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Sr^{2+} Stimulation of α-amylase and RNAse in Wheat Aleurone Layer

Tae Wan Kim
Department of Plant Resources Science, Hankyong National University, Ansung 456-749, Korea
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ABSTRACTS : To measure an effects of strontium on secretion of α-amylase and RNase, wheat aleurone layers were isolated after the pre-incubationin a solution with or without 10 mM $SrCl_2$ or $CaCl_2$ for 3 days at 25℃ in the dark under aseptic conditions. The secretion of α-amylase reached a maximum at 72 h after incubation. Sr^{2+} induced more effectively secretion of α-amylase than Ca^{2+}. The α-amylase secretions by Sr^{2+} or Ca^{2+} ware about 2 (Ca^{2+}) to 2.5 (Sr^{2+}) fold higher than it without divalents. When aleurone layers were incubated without divalent ions, however, the α-amylase was remarkably retained in the tissues. Total α-amylase synthesis (*i.e.* tissues + media) was slightly lowered by 10 mM $SrCl_2$ addition. It seemed that the RNase secretion begins at 18 h after incubation. This meaned that the RNase secretion may process slower than α-amylase secretion. Ca^{2+} effect on RNase secretion is stronger than Sr^{2+} unlikely to α-amylase. The secretion process is likely to be suddenly induced between 72 h and 96 h. These results suggested that the secretion was enhanced after the accumulation in aleurone layers.

Key words: aleulone layer, α-amylase, RNase, secretion, strontium, wheat.

INTRODUCTION

Plant development and ecosystems have been in fatal danger from pollutants such as Cd^{2+}, Hg^{2+}, and Pb^{2+} from industrial factories, and from radioactive waste materials (^{90}Sr, ^{137}Cs, *etc.*). The accident in Chernobyl resulted in the accumulation of radionuclides such as ^{137}Cs and ^{90}Sr in milk and crop plants through the whole of Europe[1-4]. Hamilton and Minski[5] reported that the high Sr^{2+} content in human bone in southeast and northwest England is associated with the distribution of calcareous rocks, where natural apatites occur, containing up to 7.3% Sr^{2+}, and it is caused by dietary intake. The radioactive isotope ^{90}Sr (half-life = 28.5 years) has been studied because of the possibility of long-term soil contamination after nuclear explosions which could lead to an incorporation into human cells through the food chain. Through ecosystematic food chain relations between plants and livestock it may always be possible to transfer toxic heavy metals and radionuclides into humans. ^{89}Sr and ^{90}Sr emit β-ray, and their radiation are caused by the surplus of neutrons. The conversion of a neutron into a proton results in electron expulsion. According to Lang[6], human hair contains 0.05 g strontium/g, and human organs take up about 2 mg/day. This Sr^{2+} could be perspired. However, the accumulation of radioactive strontium is dangerous and harmful. It is known that β-rays could result in leukemia, pneumonia, and infant mortality[7,8].

Calcium as a macronutrient is present in plants plentifully. The adaptation of plants for Ca^{2+} seems to be dependent on the plant species. The translocation of Ca^{2+} and Sr^{2+} through the transpiration stream in the xylem is well known, but in the phloem both Ca^{2+} and Sr^{2+} are immobile[9]. Thus, the Sr^{2+} uptake is competitively related to Ca^{2+} which has a pivotal role in intracellular processes[10,11]. Calcium has an effect on the α-amylase secretion during rice[12] and barley germination[13]. The enhancement of enzyme secretion by Ca^{2+} in the plant cell was reported first by Chrispeels and Varner[14]. Over the subsequent years, a universal secretion pathway was elucidated as following: rER → Golgi → secretion vesicles plasma membranes. Since α-amylase regulation is important both to the agriculture and to the brewery industry, it has attracted many agricultural or food scientists. It is clear that the GA_3-induced changes in the rER and Golgi apparatus, the synthesis of α-amylase and of other enzymes (*e.g.* RNase, polyphenol oxidase, and peroxidase),

*Corresponding author:
Tel: +82-31-670-5081 Fax: +82-31-670-5499
E-mail: taewkim@hnu.hankyong.ac.kr

and their subsequent secretion are all tightly correlated[12,15]. Alpha-amylase is known to be a metalloenzyme[16].

The effect of Sr^{2+} (at 20 mM) on secretion in barley aleurone layers was also reported[17]. However, there are only a few observations on the Sr^{2+}-action in wheat aleurone layers. A Sr^{2+} or Ca^{2+} concentration of 20 mM[17] is extremely high, resulting in inhibition of germination *per se*. The objectives of this study are to investigate the secretion of hydrolytic enzymes, *i.e* α-amylase and RNase, induced by strontium.

