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Response of β-Glucanases to GA_3 in Barley Aleurone Layers

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보리 호분층에서 (1-3)-β-glucanase와 (1-3,1-4)-β-glucanase의 GA_3에 대한 반응

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ABSTRACT : Isolated barley aleurone layers were used to examine response of (1-3)- and (1-3,1-4)-β-glucanases to GA_3. Protein content and levels of (1-3)- and (1-3,1-4)-β-glucanases increased in the presense of added GA_3. However, (1-3)- and (1-3,1-4)-β-glucanases showed different response to GA_3 in their production and secretion patterns. (1-3,1-4)-β-glucanase showed higher increase in enzyme activity than (1-3)-β-glucanase in the early stage of GA_3 treatment. Secretion of enzyme by GA_3 into the surrounding medium was more enhanced in (1-3,1-4)-β-glucanase than in (1-3)-β-glucanase. The differential response of the enzymes might be related to the physiological role of the enzymes in germination of barley grain.

Key words : Aleurone layer, (1-3)-β-glucanase, (1-3,1-4)-β-glucanase, Gibberellic acid (GA_3)

The hormonal regulation of hydrolases involved in the enzymic degradation and mobilization of endosperm storage macromolecules in germinating cereal grain has been investigated intensively. For many of these investigations isolated barley aleurone layers have been used as a model system because they synthesize and secrete many hydrolases in response to exogenously applied gibberellic acid (GA_3) in vitro. Using this system the synthesis and secretion of α-amylases, (1-3,1-4)-β-glucanases, and proteases have been shown to be enhanced by GA_3 [2,6,10].

Aleurone layer of barley grain which is derived from the triploid nucleus after fertilization overlies the starchy endosperm where storage macromolecules are located. Walls of the aleurone and starchy endosperm contain large amount of (1-3,1-4)-β-glucans (20-29% of the cell wall components by weight)[3]. (1-3,1-4)-β-glucans are hydrolized by (1-3,1-4)-β-glucanases(E.C.3.2.1.73) in the early stage of barley grain germination to allow starch- and protein-degrading enzyme penetration into endosperm cells[7]. Therefore, rapid synthesis and secretion of (1-3,1-4)-β-glucanases are very important in barley endosperm mobilization. (1-3)-β-glucanases (E.C.3.2.1.39) are also expressed in germinating barley grain. However, little amount of (1-3)-β-

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glucans, substrates of (1-3)-β-glucanases, are found in barley grain. Possible physiological role of (1-3)-β-glucanase in germinating grain is to provide protection against pathogen infection during germination[4].

Physiological role of the enzymes may be related to the temporal regulation of their gene expression, post translational modification and secretion of the expression products. We attempted to investigate this possibility by observing the production and secretion of (1-3)- and (1-3,1-4)-β-glucanases in barley aleurone layers in response to exogenously applied GA_3.

Materials and Methods

Preparation and incubation of aleurone layers

Aleurone layers were isolated from the malting barley (*Hordeum distichum* L. var. Jinkwang) essentially as described[2]. Embryo-less half-grains were sterilized with 0.2% (w/v) silver nitrate solution for 20 min at room temperature, rinsed several times in sterile water and preincubated on moist sterile vermiculite in majenta boxes for 4 days in the dark. Aleurone layers were separated aseptically from the starchy endosperm with the aid of broad-pointed forceps. Aleurone layers were incubated in 20 mM sodium succinate buffer (pH 5) containing 10 μg/ml chloramphenicol with and without 1 μM GA *ml* in shaking incubator at 25°c for up to 5 days.

Enzyme extraction and assay

Enzyme activity was determined in the incubation medium and the extract of the aleurone layers. The aleurone layers were washed several times with fresh incubation medium and homogenated with 2 ml of fresh incubation medium. The homogenate was centrifuged at 10,000 x*g* for 15 min and the supernatant was used for enzyme assay and protein quantitation. Protein content was determined according to Bradford[1]. (1-3)-β- and (1-3,1-4)-β-glucanase activities were assayed as described[11,12].

