Study of the Incorporation of Selenium into Peroxidase Isozyme of Wheat Seedling

A. PENG,* Y. XU, AND Z. J. WANG

*SKLEAC, Research Center for Eco-environmental Sciences,
Chinese Academy of Sciences, Beijing, 100085,
People's Republic of China*

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ABSTRACT

The effects of selenium on the activity of peroxidase (POD) of wheat seedling and its isozyme pattern were studied using a greenhouse hydroponic experiment. The results show that the activity of POD is increased in response to higher Se concentration (approx 5.0 mg/L) in culture medium. The electrophoretic pattern of the POD isozyme was altered by growth of the wheat in a selenium medium. Se could incorporate into some POD isozymes during either seed germination or the seedling growth period. There is a dose-responsive incorporation of selenium in isozyme of POD and selenium content in the isozyme increase along with the increase of selenium concentration in the medium.

Index Entries: Selenium; peroxidase incorporation; peroxidase isozyme; hydroponic experiment.

INTRODUCTION

Both nutritional and toxic effects of trace elements, such as selenium (1,2), on plants have been previously observed by many investigators. The harmful effects of these trace elements have been detected at the sub-cellular level in plant tissues. In particular, studies of some enzymes in grain seedlings revealed that many isozymes have altered their expression patterns and levels in response to adverse growth conditions. As an example, peroxidase (POD), an important enzyme for growth and devel-

*Author to whom all correspondence and reprint requests should be addressed.

opment of plants, has been shown to increase its activity and change its isozyme spectrum when the plants were growth in harmful environment (3) or were exposed to toxic elements (e.g., mercury) (4).

In this article, we analyzed the effects of Se on the activity of POD and its isozyme pattern. We attempted to determine whether Se could stimulate the activity of POD and whether Se exists in any POD isozymes during wheat seedling growth in a Se environment. Our data suggest that the activity of POD is increased in response to higher Se concentration in culture medium. Se could incorporate into some POD isozymes during either seed germination or seedling growth period. The incorporation during seed germination is higher than that during the seedling growth period.

MATERIALS AND METHODS

Growth Experiment of Wheat

Wheat seeds (*Triticum aestivum* L., H-65) were germinated in a culture bottle at approximately 25°C for 3 d. The seedling were transferred to Hoagland's solution containing, if added, various concentration of selenite: 0, 0.1, 0.5, 1.0, 5.0, 10.0, and 20.0 mg Se/L during the seed germination (test 1) or seedling growth period (test 2). The growth condition was as follows: 24-h photoperiod per day; light intensities of 3500 lx, and temperature of 20°C. On the seventh day (for assay activity of POD of 14 d), the seedlings were harvested for detection of POD activity and electrophoresis separation.

Electrophoresis

The isozyme of POD was separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The 0.5-g seedling samples were cut into pieces and placed in a chilled mortar along with 2 mL of buffer solution (Tris–HCl buffer, pH=6.8). The homogeneous solution obtained was centrifuged in the cold at 11,400 g for 20 min at 22°C. The supernatant was used to separate POD isozymes by electrophoresis on SDS-PAGE as described by Davis (5). The 50-mL test solution was concentrated in 2.5% concentrated gel under 15–20 mA current and 7.5% separated gel under a constant current of 30 mA for 5 h. At the end of the electrophoresis, the gels were stained with dibenzylamine–ascorbic acid for 10 min and destained with 5% acetic acid. A part of the band was scanned at 420 nm by using a CS-910 Shimadzu (Japan) thin layer scanner. Meanwhile, the visualized isozyme bands of the same width were sliced separately and the sliced bands were used for the determination of Se.

Peroxidase Activity Assay

The assay was performed as described by Zhu (6). The harvested seedling as the sample to be assayed was weighed and placed in a

chilled mortar along with a volume of cold buffer solution equal to the weight of tissue. The composition of the buffer was 2 mL of 0.1 M Tris-HCl. Tissues were macerated with a pestle until a relatively smooth suspension was obtained. The suspension was centrifuged in 0°C at 11,400 g for 20 min. The supernatant was used for the enzyme assay. Then, 2.9 mL of 0.05 mol/L phosphate buffer solution, including 1.0 mL of 2% H₂O₂ and 1.0 mL of 0.05 mol/L guaiacol, was mixed with 0.1 mL supernatant. The two substrates were prepared fresh just before use. The system was followed by measuring the change in absorbency at 470 nm in a Beckman (US) DU 650 spectrophotometer and counted the time immediately. The activity were calculated by measuring the ratio A at 470 nm/min = 0.01, which is defined as 1 unit of activity.

Determination of Selenium

Two methods have been used to determine the Se content of the isozyme bands: (a) neutron activation techniques (used in test 1) and (b) the high-performance liquid chromatography-flame-ionization detector (HPLC-FID) method developed in our laboratory (used in test 2)(7).

