Note

An Arabinogalactan-rich Protein Containing 3-*O*-Methylrhamnose (Acofriose) in Young Plants of *Osmunda japonica*

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Certain polysaccharide-proteins from plants inhibit hemagglutination by human blood specific antisera or lectins.^{1~6)} Here we report the occurrence in young ferns of 3-*O*-methylrhamnose (acofriose) containing an arabinogalactan-rich protein (AGRP) which strongly inhibits hemagglutination by human blood-group specific reagents.

AGRP was extracted from young plants of *Osmunda japonica* by the method described previously,⁴⁾ and was purified by ethanol precipitation (50~80%, v/v), then gel-filtration on a Toyopearl HW-55 (Toyo Soda Manufacturing Co., Ltd.) column (2.5 cm × 60 cm) equilibrated with distilled water. The purified AGRP was eluted as a single peak of carbohydrate which was monitored by the phenol-H₂SO₄ method⁷⁾ from a Sepharose CL-6B (Pharmacia Fine Chemicals) column (0.9 cm × 100 cm)

equilibrated with 0.015 M sodium phosphate, pH 7.2, containing 0.15 M NaCl, and the maximum blood-group H activity that was measured by the published method¹⁾ was also eluted at the same elution volume as the peak. AGRP was hydrolyzed *in vacuo* at 115°C for 22, 48, or 72 hr with 4 N methanesulfonic acid containing 0.2% 3-(2-aminoethyl)indole by the method of Simpson *et al.*,⁸⁾ and its amino acids were analyzed on an Atto amino acid analyzer Type MLC-703. The major amino acids were glutamic acid, aspartic acid, serine, threonine, and hydroxyproline in the molar proportions of 19, 12, 11, 11, and 9%, respectively. The amino acid residues accounted for 5~6% (w/w) of AGRP. The carbohydrate content of AGRP was estimated to be 94~95% (w/w) by the phenol-H₂SO₄ method,⁷⁾ and a large amount of uronic acids (55~57% of total carbohydrate) was also found in AGRP by a colorimetric assay.⁹⁾ The neutral sugars of AGRP were analyzed on a Shimadzu gas-liquid chromatograph (GLC) Type GC-R1A with a flame ionization detector. AGRP was hydrolyzed in a sealed glass tube with 2.5 M trifluoroacetic acid for 8 hr at 100°C. Alditol derivatives of the corresponding neutral sugars in the hydrolyzate were separated in glass columns (3 mm × 2.1 m) packed with 3% Silicone OV-225 on Gaschrom Q (170~210°C, 2°C/min) and 2% Silicone GEXF-1150 on Gaschrom P (95~170°C, 2°C/min). The molar proportions of predominant neutral sugars in AGRP were an unknown sugar (10% as rhamnose), rhamnose (9%), arabinose (12%), and galactose (60%). We synthesized 3-*O*-methyl-L-rhamnose by regioselective methylation of an *n*-dibutyltin oxide (Bu₂SnO) derivative of L-rhamnose as a possible candidate for the unknown sugar by a slight modification of the method of Haque *et al.*¹⁰⁾ A qualitative analysis of the alditol derivatives of the synthesized 3-*O*-methyl-L-rhamnose and the unknown sugar was done on a Hitachi-M80 combined gas chromatograph-mass spec-

FIG. 1. Mass Spectra of Alditol Trifluoroacetates Derived from Sugars.
A, the unknown sugar in AGRP; B, the synthesized 3-*O*-methyl-L-rhamnose.

TABLE I. INHIBITION BY NEUTRAL SUGARS PRESENT IN THE POLYSACCHARIDE-PROTEIN (AGRP) OF HEMAGGLUTINATION BY EEL ANTI-H SERUM

Sugars	Minimum amount of sugars required for the complete inhibition of eel anti-H agglutinin	
	(mg/ml) ^c	(μ mol/ml)
L-Fucose ^a	0.08	0.47
3-O-Methyl-L-rhamnose ^b	0.02	0.11
L-Rhamnose	> 5	> 30.5
L-Arabinose	> 5	> 33.3
D-Galactose	> 5	> 27.7

^a L-Fucose was used as the positive control.

^b 3-O-Methyl-L-rhamnose was synthesized and other sugars were obtained from commercial sources.