Results and Discussion

Amount of total protein and protein secreted into the medium increased by the treatment of aleurone layers with 1μM of GA_3. In the presence of GA_3 protein content in the medium increased greatly. Sixty-one and 78% of the total protein was secreted into the medium 3 and 5 days after incubation, respectively. Less amount of protein was secreted into the medium in the absence of GA_3. After 3 to 5 days of incubation only about 20% of the total protein was secreted into the medium. Total protein increased up to 17% by GA_3 treatment(Fig. 1).

In the presence of GA_3 Synthesis and secretionof protein in barleyaleurone layers. Aleurone layers were incubated in 20 mM sodium succinate buffer with or without 1 μM GA_3. (1-3)-β-glucanase activity increased drastically. After 3 days of incubation total enzyme activity increased over 2-fold, and over 70% of the total (1-3)-β-glucanase activity was found in the medium. On the contrary 70% of the total enzyme activity was present in the aleuronelayers in the absence of GA_3(Fig. 2). Specific enzyme activity was much higher in the aleu-

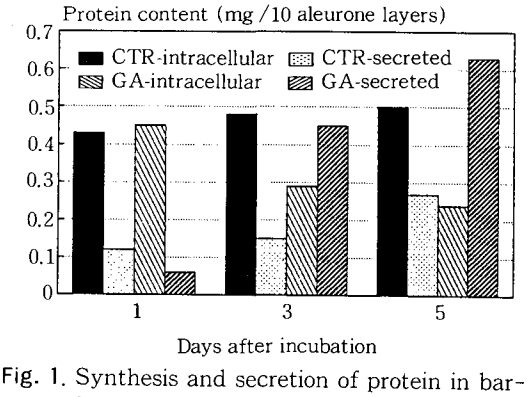

Fig. 1. Synthesis and secretion of protein in barley aleurone layers. Aleurone layers were incubated in 20 mM sodium succinate buffer with(GA) or without(CTR)1 μM GA_3.

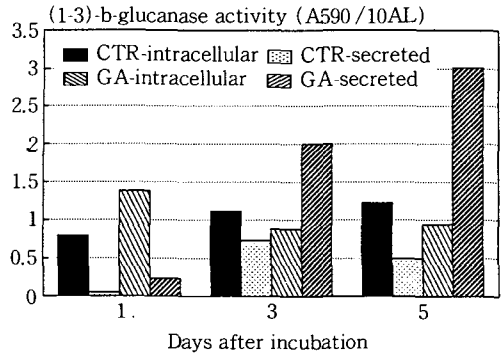

Fig. 2. Production and secretion of (1-3)-β-glucanase activity in barley aleurone layers in the presence(GA) or absence(CTR) of 1 μM GA_3.

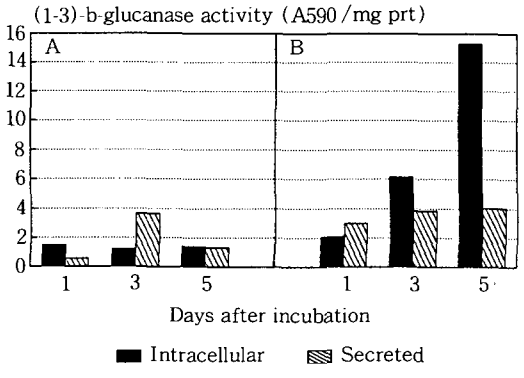

Fig. 3. Changes in specific (1-3)-β-glucanase activity in the absence(A) or presence (B) of 1 μM GA_3.

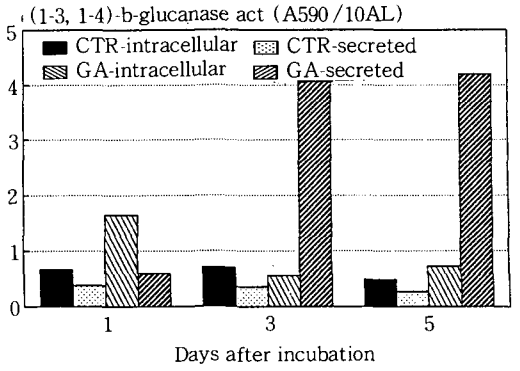

Fig. 4. Production and secretion of (1-3,1-4)-β-glucanase activity in the presence(GA) or absence(CTR) of 1 μM GA_3.

