Short Communication

Immunochemical Comparison of Carbohydrates with Blood Group ABH-Activities Contained in Vegetable Foods: A Preliminary Study

Shigeru YAMAMOTO

Laboratory of Serology and Biochemistry,
National Research Institute of Police
Science, 6, Sanban-cho, Chiyoda-ku,
Tokyo 102, Japan

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The occurrence of blood group H-, A- and B-like substances in higher plants and a higher fungus (30 families, 75 species) has been reported by the present author.^{1~3)} Most materials were from wild and not horticultural plants. Later, fucose-containing polysaccharides and glycoproteins with high H-activity were isolated from fruit-bodies of *Collybia (Flammalia) velutipes*⁴⁾ and seeds of *Euonymus Sieboldiana*.^{5,6)} In the course of further screening of about 150 kinds of vegetables, fruits and spices, ABH-active substances were found in 12 families, 19 species, and were seemingly abundant in two families, the Rosaceae and Cruciferae. Some of them were isolated as carbohydrate-rich compounds through subsequent fractionation with ethanol and gel filtration. This paper presents preliminary data on the immunochemical characteristics of carbohydrates possessing blood group activities which were obtained from 9 vegetables selected as being representative.

Various kinds of vegetables commercially available were homogenized in 5 volumes (for fresh materials) or 10 volumes (for dried materials and powders) of 0.145M phosphate-buffered saline (PBS), pH 7.2, heated for 30 min at 100°C, and centrifuged at 4,000 rpm for 30 min at room temperature, except buckwheat powder which was extracted at room

temperature. The resulting supernatants were used as crude extracts. Blood group activities were assayed by the agglutination inhibition technique using 4 kinds of agglutinins adjusted to 1:8 in titer, eel anti-H, chicken anti-H, human anti-A and human anti-B, as described previously.¹⁾

Table I shows the blood group H-, A- and B-activities of the crude extracts of 19 plant food-stuffs. Blood group A- and B-activities were detected in 4 species, raisins, prunes, buckwheat powder and leaves of tangle (Konbu). H-Activity was detected in 15 other species, and was especially high in fresh leaves of radishes. Although the Cruciferae appeared to be characterized by the presence of H-activity, there was nothing in common among the Rosaceae, so far as the species assayed were concerned. Apart from the question of individual blood groups, ABH-activities are widely distributed in various edible plants from the Angiospermae to the Phaeophyceae, including the mushroom, *C. velutipes*,²⁾ and are not unusual localized components in wild and particular plants.

To confirm the inhibitory activities, large volumes of crude extracts of 9 vegetables were submitted to fractionation with ethanol, divided into 3 fractions, 0 to 50% precipitates, 51 to 90% precipitates and the 90% soluble portion, and lyophilized. The resulting blood group-active fractions are listed in Table II together with data on their yields. Each of the blood group-active ethanol fractions from radishes, turnips and cabbage was separately applied to a 1.5 cm × 90 cm column of Sephacryl S-200 which was pre-equilibrated and eluted with PBS. Each one from 6 other species and ethanol 90% precipitates from cabbage were also separately applied to a 1.5 cm × 90 cm column of Bio-Gel P-10, pre-equilibrated and eluted with PBS. The eluates were monitored at 280 nm for protein content and at 480 nm for total carbohydrate by the phenol-sulfuric acid method.⁷⁾ Fractions were pooled on the basis of the protein and carbohydrate elution profiles, and each pooled sample was assayed for blood group activities after lyophilization.

TABLE I. INHIBITORY ACTIVITIES OF CRUDE EXTRACTS OF 19 VEGETABLE FOOD-STUFFS

Family	Plant name (Taxonomical order) Species	Source material	Inhibitory activities against agglutinins (1:8)			
			Anti-H (Eel)	Anti-H (Chick.)	Anti-A (Hum.)	Anti-B (Hum.)
Cucurbitaceae	<i>Cucurbita moschata</i> (Pumpkin)	Fruit ^a	L ^c	M	In	In
Solanaceae	A kind of <i>Caspium annuum</i> (Paprika)	Powder ^b	M	In	In	In
Labiatae	<i>Salvia officinalis</i> (Sage)	Powder ^b	L	In	In	In
Actinidiaceae	<i>Actinidia chinensis</i> (Kiwi)	Fruit ^a	M	L	In	In
Vitaceae	<i>Vitis labrusca</i> (Fox grape)	Fruit ^b	M	L	L	L
Rutaceae	<i>Citrus Junos</i> (Yuzu : Citron)	Fruit ^a	L	In	In	In
Ru.	<i>Zanthoxylum piperitum</i> (Jpn. Pepper)	Powder ^b	L	L	In	In
Rosaceae	<i>Pyrus pyrifolia</i> (Jpn. pear)	Fruit ^a	L	In	In	In
Ro.	<i>Malus domestica</i> (Apple)	Fruit ^a	L	L	In	In
Ro.	<i>Prunus salicina</i> (Plum)	Fruit ^b	M	L	M	L
Ro.	<i>Fragaria chiloensis</i> var. <i>ananassa</i> (Strawberry)	Fruit ^a	L	In	In	In
Cruciferae	<i>Raphanus sativus</i> var. <i>hortensis</i> (Radish)	Leaves ^a	H	In	In	In
Cr.	<i>Brassica Rapa</i> var. <i>glabra</i> (Turnip)	Leaves ^a	M	In	In	In
Cr.	<i>Brassica oleracea</i> var. <i>capitata</i> (Cabbage)	Leaves ^a	L	In	In	In
Cr.	A mixture of <i>Brassica</i> (Mustard)	Paste	M	In	In	In
Polygonaceae	<i>Fagopyrum esculentum</i> (Buckwheat)	Powder ^b	M	L	L	L
Liliaceae	<i>Allium Cepa</i> (Onion)	Tuber ^a	In	L	In	In
Tricholomata- ceae	<i>Lentinus edodes</i> (Shimeji : Mushroom)	Fruit- bodies ^a	L	In	In	In
Laminariaceae	<i>Laminaria japonica</i> (Konbu : Tangle)	Leaves ^b	L	L	L	L