^c Inhibitory activity is given as the concentration of sample (mg/ml) before addition of agglutinin and erythrocyte suspension.

trometer. The chemical ionization (CI) mass spectra of alditol trifluoroacetate derivatives of the two sugars were recorded under the following conditions: electron energy, 100 eV; ionizing current, 110 μ A (Fig. 1). The CI mass spectrum of the alditol trifluoroacetate of the unknown sugar appeared to be the same as that of the synthesized 3-O-methyl-L-rhamnose, and the highest mass number peak at m/z 565 (MH^+) was assumed to be formed from the alditol trifluoroacetate derivative of 3-O-methyl-L-rhamnose which has molecular weight of 564. The electron impact (EI) mass spectra of the alditol acetate derivatives of the unknown sugar and the synthesized 3-O-methyl-L-rhamnose were also recorded (electron energy, 70 eV; ionizing current, 110 μ A). The EI mass spectrum of the alditol acetate of the unknown sugar, a series of mass fragments, m/z 43, 87, 101, 129, 143, 189, and 203 fully corresponded to those of 3-O-methyl-1,2,4,5-tetra-*O*-acetyl-6-deoxy-hexitol reported by Weckesser *et al.*¹¹⁾ The chromatographic properties of the unknown sugar and the synthesized 3-O-methyl-L-rhamnose were also compared. Descending paper chromatography (PC) was done on Whatman No. 1 paper using 1-butanol-pyridine-water, 6:4:3 (by volume). On PC the spots of the two sugars migrated with the same R_{Fha} value of 1.22~1.23, and the spots were not stained with $AgNO_3$ -NaOH, but

they were with aniline hydrogen phthalate, GLC analysis showed that the unknown sugar has an identical retention time to the authentic 3-O-methyl-L-rhamnose. From these data the unknown sugar in AGRP was identified as 3-O-methylrhamnose. It has been established that the terminal L-fucosyl residue is a prerequisite for both blood-group H and Le^a activities of human and animal blood-group substances.^{12,13)} Since the blood-group activities were found by a hemagglutination inhibition assay in AGRP regardless of its low fucose content (less than 1 mol%), it is suggested that other sugar residues might be responsible for the blood-group activities of AGRP. Among four major neutral sugars found in AGRP, only 3-O-methyl-L-rhamnose strongly inhibited eel anti-H agglutinin (Table I). This suggests that the 3-O-methylrhamnose is involved at least in the blood-group H activity of AGRP. 3-O-Methyl-L-rhamnose (acofriose) containing AGRP which shows human blood-group activity has not hitherto been reported in a non-flowering plant, *osmunda*.

REFERENCES

- 1) G. F. Springer and P. K. Desai, *Biochemistry*, **10**, 3749 (1971).
- 2) S. Yamamoto, *J. Immunogenet.*, **2**, 197 (1975).
- 3) S. Yamamoto, I. Sakai and S. Iseki, *Immunol. Commun.*, **10**, 215 (1981).
- 4) K. Nakamura, Y. Tsumuraya, Y. Hashimoto and S. Yamamoto, *Agric. Biol. Chem.*, **48**, 753 (1984).
- 5) Y. Tsumuraya, Y. Hashimoto, S. Yamamoto and N. Shibuya, *Carbohydr. Res.*, **134**, 215 (1984).
- 6) Y. Tsumuraya, K. Nakamura, Y. Hashimoto and S. Yamamoto, *Agric. Biol. Chem.*, **48**, 2915 (1984).
- 7) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1965).
- 8) R. J. Simpson, M. R. Neuberger and T. -Y. Liu, *J. Biol. Chem.*, **251**, 1936 (1976).
- 9) T. Bitter and H. M. Muir, *Anal. Biochem.*, **4**, 330 (1962).
- 10) M. E. Haque, T. Kikuchi, K. Yoshimoto and Y. Tsuda, *Chem. Pharm. Bull.*, **33**, 2243 (1985).
- 11) J. Weckesser, H. Mayer and G. Drews, *Eur. J. Biochem.*, **16**, 158 (1970).
- 12) W. M. Watkins, "The Glycoprotein," B. B. A. Library, Vol. 5, Elsevier, Amsterdam, 1972, p. 830.
- 13) R. Oriol, J. Le Pendu and R. Mollicone, *Vox Sang.*, **51**, 161 (1986).