ROLE OF MOLYBDENUM IN THE BIOLOGICAL FUNCTION OF SULFITE OXIDASE AND SULFUR DIOXIDE TOXICITY

RABIA GEELANI, SHAJRUL AMIN, MINU BALKHI AND AKBAR MASOOD*

Department of Biochemistry, The University of Kashmir, Srinagar 190 006, J&K, India

Key words: Sulfite oxidase, Molybdenum, Spinacea oleracea, Sulfur dioxide.

ABSTRACT

Sulfite oxidase catalyses the physiologically vital oxidation of sulfite to sulfate in animals, plants and bacteria. Sulfite oxidase isolated from animal sources has been shown to contain molybdenum as cofactor. Tungsten is able to replace molybdenum in Mo-enzymes, forming catalytically inactive (or possessing very low activity) analogs. Measurable sulfite oxidase activity was observed in Spinacea oleracea grown under normal environmental conditions in absence of any external stress. The tungsten irrigated plants were apparently healthy, but were more susceptible to aqueous sulfur dioxide toxicity. Such plants showed concentration dependent loss of sulfite oxidase activity. A greater decrease in chlorophyll, amino acid and protein content was observed in these plants on treatment with aqueous sulfur dioxide. It is found that sulfite oxidase in Spinacea oleracea like the animal enzyme is a Mo- enzyme and is instrumental in counteracting the toxic effects of aqueous sulfur dioxide in plants.

INTRODUCTION

Sulfite oxidase is a vital enzyme catalyzing the oxidation of sulfite to sulfate. The terminal reaction in the oxidative degradation of the sulfur containing amino acids cysteine and methionine is catalysed by this mitochondrial enzyme. It belongs to the molybdenum cofactor (Mo-Co) containing family of enzymes.

E-mail: akbar masood [akbar_masood@hotmail.com]
It has long been known that the rare transition element molybdenum is an essential micronutrient for plants, animals and microorganisms (Bortels et al. 1930). Molybdenum and tungsten are the only second and third row transition metals that are required for the growth of at least some organisms; molybdenum in particular is an essential trace element for most living systems, including microorganisms, plants, and animals (Stiefel, 1993). X-ray crystallographic analysis of Mo-enzymes reveal that the cofactor is not located on the surface of the protein, but it is buried deeply within the interior of the enzyme and a tunnel-like structure makes it accessible to the appropriate substrates (Kisker et al. 1997). Once Mo-co is liberated from the holoenzyme, it loses Mo and undergoes rapid and irreversible loss of function due to oxidation (Rajagopalan and Johnson, 1992). The demolybdo-forms of Mo-enzymes are catalytically inactive (Kisker et al. 1997). Elevated amounts of the Mo-antagonist, tungsten, were shown to inhibit the activity of Mo-enzymes by replacing Mo as a ligand of MPT (Wray and Filner, 1970; Kisker et al. 1997).

The best characterized sulfite oxidizing enzymes from the sulfite oxidase family are those from avian and mammalian sources (Rajagopalan, 1980), that are homodimers containing heme b and molybdenum coordinated via an MPT-type molybdenum pterin cofactor and a conserved cysteine residue. However, the occurrence of sulfite oxidase in plants was a matter of slight controversy for a long time, because this enzyme would catalyze a reaction that counteracts sulfate assimilation. In animals, however, it is a well-studied enzyme involved in the degradation of sulfur-amino acids (Eilers et al. 2000). However, various scientists using different technical procedures observed its presence in plants (Ganai et al. 1997, Eilers et al. 2000) but the presence of molybdenum as cofactor of sulfite oxidase has not been demonstrated as yet. In order to deal with the same objective Spinaceae oleraceae plant was selected to check for the role of molybdenum in the activity of sulfite oxidase.

MATERIALS AND METHODS

Plant Material

Twenty-four earthen pots, each of 15cm diameter were selected for sowing of Spinaceae oleraceae seeds. Pots were divided into four sections with six pots in each section. Seeds were equally divided in each section. Pots were kept under natural environmental conditions. Plants in each section were daily treated with 10, 100, 1000 ppm of sodium tungstate solution respectively. Plants grown in fourth section (control) were treated with 200mL of water.

Sampling of Tissue - Leaves from all four sections were collected separately, cut into small pieces, weighed for fresh and dry weight and kept for preparation of homogenate.

Dry Weight Determination - The weighed samples were kept for drying at 105°C in an oven for two hours and then at 70°C till a constant weight was attained.

Preparation of Homogenate - A 10% (W/V) homogenate was prepared. Weighed sample of tissue was homogenized in chilled water using pestle and mortar. The homogenate was filtered through eight layered muslin cloth. The
muslin filtered homogenate was centrifuged in cold at 500 × g for 10 minutes. The supernatant was subsequently used for enzyme assay and other estimations.

**Sulfite oxidase assay** - Sulfite oxidase was assayed at pH 7.8, essentially by the method described by Macleod et al. (1961), using sodium sulfite as the substrate. The activity of the enzyme was indicated by decrease in absorbance of potassium ferricyanide at 420 nm that was used as an indicator substrate in the coupled assay.

