Note
Contamination of Chitin Oligosaccharides in a Laminarioligosaccharide Preparation Can Cause a Confused Interpretation of Its Elicitor Activity

Yoshitake Desaki, Ippei Otomo, and Naoto Shibuya

Department of Life Sciences, School of Agriculture, Meiji University, 1-1-1 Higashi-Mita, Tama-ku, Kawasaki, Kanagawa 214-8571, Japan

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Chitinase treatment of a commercial laminarioligosaccharide preparation from a mushroom resulted in a loss of previously reported elicitor activity in rice cells, indicating that the activity was attributable not to the laminarioligosaccharide but rather to the contaminating chitin fragments. This suggests that the elicitor activity of laminarioligosaccharides from such sources containing chitinaceous substances should be carefully interpreted.

Key words: microbe-associated molecular pattern (MAMP); β-glucan; chitin; elicitor; rice

Branched β-glucans are a typical component of the cell walls of various fungi and oomycetes, and hence are recognized as a representative MAMP (microbe-associated molecular pattern) for these microbes. In fact, their fragments, β-glucan oligosaccharides, have been found to act as potent elicitors in various plant species. A hepta-β-glucoside from Phytophthora megasperma f. sp. glycinea and a tetraglucosylglucitol from the rice blast fungus Pyricularia oryzae are two well characterized examples of elicitor-active β-glucan oligosaccharides, acting specifically on certain legumes and rice, respectively. Both of these oligosaccharides are branched at the O-3 or the O-6 positions of glucosyl residues in their 1,6-linked or 1,3-linked backbone chains and the positions of branching are critical to their biological activities. On the other hand, several papers have reported that linear laminarioligosaccharides also serve as an elicitor in some plant cells, including rice. As we did not find any elicitor activity for linear laminarioligosaccharides in rice, we tried to clarify the reason for the discrepancy, and found that contamination by minute amounts of chitin oligosaccharides in some commercial laminarioligosaccharides prepared from a mushroom caused the difference, at least in one case.

Figure 1 shows the elicitor activity of two commercial products of laminaripentaose prepared from pachyman, a 1,3-linked β-glucan from the cell walls of a mushroom, or curdlan, also a 1,3-linked β-glucan from a bacterium, on suspension-cultured rice cells. In both the generation of reactive oxygen species (ROS; Fig. 1, top) and the induction of a defense gene (Fig. 1, bottom), OsKSL4, encoding an enzyme involved in the biosynthesis of phytoalexin, only the laminaripentaose preparation from pachyman showed extensive elicitor activity comparable to that of N-acetylmuramyl-L-alanine (GN8).

Considering the reason for this difference in elicitor activity between these two products, we noticed that the one prepared from pachyman might contain a small amount of chitin oligosaccharides, because chitin is a typical cell wall component of fungal cell walls including mushrooms, whereas bacterial cell walls do not contain chitin. To examine this possibility, laminaripentaose from pachyman was treated with rice chitinase expressed in yeast and purified by affinity chromatography, and analyzed for elicitor activity. As shown in Fig. 2, the elicitor activity shown by the laminaripentaose preparation disappeared almost completely after chitinase treatment, indicating that the elicitor activity originally observed for this preparation was actually caused by the contamination of the laminaripentaose by chitin oligosaccharides. Analysis of the laminaripentaose preparation by HPAEC before and after chitinase treatment showed that there was no change in the elution profile (data not shown), indicating that the enzyme treatment was specific for chitin.

Because chitin oligosaccharides show very high elicitor activity for various plant cells, even in the subnanomolar concentration range, only a minute contamination into laminarioligosaccharides results in an apparent elicitor activity of the preparation. In fact, contamination of less than 0.001% of GN8 in the laminaripentaose preparation used in this experiment can explain the ROS generating activity observed for this preparation. Such a low level contamination cannot be detected by conventional chromatographic analyses, even with the use of the HPAEC-PAD system, which enables good resolution and sensitive detection of chitin oligosaccharides.

Thus the elicitor activity observed for the laminarioligosaccharides from pachyman in rice cells was probably attributable to the contamination by chitin oligosaccharides. On the other hand, it appears that this possibility is low in the case of laminarioligosaccharides obtained from organisms not containing chitin. Hence results for laminarioligosaccharides from laminarin, a sea weed polysaccharide, and also from curdlan might not be affected by contaminating chitin oligosaccharides.

Although laminarioligosaccharides have been used as an elicitor to induce defense responses in plants, interpretation of the results should take this consideration into account to avoid confusion.

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Fig. 1. Elicitor Activity of Two Commercial Laminaripentaose Preparations.

Top, Induction of ROS in suspension-cultured rice cells by elicitor treatment was measured by luminol assay. Forty mg of cultured rice cells were suspended with 1 mL of modified N6 medium in a 2-mL centrifuge tube and pre-incubated for 30 min at 25 °C on a thermomixer shaken at 750 rpm. After pre-incubation, 1.6 ng/mL of GN8 or 50 μg/mL of laminaripentaose was added to the medium. Ten micro-liter aliquots of the medium were transferred to a 96-well microtiter plate at various time points and measured for ROS with a luminometer (BERTHOLD TR717, Tokyo, Japan). Two commercial laminaripentaose preparations (Seikagaku Kogyo, Tokyo, Japan, made from pachyman; Megazyme International Ireland, wicklow, Ireland, made from curdlan) were used for the experiment. Bottom, induction of OsKSL4 gene expression was analyzed by quantitative real-time PCR. Rice cells were treated with the various elicitors for 3 h and collected. Total RNA was extracted using an RNasey plant mini kit (Qiagen, Valencia, CA, USA), and the cDNA was amplified using a Quantitect Reverse Transcription Kit (Qiagen). Gene expression was detected with Taqman probe technology using the ABI prism 7500 fast system (Applied Biosystems, Foster City, CA). The data were normalized to amplification of the 18S rRNA internal control. Gene-specific primers were designed as follows: OsKSL4, forward primer TCGCATTTGCGTGGCACA, reverse primer TTGGAACTTCCGACATCAGAAA, probe CTATTGCGGTCACACTTGTGGCCGA; 18S rRNA, forward primer CAGATACCGTCCTAGTCTCC, reverse primer CGCGCGAGTCTAGTAAAGCAACAT, probe AAAAACTGCGCCGACACAGGATCG.

Fig. 2. Elicitor Activity of Laminaripentaose Prepared from Pachyman Was Eliminated by Chitinase Treatment.

Laminaripentaose (from pachyman, 1 mg/mL) or GN8 (1 μg/mL) was dissolved in 30 mM sodium acetate buffer (pH 5.0). Five μg of the purified chitinase was added to each solution and incubated for 18 h at 37 °C. After incubation, the chitinase in the samples was inactivated by boiling for 30 min. The chitinase treatment was repeated twice. The original and chitinase-treated samples were used for the treatment of rice cells (laminaripentaose, 50 μg/mL; GN8, 1.6 ng/mL). Top, Analysis of ROS generation. Bottom, Analysis of OsKSL4 expression.

References