^a Fresh.^b Dried.^c L, low activity (above 10 mg/ml); H, high activity (below 1.25 mg/ml); M, intermediate between L and H; In, inactive.

Figure 1 shows the gel filtration pattern of ethanol 50% precipitates (100 mg) from turnips where the H-activity was condensed in first major carbohydrate peak, and its approximate molecular weight ranged between 150,000 and 50,000, based on Dextran standards (Pharmacia). Every ethanol 50% precipitate from radishes and cabbage gave similar elution profiles, the H-activity of which is indicated by a bar in the same figure. Figure 2 shows the gel filtration pattern of ethanol 90% precipitates (70 mg) from pears, together with each blood group-active elution range of pear and other ethanol fractions, and their approximate molecular weights were all about 3,000.

The 10 blood group-active and carbohydrate-rich fractions were analyzed for neutral sugars by gas chromatography, as trifluoroacetyl derivatives as described previously.⁶⁾ Fucose was detected in each specimen to a lesser extent, irrespective of the intensity of the H-activity against eel anti-H agglutinin, as shown in Table II. The kind of predominant sugar(s) was somewhat different among the specimens, presumably reflecting genus or family specificities, such as arabinose and galactose in the high molecular carbohydrates of the Cruciferae. Carbohydrate sequences with blood group ABH-activities exist in glycoproteins and glycolipids, and are characterized by the presence of fucose res-

TABLE II. NEUTRAL SUGAR COMPOSITIONS OF BLOOD GROUP-ACTIVE CARBOHYDRATES PARTIALLY PURIFIED ON GEL FILTRATION

Plant name	Blood group-active fractions				Neutral sugars detected by gas chromatography									
	Ethanol	(Yields) ^a	Gel filt.	(I.A.) ^b										
Grape	90% sol ^c	(1.15)	P-10 ^d	(2.5)	—	, Fuc	, Rib	, Ara	—	, Man	—	, [Gal] ^e		
Pear	90% ppt.	(2.41)	P-10	(2.5)	—	, Fuc	, Rib	, [Ara]	—	, —	, Glc	, [Gal]		
Apple	90% ppt.	(1.43)	P-10	(2.5)	—	, Fuc	, Rib	, Ara	, Xyl	Man	, [Glc]	, Gal		
Plum	90% ppt.	(9.44)	P-10	(1.25)	[Rha]	, Fuc	, [Rib]	, Ara	, [Xyl]	—	, Glc	—		
Strawberry	90% ppt.	(0.08)	P-10	(2.5)	—	, Fuc	, Rib	, Ara	, Xyl	Man	, [Glc]	, [Gal]		
Radish	50% ppt.	(0.19)	S-200	(0.02)	Rha	, Fuc	, Rib	, [Ara]	, Xyl	Man	, Glc	, [Gal]		
Turnip	50% ppt.	(0.16)	S-200	(0.08)	—	, Fuc	, Rib	, [Ara]	, Xyl	—	, Glc	, [Gal]		
Cabbage	50% ppt.	(0.18)	S-200	(0.08)	—	, Fuc	, Rib	, [Ara]	—	, Man	, Glc	, [Gal]		
Cab.	90% ppt.	(0.12)	P-10	(1.25)	—	, Fuc	, Rib	, Ara	, Xyl	Man	, [Glc]	, [Gal]		
Buckwheat	90% ppt.	(6.86)	P-10	(1.25)	—	, Fuc	, Rib	, Ara	—	, Man	, [Glc]	, Gal		

^a Yields, g/100 g starting (fresh or dried) material.

^b Inhibitory activity (mg/ml) against eel anti-H agglutinin (1:8).

^c sol., soluble; ppt., precipitates.

^d Bio-Gel P-10 or Sephacryl S-200.

^e [], major sugars.

FIG. 1. Sephacryl S-200 Gel Filtration of Ethanol 50% Precipitates from Turnip Leaves.

Arrows indicate the maximum elution volumes of molecular weight markers.

—, elution volume range of blood group activities obtained from each ethanol fraction (Table II) of the indicated plants.

FIG. 2. Bio-gel P-10 Filtration of Ethanol 90% Precipitates from Japanese Pear Fruits.

Arrows and —, as in Fig. 1.

idues as non-reducing terminals.⁸⁾ To clarify the nature of such ABH-active carbohydrates of vegetable origin, separation, chemical analysis and identification of the determinant sugars of these compounds are now in progress. A part of the results will be reported soon.⁹⁾

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