The neutron activation techniques was described by Sun et al. (8). The sliced gel bands from SDS-PAGE were cut in 3-mm × 7-mm sections, placed into a quartz tube (6 mm × 40 mm), dried at 50°C, and sealed up for radiation.

With regard to HPLC-FID the sliced gel bands from SDS-PAGE were digested in a 20 mm × 200 mm digestion tube with a mixture of nitric acid and perchloric acid (7:2 v/v) at 170°C for 4 h and 220°C for 1 h. After digestion, the solution was cooled to room temperature, treated with 4 N HCl at 50°C for 30 min, and the pH adjusted to 2.0 with ammonium hydroxide (1:1 v/v). The 2,4-diaminonaphthalene (Fluka AG, Switzerland) derivative of selenite was extracted into the cyclohexane phase. A portion of the organic phase was injected into normal-phase HPLC (Waters 201 HPLC W/510 with a Shimadzu (Japan) RF-535 fluorescence detector and *m*-porosil C₁₈-NH₂ column.). The determination of every sample was repeated three times, and their average value was calculated. The accuracy of the determination of Se content was checked by a standard reference material (rice flour, Japan NIES-10).

RESULTS AND DISCUSSION

Effect of Se on the POD Activity

The POD activities from 7- and 14- d-old wheat seedlings grown in various Se concentration mediums are shown in Fig. 1.

As shown in Fig. 1, an increase of POD activity was observed when Se in the medium was greater than 5.0 mg/L. POD activities at 7 and 14 d incubation has been found to be 124% and 125% of the control at 10

Fig. 1. Influence of Se(IV) on POD activities of wheat seedling.

and 20 mg Se/L ($p < 0.05$), respectively. The POD activities at 14 d incubation were higher than that at 7 d incubation.

Recent studies (9) of barley seedlings has revealed that POD activity increased with an increase of Se concentration. Ishida (10) reported on the increase of POD activity when the cell of plant was decrepit. Van Hasselt (11) indicated that POD-H₂O₂ system could result in the degradation of chlorophyll. It was also shown in our previous study (12) that the chlorophyll content was reduced when the Se concentration in medium-induced and incubation time was prolonged. Meanwhile, the increase of POD activity increased the rate of degradation of chlorophyll and accelerated harm to the plant. Accordingly, the influence of Se on POD activity was characteristic of a stress environment. The same result has been found in a study of mercury (4); the POD activity of wheat was increased with an increase of Hg concentration.

Effect of Se Concentration on the POD Isozyme Spectrum

The scanning micrograph of a peroxidase isozymogram of a wheat seedling, grown after the seed was germinated (test 1) in different Se media, is given in Fig. 2.

It indicates that there were 10 isozyme bands for the control group, among them bands III and V₁ were relatively lower. However, in the relative mobility (R_f) 0.18–0.24 region, a new isozyme band (IV₁) was found in the presence of adequate Se concentration (approx 0.5–1.0 mg/L). Its height increased with an increase of Se concentration in culture

Fig. 2. Scanning micrograph of peroxidase isozymogram of a wheat seedling to Se supplied during seed germination (7 d).

Table 1
Se Contents in bands of POD Isozyme of Wheat Seedling to Se Supplied
During Seed Germination (ng/band)

POD isozyme Band	MW KDa	Se conc. in culture medium, mg.L ⁻¹						
		0(CK)	0.1	0.5	1	5	10	20
I	117.4	0.30	0.57	1.47	2.02	3.56	2.13	2.65
II	113.3	0.35	0.28	0.67	2.28	2.80	3.92	2.39
III	109.3	ND*	ND	0.15	0.19	0.43	0.50	0.52
IV	101.1	0.72	0.08	0.23	0.30	0.51	0.83	0.74
V	88.9-94.3	ND	0.09	0.24	0.46	0.73	0.71	0.43

*ND: not detected.

medium. The result shows that the change of POD isozyme main spectrum was expressed in four band, such as II (113 kDa), III (109 kDa), IV (101 kDa), and V (89–94 kDa) principally. The change of the bands' heights, except band II, tended to "higher-lower-higher" with an increase of Se. The Se contents of these bands are shown in Table 1.

It can be seen that Se contents in these bands have the trend of first increasing, then decreasing, and, finally, rapidly increasing to their

Fig. 3. Scanning micrographs of a POD isozymogram of wheat seedling to Se supplied during seedling growth (7 ds).