MATERIALS AND METHODS

Isolation of aleurone layer

The seeds of wheat (*Triticum aestivum*) were cut and the embryo containing halves were discarded. After sterilizing the seed halves without the embryo for 15 to 20 min in 1% $NaOCl_4$, the seeds were rinsed several times with distilled water and then preincubated on moist sterile Whatman paper in 10 cm autoclaved Petri-dishes. Each petri-dish contained 100 half seeds. The preincubation was performed for 3 days at 25℃ in the dark (wrapped in aluminum foil) under aseptic conditions, and then endosperms were removed immediately with two small spat- ulas under sterile conditions. The aleurone layers were put in 10 mL Erlenmeyer flask containing 2 mL incubation medium at 25℃ for the required time, shaking at 40 oscillations per minute. The incubation media consisted of 1 mM acetate -NaOH buffer (pH 4.8), 10 mM $CaCl_2$ or $SrCl_2$, 1 M GA_3, and 0.5 mg chlora- mphenicol per mL medium. One mL of water was added at the end of the incubation period and the medium was decanted. The aleurone layers were briefly rinsed with 2.5 mL of water and then the rinsing and the decanted media were combined. Aleurone layers were homogenized with a mortar pestle. After centrifugation at 12,000 g for 10 min, the supernatants and the combined media were used for the measurement of α-Amylase and RNase activity. The activity was measured, using a conversion factor of 2.7 g of α-amylase per unit of optical density change by Shuster and Giffords method[18].

α-Amylase activity

Five hundred L tissue or media extracts were mixed with 1.5 mL 1 mM acetate buffer and starch solution. After incubating for 2 min at 20℃, 1.0 mL of an iodine reagent was added. The mixture was diluted with 4.0 mL double distilled water and read at 620 nm[14,18]. The iodine reagent consisted of 1 to 100 times diluted solution (with 0.05 N HCl) of 6.0 g KI and 600 mg I in 100 mL double distilled water. The starch solution was made as following:

boil 150 mg starch for 1 min
↓
2,000 x g centrifugation for 10 min
↓
mix with 600 mg KH_2PO_4, 200 M $CaCl_2$
↓
dissolve in 100 mL double distilled water.

The measurements of absorbance changes were continued until there was a 30 to 75% decrease in the initial values (about 1.35).

RNase activity

RNase activity was measured according to Lee et al.'s method[19]. The reaction mixture contained 1.0 mL of 0.4 M sucrose, 500 μM $MgSO_4$, 5 mM KCl, and 1 mg/mL Sigma type III yeast RNA in 200 mM acetate buffer (pH 6.0). The reaction was started by the addition of 400 μL enzyme extract. After 15 to 30 min at 30℃, 4 mL cold EtOH were added. Enzyme activity was related to the increased absorbance at 260 nm in the clear filtrate, compared with a control where EtOH was added immediately after reaction start. The activity of RNase was calculated using a constant of E_{260} = 90.1/μM/cm.

RESULTS

α-Amylase activity

Fig. 1 shows the changes in α-amylase activity by Ca^{2+} and Sr^{2+} in the presence of GA_3. α-amylase synthesis in wheat aleurone layer tissue begin 3 h after incubation whether with divalent ions or without. α-amylase secretion appeared to be induced 6 h after incubation. Sr^{2+} and Ca^{2+} clearly increased the secretion of α-Amylase. The secretion reached a maximum at 72 h after incu-

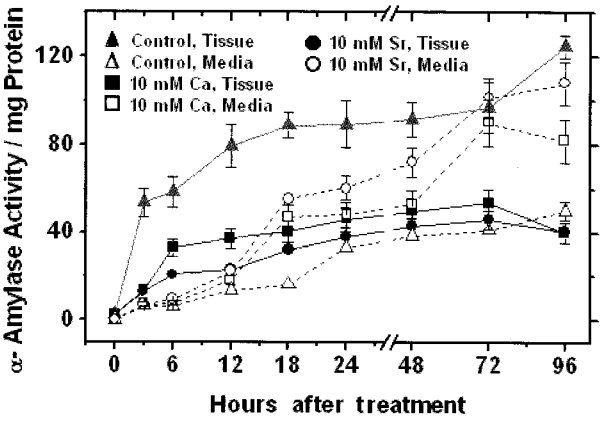

Fig. 1. The changes in α-amylase activiy. The dissected aleurone layers were incubated in 1 mM acetate-NaOH buffer (pH 4.8), 10 mM $CaCl_2$ or $SrCl_2$, 1 μM GA_3, and 0.5 mg/mL of chloramphenicol.

