Note

Measurement of Oligosaccharides Derived from Tamarind Xyloglucan by Competitive ELISA Assay

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Xyloglucan oligosaccharides, which were derived from tamarind xyloglucan by cellulase digestion, were measured by a competitive ELISA method using antibodies raised against a complex of BSA and xyloglucan nonasaccharide. By this method, tamarind xyloglucan oligosaccharides could be measured in the range of 0.1 to 40 nmol/well.

The xyloglucan of tamarind seed (Tamarindus indica), which is called tamarind gum, is commercially available as a food additive for improving the viscosity and texture of processed foods. It is a branched heteropoly saccharide having a β-(1→4)-linked β-glucan backbone chain that is partially substituted at the O-6 position of the β-glucopyranosyl residues with α-D-xylopyranose or with 2-O-β-D-galactopyranosyl-α-D-xylopyranose. In the course of our studies on its fine chemical structure, we obtained hepta saccharide (XG07, Glc: Xyl = 4:3), octasaccharide (XG08, Glc: Gal: Xyl = 4:1:3), and nonasaccharide (XG09, Glc: Gal: Xyl = 4:2:3) in fairly good yield by cellulase digestion of tamarind gum. Through our investigation on the physiological activities of the oligosaccharides derived from tamarind xyloglucan on the animal digestive system, we found that the octasaccharide (XG08) and nonasaccharide (XG09) bearing 2-O-β-D-galactopyranosyl-α-D-xylopyranosyl unit inhibited the absorption of 3-O-methyl-D-glucose by everted sacs from rat small intestine. Especially, XG09, which bears two 2-O-β-D-galactopyranosyl-α-D-xylo pyranosyl units, inhibited the absorption more effectively than XG08, suggesting that XG09 could be used as a food additive as a soluble dietary fiber with a small molecular weight. In addition, Fry et al. reported that the very low concentration of plant cell wall xyloglucan oligosaccharides produced by the action of endo-(1→4)-β-glucanases can affect the growth of plant tissues. These facts suggested that the xyloglucan oligosaccharides are biologically attractive materials and have demanded a reliable and specific method for measuring plant xyloglucan oligosaccharides using only small amounts of materials. In this context, we have already reported the estimation of xyloglucan in vegetables by an ELISA method using plant xyloglucan-recognizing antibodies. In this paper, we report the measurement of the xyloglucan oligosaccharides at nanomolar level by competitive ELISA method. Preparation and characterization of XG07, XG08, and XG09 of tamarind xyloglucan, the glycoconjugate of bovine serum albumin (BSA) and XG09 (BSA-XG09), and the IgG fraction of antiserum raised against BSA-XG09 (anti-XG09) are described in our previous papers. Alkaline phosphatase-labeled goat anti-rabbit IgG H+L (AP-anti-rabbit IgG) was purchased from Miles Scientific. ELISA plates were from Corning.

Competitive ELISA procedures were done based on the competitive reaction of the oligosaccharide and BSA-XG09 (fixed on the microtiter well) toward anti-XG. For competitive ELISA, ELISA plate was coated with 100 µl of BSA-XG09 (10 µg/ml in PBS). The glycoconjugate solution was allowed to bind overnight at 4°C. The plate was washed three times with PBS and then 200 µl of 4% powdered skim milk in PBS was added to block the residual protein binding sites on the well. After standing at room temperature for 1 h, the plate was washed with PBS. To the washed plate, solutions of oligosaccharide were added at 2 x the final concentration, 50 µl/well, serially diluted in PBS containing 10% FCS (PBS-FCS). The anti-XG09 solution (2.3 µg as IgG, 50 µl in PBS-FCS) was then added. Several wells were used as reference wells, where 50 µl of PBS-FCS was added in place of an oligosaccharide solution. These wells were used to calculate the 0% inhibition value. The plate was left at 30°C for 2 h and then washed six times with PBS containing 0.02% Tween 20 (PBS-Tween). APC-anti-rabbit IgG was added, 100 µl/well, diluted 1:1000 in PBS-FCS and left at 30°C for 2 h. After incubation, the plate was washed six times with PBS-Tween and developed by the procedure described previously. The inhibition ratios were calculated by the following equation: Inhibition (%) = [(A - B)/A] x 100, where A is the average absorbance at 410 nm of the reference wells, and B is that of the wells with xyloglucan oligosaccharide.

Figure shows typical calibration curves for measurement of xyloglucan oligosaccharides by this competitive ELISA method. It is apparent that XG09 could be measured in the range of 0.1 to 10 nmol/well. Other oligosaccharides, XG07 and XG08, showed no inhibitory activities at concentrations of less than 1 nmol/well, while these oligosaccharides could be measured in the range of 3 to 40 nmol/well. Concerning the specificity of anti-XG, amounts of XG07, XG08, and XG09 required for 50% inhibition were 40 nmol, 20 nmol, and 1 nmol, respectively. This result coincides with the inhibitory reaction of the oligosaccharides on
the precipitation reaction between tamarind xyloglucan and anti-XG as described previously, indicating that anti-XG is specific to the sugar units containing 2-O-β-D-galactopyranosyl-α-D-xylopyranosyl groups. In this competitive ELISA system the antigen fixed on the well is BSA-XGO9, therefore, it is most sensitive to XGO9. For practical use, we can apply this method to a rapid detection and estimation of total xyloglucan oligosaccharides in specimens. The detailed results of the application of this method will be reported elsewhere.

References
2) P. Kooman, Recueil, 80, 849-865 (1961).