Table 2
Se Contents in Bands of POD Isozyme of Wheat Seedling to Se Supplied During Seedling Growth

POD isozyme Band	MW KDa	Se conc. in culture medium, mg.L ⁻¹						
		0(CK)	0.1	0.5	1.0	5.0	10.0	20.0
I	117.4	0.040	0.090	0.070	0.085	0.308	0.230	0.205
II	113.3	ND*	ND	ND	ND	0.145	0.081	0.135
III	109.3	ND	ND	ND	0.016	0.035	0.055	0.084
IV	101.1	0.045	0.025	0.050	0.064	0.052	0.270	0.295
V	88.9-94.3	0.025	0.028	0.125	0.143	0.144	0.330	0.282

*ND: not detected.

higher levels. In the control group, band IV (101 kDa) has obvious Se content, but the higher incorporation of Se varied with the Se level in the culture medium. In the high-Se medium, the greatest amount of Se was with band II (113 kDa), followed by band I (117 kDa) in Se concentration

in 10 and 5 mg/L, media, respectively. However, the incorporation was reduced when the Se concentration in the medium was greater than 5 mg/L (bands I and V) and 10 mg/L (bands II, III, and IV), indicating its poisonous function at these Se concentration.

Effect of Cultivated Method on Se Incorporation into POD Isozyme

In this experiment (test 2), the wheat seeds were germinated in normal medium, and the seedlings were transferred to Hoagland's solution containing various concentrations of Se. Because the difficulty of determining selenium content is the result of the very low concentration in the spectrum band of electrophoresis, neutron activation analysis and HPLC-fluorometry provided the Se determination; the sensitivity of the latter is higher than the former. Therefore, in this test, the HPLC-FID method was used to determine Se content. Scanning micrographs of a POD isozymogram are shown in Fig. 3. The results also indicate eight bands' five main bands (I, II, III, IV, and V) and two lower bands (VIII, IX), but a new band was also observed in IV, (Fig. 3). The change of spectrum was not obvious when Se content in medium was less than 0.5 mg/L. A new band (IV₁) and bands VI and VII were raised when the Se concentration was greater than 1.0 mg/L. The height of band VII was increased with increasing Se applied in the culture medium, but the change in band VI and VII was not found. The height of bands II, IV, V, VIII, and IX increased with an increase of Se concentration.

The Se content in bands is shown in Table 2. The Se contents of the bands in both tests 1 and 2 under the same dosage were higher in bands I, II, VI, and V, but the Se contents of the bands in test 2 were much lower than for test 1. For bands I and II and bands IV and V the highest Se content appeared in 5mg Se/L and 10 mg/L, respectively. The Se content of band I (117 kDa) was significantly higher than in the control group; there was no change in the presence of 0.1, 0.5, and 1.0mg/L Se, but increased when the Se concentration was increased to 5mg/L, then decreased for 10 and 20 mg/L of Se. The Se contents of band IV (101 kDa) was similar to band I. The results show that Se could incorporate into the POD isozyme and the toxic concentration of Se is 5.0–10.0mg Se/L, which agreed with the results of the above experiments.

Because trace Se could be detected in 117, 101, and other kDa bands of the POD isozyme in the control group, it has been determined that the POD isozyme may contain Se, but whether a Se-containing enzyme exists in the POD isozymes of wheat seedlings needs to be studied further.

Peroxidase is extremely sensitive to adverse circumstances. Conditions such as temperature, salinization (3), presence of heavy metal (4), and plant disease can all change the enzyme spectrum. This type of regulation was accomplished by either inducing the production of new isozymes or by inhibiting the synthesis of certain existing isozymes.

There are multiple types of POD isozyme, and the amount of a particular isozyme differs in different species. It also varies in different organs, or in different developmental stages of the same plant. The results of this article indicate that the electrophoretic pattern of isozymes of POD was altered by both germination of seed and seedling growth of the wheat in various concentrations of selenium medium. New band such as bands IV₁, and I₁ have been found when the Se concentration in medium was greater than 1.0 mg Se/L and 20 mg Se/L, respectively. The appearance of new IV₁ and I₁ bands and inducing of activity of POD could be treated as a response of early Se poison. It may be stated that the wheat had suffered from Se poisoning in the early stage and could be an early index of evaluation.

CONCLUSIONS

1. Peroxidase activity is sensitive to Se stress in the environment and could be increased with increased Se concentration in culture medium. The electrophoretic pattern of the POD isozyme was altered by the growth of the wheat in a selenium medium.
2. Selenium can be incorporated into the peroxidase isozyme by adding Se in hydroponics culture during the wheat seed germination or during the growth of the seedling. There is a dose-responsive incorporation of Se in the POD isozyme, and the Se content in the isozyme increases along with the increase of Se concentration in the medium.
3. The abnormal phenomena in the POD isozymes could be found if the Se concentration was higher than 5 mg/L and 10.0 mg/L, indicating its poisonous function in these Se concentration during growth of the wheat seedling.

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