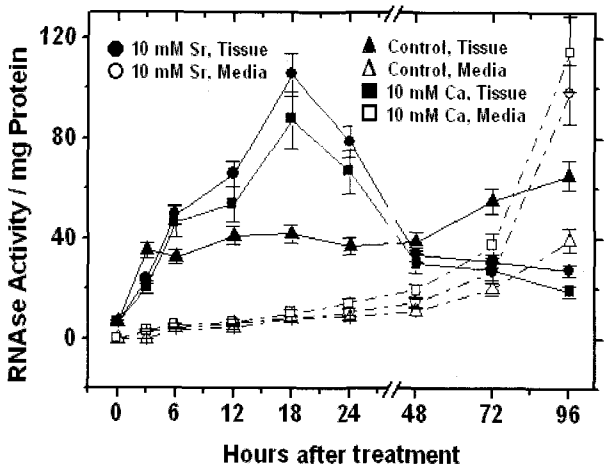

Fig. 2. The changes in RNase secretion. Aleurone layers were incubated in the presence of 10 M GA_3 and divalent ions.

bation. Sr^{2+} induced secretion of α-amylase more effectively than Ca^{2+}.

Thus, the α-amylase secretion induced by the divalents Sr^{2+} and Ca^{2+} were likely to be a common phenomenon. The α-amylase activities secreted by Sr^{2+} or Ca^{2+} were about 2 (Ca^{2+}) to 2.5 (Sr^{2+}) fold higher at 18 h after incubation than it without divalents, whereas when aleurone layers were incubated without divalent ions, the α-amylase were remarkably retained in the tissues. However, total α-amylase synthesis (*i.e.* tissues + media) was slightly lowered by addition of 10 mM $SrCl_2$.

RNase secretion

As shown in Fig. 2, the RNase was not actively secreted until 72 h after incubation. This means that the RNase secretion may process slower than α-amylase secretion. RNase secretion by Sr^{2+} was not active compared to Ca^{2+}. The secretion process was likely to be suddenly induced between 72 h and 96 h. These results suggested that the secretion of Rnase may need a lag period to efflux out aleurone layer. For α-amylase, the synthesis and secretion occured at the same time without an accumulation in aleurone layer.

DISCUSSION

It is well known that α-amylase secretion in barley and rice aleurone layers requires external mM Ca^{2+}concentrations for saturation[12,15,20,21]. In this work, the concentration of 10 mM is far higher than that for germination without stress. In fact, Sr^{2+} above 10 mM is extremely toxic for germination. Since Chrispeels and Varner[14], many experiments using their classical method have given evidence that other divalent ions (Sr^{2+}, Ba^{2+}, sometimes Mg^{2+} can partly replace Ca^{2+}, indicating that the effects are related to a more general quality of the ions and might not be due to those ions entering the cell to exert an effect as a rather specific second messenger[15,17].

It has also been reported that one physiological role of Ca^{2+} in plants is to maintain the normal integrity of the membrane[22]. Furthermore, Kiyosawa and Adachi[23] have found that Sr^{2+} can be substituted for Ca^{2+} in terms of survival of *Chara* internode cells in an electrolyte solution. With Fujii's recent report[24] that Sr^{2+} and spermine also prevented the leakage of intracellular amino acids and glycerol in algae, and with Naik and Srivastava's finding[25] that polyamines were effective in decreasing betacyanin efflux from discs of beet root, the above mentioned observations may imply that the hydrolytic enzyme secretion out of aleurone layers in wheat is an ionic effect of Sr^{2+}.