**Estimation of proteins** - Proteins were estimated by the method of Lowry et al (1951).

**Estimation of amino acids** - Estimation of total amino acids was carried out by the method of Lee and Takahashi (1960).

**Generation and estimation of sulfur dioxide** - Aqueous sulfur dioxide was generated by reducing hot concentrated

\[ \text{H}_2\text{SO}_4 \text{ with copper turnings} \]

\[ \text{Cu + 2H}_2\text{SO}_4 \rightarrow \text{CuSO}_4 + 2\text{H}_2\text{O} + \text{SO}_2 \]

Sulfur dioxide was estimated according to West and Gaeke (1956).

**Treatment of leaf discs from each section with aqueous sulfur dioxide and preparation of homogenate** - Leaf discs of 1 cm diameter from each section (10, 100, 1000 ppm sodium tungstate treated) were treated with 100 ppm of aqueous sulfur dioxide for four hours in petridishes (15 × 20 mm) under light which was provided by a 100 W electric bulb. Treatment conditions were kept similar for each section. After 4 hours leaf discs were separated, washed, patted dry, weighed and homogenized.

**RESULTS**

**Effect of sodium tungstate treatment on the activity of sulfite oxidase**

The change in absorbance of sulfite oxidase is shown in Fig. 1. The activity was measured in terms of decrease in absorbance at 420 nm for 4 minutes. Fig. 2 shows change in absorbance of sulfite oxidase in sample treated with 10 ppm of sodium tungstate. Sulfite oxidase activity got decreased to 89.85% (Table 1). Fig. 3 shows change in absorbance of sample treated with 100 ppm of sodium tungstate where activity got decreased to 98.1% (Table 1). No sulfite oxidase activity was seen in plant treated with 1000 ppm of sodium tungstate.

**Table 1**

<table>
<thead>
<tr>
<th>Treatment with sodium tungstate</th>
<th>Enzyme activity in U*/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>0.897 ± 0.171</td>
</tr>
<tr>
<td>10 ppm</td>
<td>0.091 ± 0.012</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.017 ± 0.0009</td>
</tr>
</tbody>
</table>

Data represented is the average of six replicates ± S.E
tungstate. Tungsten treatment decreased the activity of sulfite oxidase to 100%.

Effect of sodium tungstate treatment on protein content of *Spinacea oleracea*

No significant decrease in the concentration of proteins was observed on treatment with sodium tungstate (Table 2).

Effect of SO$_2$ exposure on proteins of *Spinacea oleracea* treated with 100 ppm sodium tungstate

The effect of SO$_2$ exposure on proteins of *Spinacea oleracea* treated with 100 ppm sodium tungstate is shown in Table 3. The plant shows a decrease of 73.3% in protein concentration on exposure to 100ppm sulfur dioxide. However, a decrease of only 44.8% is observed in tungsten untreated plants on exposure to the same concentration of sulfur dioxide.

Effect of SO$_2$ exposure on amino acids of *Spinacea oleracea* treated with 100 ppm sodium tungstate

Table 4 shows decrease of 23.9% in amino acid concentration in *Spinacea oleracea* plant on exposure to 100ppm of sulfur dioxide, while a decrease of

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total protein (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>0.41±0.06</td>
</tr>
<tr>
<td>10 ppm</td>
<td>0.41±0.02</td>
</tr>
<tr>
<td>100 ppm</td>
<td>0.40±0.031</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>0.398±0.015</td>
</tr>
</tbody>
</table>

Data represents the average of six samples analyzed separately ± S.E.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total proteins (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>0.562±0.06</td>
</tr>
<tr>
<td>100 ppm of SO$_2$</td>
<td>0.310±0.020</td>
</tr>
<tr>
<td>100ppmSO$_2$+100 ppm sodium tungstate</td>
<td>0.150±0.016</td>
</tr>
</tbody>
</table>

Data represents the average of six samples analyzed separately ± S.E

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total amino acids (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>0.046±0.005</td>
</tr>
<tr>
<td>100 ppm SO$_2$</td>
<td>0.035±0.001</td>
</tr>
<tr>
<td>100 ppm SO$_2$ +100 ppm sodium tungstate</td>
<td>0.019±0.002</td>
</tr>
</tbody>
</table>

Data represents the average of six samples analyzed separately ± S.E.
Role of molybdenum in the biological function

58.6% is observed in plants treated with sodium tungstate on exposure to same concentration of sulfur dioxide.

Effect of SO₂ exposure on photosynthetic pigments of *Spinacea oleracea* treated with 100 ppm sodium tungstate

*Spinacea oleracea* shows a decrease of 29.9% in chlorophyll content on exposure of 100 ppm of SO₂ while a significant decrease of 60.1% was observed in plants treated with 100 ppm of sodium tungstate on exposure to same concentration of sulfur dioxide. Pheophytins show a decrease of 37.5% when the plants were exposed to 100 ppm of SO₂ while a significant decrease of 71.85% was found in plants treated with 100 ppm of sodium tungstate on exposure to same concentration of sulfur dioxide (Table 5).

