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Inhibitory Effect of Pectin from the Segment Membrane of Citrus Fruits on Lipase Activity

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Summary Segment membranes from 4 citrus species selected from 4 sections were treated with water to obtain polysaccharides containing pectin. The extracts, which inhibited pancreatic lipase activity in a concentration-dependent manner, were divided into high molecular weight fractions [molecular weight (M.W.) >300,000], which inhibited the activity strongly, and low molecular weight fractions (M.W. <300,000), which did not show such strong inhibition. The high molecular weight fractions were composed mainly of a characteristic sugar of pectin, namely, galacturonic acid. A galacturonic acid-rich fraction purified by anion exchange chromatography from a water extract also strongly inhibited the activity. The inhibitory activity of the high molecular weight fraction was much stronger than that of commercial citrus pectin. The results suggest that pectin from segment membranes of citrus fruits might be useful as a functional food, especially as a fat-reducing material.

Key Words citrus, pectin, pancreatic lipase, triacylglycerol digestion, inhibition

Though citrus is a major source of commercial pectin, a large amount of citrus pulp is disposed of as industrial waste after processing. Citrus pulp is composed mainly of peel, albedo and segment membrane, and their major chemical components are polysaccharides, cellulose, hemicellulose and pectin (*1*). In general, peels and albedo are not suitable as food, while segment membrane can be a foodstuff (*2*). If segment membrane can be isolated directly and easily from citrus fruits during citrus juice production, the membrane may become a useful foodstuff. Segment membrane is already isolated in the process of making canned oranges, in which oranges are mechanically peeled to isolate the segments (*3*). If such segment membranes can be provided consistently to the market from food-processing companies and their availability as functional foods is confirmed, citrus fruits may be a valuable source. Pectin of segment membranes, however, has not been studied in detail.

Commercial pectins lowered weight gain, and plasma and liver cholesterol as well as reduced total cholesterol and LDL cholesterol concentrations (*4–12*). Administration of such pectins, however, did not affect pancreatic lipase activity (*4*). Dosage of pectin to human beings failed to lower cholesterol (*13*). Inhibitory effect of pectin on pancreatic lipase was weaker than other dietary fibers such as alfalfa, oat bran, wheat bran and xylan (*14*). The binding of porcine pancreatic lipase or human bile lipids on pectin was weaker than wheat bran (*15*). In our previous research, low-molecular-weight fractions from commercial citrus pectin [in particular, molecular weight (M.W.): 90,000] have been shown to associate with substrates and inhibit pancreatic lipase activity (*16*). Therefore, studies with appropriate pectin will clarify the cause of their dietary effects. As segment membrane is looked upon as a foodstuff, it may be a practical source of such pectin.

Generally speaking, commercial pectin is prepared by boiling citrus peel or apple pomace in acidic water (*17*). During such treatments, most of the regions except linear chains consisting mainly of galacturonic acid residue are degraded. Therefore, we tried to develop a mild and simple procedure for the preparation of citrus pectin because the acid-labile regions of the pectin might have different effects on lipase activity. Selection of the pectin's source and preparation procedure should be considered with an aim of preparation of functional foods. In this paper, segment membranes were prepared from citrus fruits of different species, and their glycosyl

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Botanical name: Allen Eureka, *Citras limon* (L) Burm; Hassaku, *Citrus hassaku* Hort ex Tanaka; Tosa-buntan, *Citrus grandis* (L) Osbeck; Grapefruit, *Citrus pradisi* Mocf.; Miyauchi-iyo, *Citrus iyo* Hort ex Tanaka; Morita-nable, *Citrus sinensis* (L) Osbeck var. *brasiliensin* Tanaka; Hyuganatsu, *Citrus tamurana* Tanaka; Orange, *Citrus sinensis* (L) Osbeck; Amanatsu, *Citrus natsudaidai* Hayata; Yuzu, Citrus junos Sieb. ex Tanaka; Ehime-nakate-unshiu, *Citrus unshiu* Mark; Shikuwasha, *Citrus depressa* Hayata; Tachibana, *Citrus tachibana* (Marc.) Tanaka; Ponkan, *Citrus reticulata* Blanco; Dekopon, kiyomi (*Citrus unshiu* Marc. × *C. sinensis* Osbec) × ponkan (*C. reticulate* Blanco); Kiyomi, *Citrus unshiu* Marc. × *C. sinensis* Osbeck; Nanka, *Citrus unshiu* Mar. × *C. reticulata* Blanco; Sweet spring, *Citrus unshiu* Marc. × *C. hassaku* Hort ex Tanaka.

