0 ± 0.04*</td>
<td>1.1 ± 0.03</td>
<td>1.1 ± 0.02</td>
<td>1.1 ± 0.02</td>
<td>1.7 ± 0.03d</td>
<td>1.7 ± 0.03d</td>
<td>1.7 ± 0.03d</td>
<td>1.7 ± 0.02d</td>
<td>-</td>
<td>&lt;0.01</td>
<td>0.48</td>
<td>0.45</td>
</tr>
<tr>
<td>AP-2 DNA-binding activity</td>
<td>1.0 ± 0.02*</td>
<td>0.90 ± 0.02c</td>
<td>0.80 ± 0.02b</td>
<td>0.98 ± 0.03b</td>
<td>0.90 ± 0.02c</td>
<td>0.83 ± 0.03d</td>
<td>0.74 ± 0.03d</td>
<td>0.60 ± 0.03d</td>
<td>0.84 ± 0.02d</td>
<td>0.02</td>
<td>&lt;0.01</td>
<td>0.26</td>
</tr>
<tr>
<td>MnSOD-BP RNA-binding activity</td>
<td>1.0 ± 0.01*</td>
<td>1.1 ± 0.02c</td>
<td>1.1 ± 0.03b</td>
<td>1.6 ± 0.02c</td>
<td>1.5 ± 0.03b</td>
<td>1.3 ± 0.01df</td>
<td>1.4 ± 0.01df</td>
<td>1.8 ± 0.02d</td>
<td>1.7 ± 0.02d</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1 Data are means ± SE, \( n = 6 \). *Different from all Mn-supplemental groups, \( P < 0.05 \). Labeled means for a dose without a common letter differ, \( P < 0.05 \); \( d \) different from the corresponding 100 mg Mn/kg group, \( P < 0.05 \).
Discussion

Results from this study revealed a positive relationship between dietary supplemental Mn level, heart Mn concentration, and MnSOD mRNA level. This confirmed that dietary Mn modulates MnSOD gene expression in heart tissue of broilers at the transcriptional level. We analyzed the first 100-bp 5’-flanking region in the chicken MnSOD gene promoter using transcription element search software and found 3 GC-rich elements for Sp1 (−44 to −36, −56 to −51, and −73 to −64) and 1 GC-rich element for AP-2 (−26 to −17). This was consistent with studies in humans (20), bovines (21), and mice (22), indicating that the promoter sequences of the chicken MnSOD gene contained multiple Sp1 and AP-2 binding sites and the transcription of the MnSOD gene might be controlled by these regulatory elements. The result of the positive correlation between heart Sp1 DNA-binding activity and MnSOD mRNA level indicated that Sp1 upregulated MnSOD gene expression in chickens, as previously reported in humans (23). Increased Sp1 DNA-binding activity might be responsible for the dietary Mn-mediated increase in MnSOD mRNA level, which contributes to one molecular mechanism for the transcriptional regulation of the MnSOD gene. The reverse correlation between heart AP-2 DNA-binding activity and the MnSOD mRNA level suggested a down-regulating role of AP-2 in the transcriptional regulation of the chicken MnSOD gene. Enhanced MnSOD mRNA level by dietary Mn could result from the suppression of AP-2 DNA-binding activity, which might be another molecular mechanism for the transcriptional regulation of the chicken MnSOD gene by dietary Mn. The modulations of Sp1 and AP-2 DNA-binding activities by dietary Mn could be due to the oxidant-sensitive cysteine residues in their DNA-binding regions (24,25). The above 2 molecular mechanisms of transcriptional regulation of MnSOD gene expression by dietary Mn have not been reported in other animals.

Both the MnSOD-BP RNA-binding activity and the 3’ untranslated region cis element are required for the efficient translation of MnSOD mRNA (19,26). The MnSOD-BP participates in formation of the translation initiation complex (27,28) to initiate mRNA translation. Considering the synthesis and activation of MnSOD protein in Saccharomyces cerevisiae (29), inserting Mn into a newly synthesized polypeptide is possible. Furthermore, the nascent polypeptide synthesis appears closely coupled to its import into mitochondria (29). In the present study, enhanced MnSOD-BP RNA-binding activity as well as increased MnSOD mRNA level, MnSOD protein concentration, and enzymatic activity due to dietary Mn addition suggests that MnSOD-BP might be an enhancer in promoting MnSOD protein translation as it is in rats (26). Dietary Mn may regulate MnSOD protein translation through MnSOD-BP, which might be a molecular mechanism for the translational regulation of the chicken MnSOD gene expression by dietary Mn. This mechanism for the translational regulation of MnSOD gene expression by dietary Mn has not been reported in other animals.

Our findings from the present study indicate that the organic Mn source with moderate chelation strength was more effective than other Mn sources in activating MnSOD gene expression at both the transcriptional and translational levels. This might be due to greater intestinal absorption and/or tissue utilization of the organic Mn source with moderate chelation strength in broilers. Compared with inorganic Mn sulfate and the organic Mn source with weak chelation strength, the organic Mn sources with moderate and strong chelation strengths significantly increased transcriptional expression of divalent metal trans-

There are 2 hypotheses regarding the absorption and utilization mechanisms of mineral complexes (32). One is that the organic mineral complex or chelate with the optimal chelation strength could resist interference from dietary antinutritional factors in the digestive tract and directly reach the intestinal brush border, where it is hydrolyzed and absorbed as an ion into the blood, resulting in a higher bioavailability of the complexed or chelated than the inorganic form of the metal. Another hypothesis is that the organic mineral complex or chelate with the optimal chelation strength could maintain its structural integrity in the digestive tract and arrive at absorptive sites in the small intestine as the original intact molecule, then be absorbed and metabolized as such, rendering the organic supplement superior in bioavailability to the inorganic source. However, until now, there has been, to our knowledge, no direct evidence supporting either of these hypotheses, mainly because of a lack of definite methods to test the organic mineral complexes or chelates. Recent findings in broilers from our laboratory have indicated that organic Mn or Zn complexes or chelates with the optimal chelation strengths could lessen the negative effects of either high calcium (33) or phytate levels (34) on the Mn and Zn absorption in the small intestine, thereby improving absorption and resulting in higher bioavailabilities of the complexes or chelates than the inorganic forms of the Mn and Zn (11,35). An antagonism between Zn and Cu occurred when the inorganic, but not organic, forms of these 2 minerals were included in a chick diet (36). Copper might be absorbed as a Cu-peptide chelate through the di- and tri-peptide transporter in the jejunum of weanling pigs (37). These findings have provided indirect evidence for the above 2 claims. In the present study, it remains unclear whether the organic Mn source with moderate chelation strength was absorbed in the small intestine of broilers and utilized in the heart tissue as an ion, as the original intact complex, or both. Therefore, further studies are needed to answer this question.

In summary, our findings from the present study suggest that dietary Mn could modulate the MnSOD gene expression in the heart tissue of broilers through Sp1, Ap-2, and MnSOD-BP at both the transcriptional and translational levels. The organic Mn source with moderate chelation strength was the most effective in activating MnSOD gene expression at both the transcriptional and translational levels. These findings provide a novel nutritional strategy for protecting tissues from oxidative damage through dietary intake of Mn using an organic Mn source with moderate chelation strength.

Acknowledgments

X.G.L., L.L., and S.F.E. designed the research; S.F.H., Y.P.W., S.F.L., L.L., L.Y.Z., and B.L. conducted research; S.F.L., S.F.H., and S.B.L. analyzed data; S.F.L., X.G.L., and K.L. wrote the paper; and X.G.L. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited


