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Detection of Hydroxyproline *O*-galactoside by LC/MS

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[Abstract] Hydroxyproline (Hyp) *O*-galactosylation is a plant-specific post-translational modification found in extracellular glycoproteins such as arabinogalactan proteins (AGPs). Hyp *O*-galactosylation is mediated by Hyp *O*-galactosyltransferase (HPGT) that catalyzes the transfer of a D-galactopyranosyl residue to the hydroxyl group of Hyp residues of peptides from the sugar donor UDP-α-D-Gal. Here we describe an LC/MS-based method for the detection of Hyp *O*-galactoside.

Materials and Reagents

1. Cotton
2. 1 ml Micropipette tip
3. Hyp *O*-galactosylated peptides or proteins
4. 0.22 M $Ba(OH)_2$
5. 0.32 M sulfuric acid
6. 1 M NaOH
7. 1 M HCl
8. 10% aqueous ammonia
9. 80% acetonitrile containing 0.1% formic acid
10. 99.9% acetonitrile (HPLC grade) containing 0.1% formic acid
11. Water (HPLC grade) containing 0.1% formic acid

Equipment

1. Heat block
2. Centrifugal evaporator
3. BT AG 50W-X8 Resin (100-200 mg resin, H+ form) (Bio-Rad Laboratories, catalog number: 143-5441)
4. Micro centrifuge
5. Micro HPLC system (JASCO International Co., model: micro21 LC-01)
6. LCQ Deca XP-plus ESI ion-trap mass spectrometer (Thermo Fisher Scientific)
7. TSK-gel Amide-80 (3 μm) column (2.0 x 150 mm) (Tosoh Bioscience LLC, catalog number: 21865)

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Procedure

A. $Ba(OH)_2$ hydrolysis
   1. Dissolve galactosylated peptide in 500 µl 0.22 M $Ba(OH)_2$ in a glass vial with cap.
   2. Incubate at 105 °C, 6 h.
   3. Incubate on ice for 5 min.
   4. Add 500 µl of 0.32 M sulfuric acid on ice.
   5. Centrifuge at 20,000 *x g* for 5 min.

B. Partial purification of $Ba(OH)_2$ hydrolysate (Figure 1)
   1. Plug a 1 ml micropipette tip with a small amount of cotton.
   2. Pack 200 mg AG 50W-X8 resin into the tip column.
   3. Wash the column with 1 ml of 1 M NaOH by gravity flow.
   4. Wash the column with 1 ml of 1 M HCl by gravity flow.
   5. Wash the column with 1 ml of water by gravity flow.
   6. Apply supernatant of the $Ba(OH)_2$ hydrolysate of the galactosylated peptide to the tip column.
   7. Wash the column with 1 ml of water by gravity flow.
   8. Elute with 1 ml of 10% aqueous ammonia by gravity flow.
   9. Evaporate the sample to dryness.
   10. Dissolve in 100 µl 80% acetonitrile containing 0.1% formic acid.

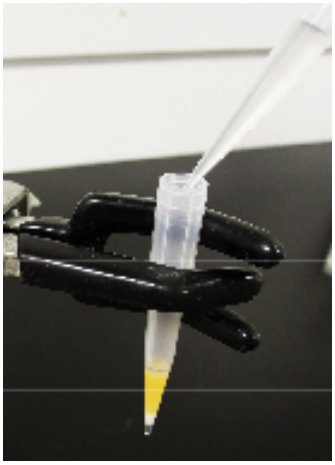

Figure 1. Partial purification of $Ba(OH)_2$ hydrolysate. Supernatant of the $Ba(OH)_2$ hydrolysate of the galactosylated peptide was applied to the tip column.

C. LC/MS analysis

   10 µl aliquots of the assay solution will be analyzed by LC-MS using a micro HPLC (high pressure liquid chromatography) system connected to an LCQ Deca XP-plus ESI ion-trap mass spectrometer. Chromatographic separation is performed by normal-phase HPLC on a TSK-gel Amide-80 (3 µm) column (2 x 150 mm).

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1. The mobile phase is composed of HPLC grade water containing 0.1% formic acid (eluent A) and HPLC grade acetonitrile containing 0.1% formic acid (eluent B). The column temperature is maintained at 25 °C.
2. The HPLC flow rate is 100 µl/min, and the elution gradient was 60 to 40% B over 10 min.
3. Subject the HPLC eluate to coupled electrospray ionization (ESI) in the positive ionization mode.
4. MS source parameters are as follows:
   a. Capillary temperature: 200 V
   b. Capillary voltage: 42 V
   c. Source voltage: 5 kV
   d. Source current: 8.5 µA
   e. Sheath gas flow: 50
   f. Aux gas flow: 0
   g. Sweep gas flow: 0
   h. The mass range: *m/z* 500-2000

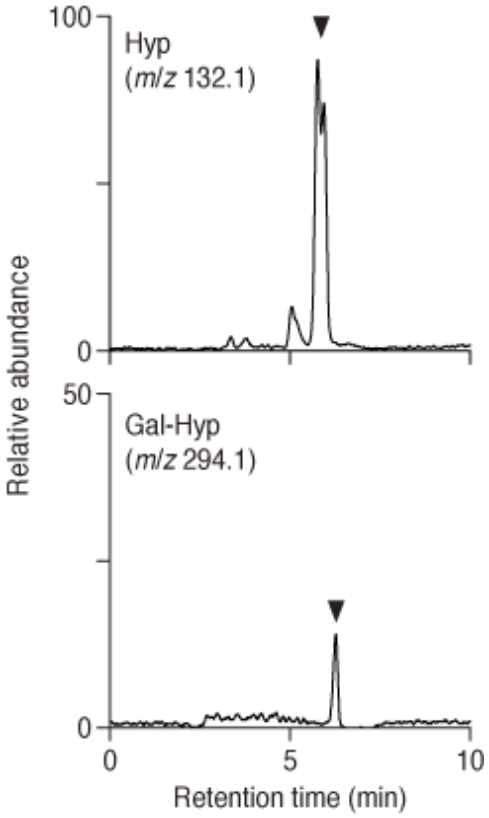

Figure 2. Detection of Hyp *O*-galactoside in $Ba(OH)_2$ hydrolysates of *in vitro* galactosylated AGP14 by LC-MS. The sample was analyzed by selected ion monitoring of Hyp (*m/z* 132.1) and Gal-Hyp (*m/z* 294.1). $Ba(OH)_2$ hydrolysis yields a diastereomeric pair of amino acids.

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5. The mass spectra are obtained by selected ion monitoring in zoom scan mode (Hyp: m/z 132.1, Gal-Hyp: m/z 294.1).

Acknowledgments

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References

1. Ogawa-Ohnishi, M. and Matsubayashi, Y. (2015). Identification of three potent hydroxyproline O-galactosyltransferases in *Arabidopsis*. *Plant J* 81(5): 736-746.
2. Ogawa-Ohnishi, M., Matsushita, W. and Matsubayashi, Y. (2013). Identification of three hydroxyproline O-arabinosyltransferases in *Arabidopsis thaliana*. *Nat Chem Biol* 9(11): 726-730.