compositions determined in order to choose the species most suitable for pectin preparation. The inhibitory effect of water extracts from the membranes and fractions obtained by ultrafiltration or anion exchange chromatography of the extracts on pancreatic lipase activity are described. Water extraction was employed, because it is a mild and simple procedure, and water-soluble pectin is thought to be useful as a food or drink additive as an inhibitor of lipid digestion.

MATERIALS AND METHODS

Materials. As citrus can be divided into 8 sections based on Tanaka's system (*18*), samples (19 species) were selected from each section with the exception of *Limonellus* Tanaka, *Paseda* Tanaka and *Psedofortuella* Tanaka and their hybrids. Most of the citrus fruits were gifts from Ehime Fruit Tree Experiment Station (Ehime, Japan). Grapefruits, Orange and Ponkan were purchased from a local store. Yuzu fruits were harvested from an author's farm. Citrus fruits were peeled to obtain segments and segment membrane was stripped from them manually. Prepared membranes were freeze-dried and milled with a coffee mill to produce a powder. Commercial citrus pectin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Unshiu (the precise species of the Unshiu was not known) pulp was obtained from a juice industry source, Ehime-Inryo Co. (Matsuyama, Japan). Trioleoylglycerol and pig pancreatic lipase (Fraction VI-S) were purchased from Sigma-Aldrich Japan (Tokyo, Japan).

Isolation of water-soluble pectin by ultrafiltration. Segment membrane powder (ca. 1 g) soaked in water (100 mL) was stirred at room temperature overnight. The mixture was centrifuged (12,000 rpm, 15 min) to remove insoluble residue. The soluble fraction was further separated into large (M.W. $>$300,000) and small (M.W. $<$300,000) molecular weight fractions by ultrafiltration (Millipore, Mass., USA). Each fraction was concentrated under reduced pressure and freeze-dried.

Anion-exchange chromatography. The extract from the segment membrane of Ehime-nakate-unshiu was further fractionated by anion exchange chromatography. DEAE-Cellulose (Whatman plc, ME, UK) was packed into a column (50 cm$\times$3.5 cm i.d.), and was equilibrated with 0.01 M sodium phosphate buffer (pH 8.0). The sample dissolved in the same buffer was loaded onto the column and the column washed with the same buffer (0 M NaCl eluate). Pectin adsorbed to the column was eluted stepwise with buffer containing 0.5 and 1.0 M NaCl (0.5 and 1.0 M NaCl eluate, respectively). These eluates were dialyzed against de-ionized water, concentrated to a small volume under reduced pressure and freeze-dried.

Glycosyl composition analysis. Glycosyl compositions were determined by derivation of sugars to TMS ethers of methyl glycosides (*19*). Samples (ca. 100 μg) were degraded with 0.25 mL of 1 M HCl in methanol (Wako Pure Chemical Industries, Ltd.) at 80˚C for 16 h. After treatment, the methanolic HCl was removed with a few drops of *t*-butyl alcohol by evaporation with a stream of air at room temperature. The products (methyl glycosides and methyl ester methyl glycosides) were silylated with 0.2 mL of piridine, hexamethyldisilazane and trimethylchlorosilane (1 : 2 : 1) at 80˚C for 20 min. Remaining silylating reagents were removed by evaporation with a stream of air at room temperature. TMS ethers of methyl glycosides were extracted with hexane and transferred to a clean test tube and the hexane evaporated. The GC analyses of the samples were performed on a Shimadzu (Kyoto, Japan) GC-9A gas chromatograph equipped with a FID detector and a fused silica DB-1 capillary column (30 m$\times$0.25 mm i.d.; J and W Scientific, CA, USA). The oven temperature was kept at 140˚C for 2 min, then increased to 200˚C at a rate of 2˚C/min, and finally the temperature was raised to 275˚C at a rate of 30˚C/min and was kept at this temperature for 10 min to condition the column.