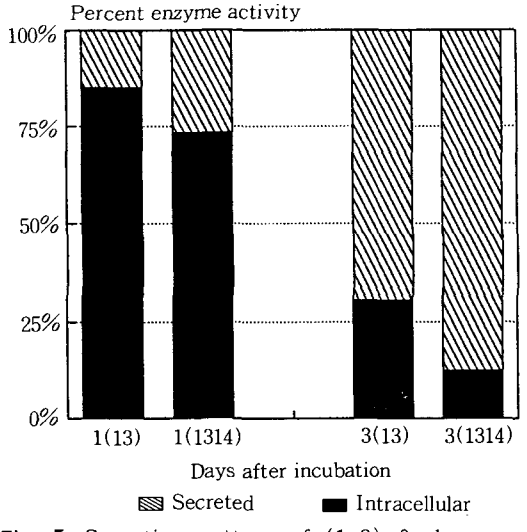

Fig. 5. Secretion pattern of (1-3)-β-glucanase (13) and (1-3,1-4)-β-glucanase(1314) activity in the presence of 1 μM GA_3.

rone layers in the presence of GA_3 indicating lower rate of secretion of (1-3)-β-glucanase than other proteins(Fig. 3).

(1-3,1-4)-β-glucanase activity also increased by GA_3. After 3 days of incubation total enzyme activity increased over 4-fold, and about 85% of the total enzyme activity was found in the surrounding medium. However, only about 35% of the enzyme activity was present in the medium in the absence of GA_3 (Fig. 4).

In the absence of added GA_3 enzyme activities were also detected in the medium but the rate of secretion and levels of activity were lower than those in the presence of added GA_3. In the presence of GA_3 synthesis and secretion rate of (1-3,1-4)-β-glucanase was much higher than those of (1-3)-β-glucanase(Fig. 2,4,5). The difference in response of the enzymes to exogenouly applied GA_3 could be explained by the different temporal and spatial regulation of the gene expression during germination. (1-3,1-4)-β-glucans are found in the cell walls of aleurone layers and starchy endosperm. Rapid depolimerization of (1-3,1-4)-β-glucans is very important for the grain germination. Therefore, rapid expression of (1-3,1-4)-β-glucanases in the early stages of germination is necessary. How-

ever, little amount of (1-3)-β-glucans, the substrate of the (1-3)-β-glucanases, are found in barley grains.

Different levels of enzymes could be a result of the differential expression of the corresponding genes. Different levels of mRNA for the α-amylase gene families and their differential response to GA_3 were reflected in the levels of corresponding isozymes[5]. Differential mRNA stability rather than transcriptional control could also account for the differential expression of the enzymes[8]. Possibility of differences in the translational efficiency can not be excluded[9]. Difference in the rate of secretion of the enzyme could be resulted by many different ways in various steps of the secretion process. One of the possibilities could be a difference in the post translational modification. Genes of both enzymes contain the GA_3 response sequenses in the upstream control region. Therefore, the differential expression of the genes with the similar structural elements also indicates the importance of the trans-acting elements in gene expression.

적 요

분리한 보리호분층을 이용하여 (1-3)-β-glucanase와 (1-3,1-4)-β-glucanase의 GA_3에 대한 반응특성을 조사하였다. GA_3 처리에 의해 호분층과 배양액 내의 단백질 함량, (1-3)-β-glucanase 및 (1-3,1-4)-β-glucanase 활성 등이 모두 증가하였다. 그러나 GA_3 처리에 의한 효소활성 및 효소분비 증가 정도는 효소에 따라 큰 차이를 보였다. (1-3,1-4)-β-glucanase가 (1-3)-β-glucanase보다 효소활성 증가 정도와 분비증가 정도 모두 컸다. 이와 같은 결과는 발아초기 (1-3,1-4)-β-glucanase의 중요한 생리작용과 관계가 있을 것으로 생각되었다.

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