REFERENCES

1. Heinrich, G. (1987) Zur radioaktiven Belastung verschiedener Pflanezen in Graz nach dem Reaktorunglck von Tschernovyl, *Mit. Naturwiss. Ver. Steiermark* 117, 7-25.
2. Heinrich, G., Mller, H. J., Oswald, K. and Gries, A. (1989) Natural and artificial radionuclides in selected Styrian soils and plants before and after the reactor accident in Chernobyl, *Biochem. Physiol. Pflanzen* 185, 55-67.
3. Heinrich, G., Wilfurth, K., Gries, A., Mller, H. J., Oswald, K. and Schulte, R. (1993) Kontamination in verschiedenen kosystemen nach Tschernobyl, In: Mller, Hj. and H. Rabitsch (eds): Untersuchungen ber die Auswirkungen des Reaktorunfalls von Tschernobyl in der Steiermark, Reaktorinstitut Graz Berichte 21, TU Graz Press, p.131-142.
4. Paunescu, N., Margineanu, R. and Iorgulescu, A. (1988) ^{90}Sr and ^{137}Cs in fallout from Chernobyl in the Bucharest -Magurele area during 1986-1987, *J. Radioanal. Nuclear Chem.* 28(4), 263-271.
5. Hamilton, E. J. and Minski, M. J. (1972)Abundence of the chemical elements in man' diet and possible relations with environmental factors, *Sci. Total Environ.* 1, 375-394.
6. Lang, K. (1974) Mineralstoffe, Spurenelemente. Eine Einfhrung fr Studierende der Medizin, Biologie, Chemie, Pharmazie und Ernhrungswissenschaften, UTB Steinkopff, p.109 -100.
7. Sterreiches ökologieinstitut für angewandte Umweltforschung. (1987) Strahlennotizien 13.
8. Cawse, P. A. (1989) The origin, transport and persistence of radionuclides, *J. Sci. Food Agr.* 49(1), 123-124.
9. Marschner, H. (1986) Mineral nutrition of higher plants, Academic Press London.
10. Haunold, E., Horak, M. and Gerzabek, M. (1986) Umwelt-

radioaktivitt und ihre Auswirkung auf die Landwirtschaft,I. Das Verhalten von Radionucliden in Boden und Pflanze, OEFZS, 4369, LA-163/86.

11. Hepler, P. K. and Wayne, R. O. (1985) Calcium and plant development, *Ann. Rev. Plant Physiol.* 36, 397-439.
12. Mitsui, T., Christeller, J. T., Hara-Nishimura, I. and Akazawa, T. (1984) Possible roles of calcium and calmodulin in the biosynthesis and secretion of α-amylase in rice seed scutellar epithelium, *Plant Physiol.* 75, 21-25.
13. Jacobsen, J. V. and Higgins, T. J. V. (1982) Characterization of the α-amylases synthesized by aleurone layers of Himalaya barley in response to gibberellic acid, *Plant Physiol.* 70, 1647-1653.
14. Chrispeels, M. J. and Varner, J. E. (1967) Gibberellic acid-enhanced synthesis and release of α-amylase and ribonuclease by isolated barley aleurone layers, *Plant Physiol.* 42, 398-406.
15. Jones, R. L. and Carbonell, J. (1984) Regulation of the synthesis of barley aleurone α-amylase by gibberellic acid and calcium, *Plant Physiol.* 76, 213-218.
16. Fisher, E. H. and Stein, E. A. (1960) α-amylase. In: the enzymes, vol 4. (eds), Boyer, P., Lardy, H. and Myrback, K. Academic Press, New York, p.313-343.
17. Jones, R. L. and Jacobson, J. V. (1983) Calcium regulation of the secretion of amylase isoenzymes and other proteins from barley alreurone layers, *Planta* 158, 1-9.
18. Shuster , L. and Gifford, R. H. (1962) Changes in 3'-nucleosidase during the germination of wheat embryo, *Arch. Biochem. Biophys.* 96, 534-540.
19. Lee, K. C., Cunningham, B. A., Paulsen, G. M., Liang, G. H. and Moore, R. B. (1976) Effects of cadimium on respiration rate and activities of several enzymes in soybean seedlings, *Physiol. Plant.* 36, 4-6.
20. Bush, D. S. and Jones, R. L. (1987) Measurment of cytoplasmic calcium in aleuron protoplasts using indo-1 and fura-2, *Cell Calcium* 8, 455-472.
21. Bush, D. S., Sticher, L., Van Huystee, R., Wagner, D. and Jones, R. L. (1989) The calcium requirement for stability and enzymatic activity of two isoforms of barley aleurone α-amylase, *J. Biol. Chem.* 264, 19392-19398.
22. Cramer, G. R., Luchli, A., and Polito, V. S. (1985) Displacement of Ca^{2+} by Na^{+} from the plasmalemma of root cells: a primary response to salt stress?, *Plant Physiol.* 79, 207-211.
23. Kiyosawa, K. and Adachi, T. (1990) Survival and death of Chara internodal cells in electrolyte solutions and calcium release from cell wall, *Plant Cell Environ.* 13, 471-476.
24. Fujii, S. (1994) Effect of Ca^{2+} on the retention of glycerol and amino acids in *Dunaliella tertiolecta*, *Plant Cell Environ.* 17, 227-230.
25. Naik, B. I. and Srivastava, S. K. (1978) Effect of polyamines on tissue permeability, *Phytochemistry* 17, 1885-1887.