### DISCUSSION

For many years tungsten was considered to be a biological antagonist of molybdenum and was used for study of the properties and functions of molybdenum in Mo-enzymes (L’vov, 1989). This was due to the fact that tungsten is able to replace molybdenum in Mo-enzymes, forming catalytically inactive (or possessing very low activity) analogs (L’vov et al. 2001). Certain microorganisms can also utilize tungsten in a similar fashion (Kisker, 1997).

Sulfite oxidase which catalyses the physiologically vital oxidation of sulfite to sulfate in animals, plants and bacteria, belongs to the molybdenum cofactor (Mo-Co) containing family of enzymes. The best characterized sulfite oxidizing enzymes from the sulfite oxidase family are those from avian and mammalian sources (Rajgopalan, 1980), that are homodimers containing heme b and molybdenum coordinated via an MPT-type molybdenum pterin cofactor and a conserved cysteine residue. However the occurrence of sulfite oxidase in plants was a matter of slight controversy for a long time, because this enzyme would catalyze a reaction that counteracts sulfate assimilation. However various scientists using different technical procedures observed its presence in plants (Ganai 1997 and Eilers et al. 2000).

Molybdenum as the second-row transition metal required by most living organisms forms the catalytically active center of all Mo enzymes, except nitrogenase (Schwarz et al. 1990) and few species that do not require molybdenum use tungsten, which lies immediately below molybdenum in the periodic table.

### Table 5

Effect of SO₂ exposure on photosynthetic pigments of *Spinacea oleracea* treated with 100 ppm sodium tungstate

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total chlorophyll (µg/mL)</th>
<th>Total pheophytins (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ppm</td>
<td>21.42±1.213</td>
<td>18.97±1.15</td>
</tr>
<tr>
<td>100 ppm SO₂</td>
<td>15.01±0.113</td>
<td>11.85±1.21</td>
</tr>
<tr>
<td>100 ppm SO₂+ 100 ppm sodium tungstate</td>
<td>8.54±0.112</td>
<td>5.34±0.11</td>
</tr>
</tbody>
</table>

Data represents the average of six samples analyzed separately ± S.E.
Fig. 1 Decrease in absorbance of sulfite oxidase with change in time

Fig. 2 Change in absorbance of sulfite oxidase in plant sample treated with 10 ppm sodium tungstate

Fig. 3 Change in absorbance of sulfite oxidase in plant sample treated with 100 ppm sodium tungstate
Because of their unique chemical versatility and similarity in structures, it is taken up by various enzymes in a similar manner as that for tungsten (Kisker et al. 1997). Measurable sulfite oxidase activity was observed in Spinacea oleracea grown under normal environmental conditions in absence of any external stress. While same plant under similar environmental conditions when treated with sodium tungstate showed no sulfite oxidase activity. Plants grown in presence of high concentration of tungsten, take up tungsten instead of molybdenum in its enzyme. The tungsten cofactor incorporated sulfite oxidase did not show any sulfite oxidizing activity. Therefore, our findings are in agreement with the above facts.

Sulfur dioxide when dissolved in water, dissociates to form sulfite, bisulfite and other ionic species. Both sulfite and bisulfite have been shown to be deleterious to plants (Winner et al. 1985). It is reported that multiple effects of sulfur dioxide and sulfite on metabolism include pigment destruction (Syanrhyeichyk and Syanrhyeichyk, 1995), interference with the activity of various enzymes (Niewiadomska et al. 1997) and destruction of various amino acids like methionine and tryptophan during aerobic oxidation of sulfite (Yang, 1973). Cowling and Koziol, 1978 observed a significant reduction in glycine and serine contents, as a result of sulfur dioxide exposure. But plants can overcome these phytotoxic effects by readily converting bisulfite and sulfite into less toxic sulfate ions by the activity of sulfite oxidase (Babich and Stotzky, 1980; Huber et al. 1987).

Plants made deficient in sulfite oxidase on treatment with sodium tungstate showed greater decrease in chlorophyll, amino acid and protein content, on treatment with aqueous sulfur dioxide. Since the replacement of molybdenum by tungsten inactivates sulfite oxidase, detoxification of sulfite and bisulfite ions is hampered, which ultimately leads to increased destruction of different components. These findings are in consistency with the conclusion that sulfite oxidase is instrumental in counteracting the toxic effects of sulfur dioxide and molybdenum acts as a cofactor in sulfite oxidase enzyme in Spinacea oleracea.

REFERENCES

Babich, H. and Stotzky, G.1980. Environmental factors that influence the toxicity of heavy metals and gaseous pollutants to microorganisms. CRC critical reviews in Microbiology. 8 : 99-145.


Hinze, H. and Holzer, H. 1986. Analysis of the energy metabolism after incubation