Enzyme assay. Lipase (EC 3.1.1.3) from porcine pancreas (100–400 units/mg protein at pH 7.7 at 37˚C) was used in the enzyme assay. Lipase activity was determined by the rate of release of oleic acid from trioleoylglycerol. Inhibition of lipase activity was estimated by the reduction of the activity brought about by sample addition. The substrate emulsion was prepared by sonicating trioleoylglyserol (80 mg), phosphatidylcholine (10 mg) and taurocholic acid (5 mg) in 0.1 M *N*-tris(hydroxylmethyl)-2-aminoethanesulfonic acid (TES) buffer (9 mL) containing 0.1 M NaCl. The assay system was comprised of the following components in a total volume of 200 μL: 25 μL enzyme solution, 50 μL sample solution, 0.5 μmol trioleoylglycerol, 0.053 μmol taurocholic acid, 0.07 μmol phosphatidylcholine, 20 μmol TES and 20 μmol NaCl. The mixture was incubated at 37˚C for 30 min. The amount of released oleic acid was estimated as described previously (*20*).

RESULTS AND DISCUSSION

Preparation of water extracts from segment membranes of different citrus species

According to Tanaka systematics (*18*), citrus can be classified into 8 sections. In this research, 19 citrus species belonging to 5 popular sections were selected, and their segment membranes prepared. Major sugars of the segment membranes of these citrus species were rhamnose (5.4–11.0%), arabinose (6.8–21.9%), glucose (25.4–55.8%) and galacturonic acid (10.5–20.6%), which indicates the presence of pectin and cellulose (Table 1). Galacturonic acid is the main component of the backbone of pectin, and detection of rhamnose indicates the presence of a ramified region of pectin (*21*). The presence of arabinose is likely due to arabinan or side-chains of pectin. Likewise the presence of galactose (2.3–11.9%) is due to galactan or side-chains of pectin. Minor sugars such as xylose (trace–11.1%) and mannose (1.1–2.8%) are probably due to the presence of the hemicellulosic polysaccharides, xylan and mannan. A comparison of the glycosyl composition of segment membranes of Ehime-nakate-unshiu (abbreviated to Unshiu hereafter) to that of

Table 1. Glycosyl compositions of segment mambranes prepared from citrus fruits.

Section and species	Glycosyl composition (mol %)								
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Gal A	Glc A
Citrophorum Tanaka									
Allen Eureka	10.7	3.2	23.5	7.7	2.3	4.8	25.4	17.9	4.6
Cephalocitrus Tanaka									
Hassaku	9.6	trace	8.1	9.1	2.1	7.3	38.5	20.6	4.8
Tosa-buntan	9.3	2.3	16.7	11.1	2.3	4.5	32.9	19.5	1.4
Grapefruits	8.8	trace	6.8	5.7	2.6	5.4	43.7	20.3	6.8
Autantium Tanaka									
Miyauchi-iyo	8.9	trace	19.7	trace	2.3	4.3	38.2	18.2	8.5
Morita-nable	9.6	1.8	15.9	4.5	1.8	5.2	48.1	11.7	1.3
Hyuganatsu	5.4	2.1	15.5	5.0	2.8	4.5	53.1	10.5	1.1
Orange	9.6	2.7	14.3	4.1	3.4	6.1	37.1	16.9	5.8
Amanatsu	10.3	2.7	15.2	8.0	2.4	4.7	31.5	20.0	5.2
Osmocitrus Tanaka									
Yuzu	5.6	1.7	21.2	5.1	2.0	11.9	32.7	18.7	1.3
Acrumen Tanaka									
Ehime-nakate-unshiu	8.5	2.7	21.9	7.3	1.9	4.9	39.8	11.5	1.5
Unshiu*	5.2	0.5	16.5	4.0	1.1	5.8	52.5	14.3	0.1
Shikuwasha	7.7	1.1	12.7	6.5	1.6	2.3	55.8	10.8	1.5
Tachibana	7.1	3.0	21.9	4.7	1.9	8.2	31.1	14.0	8.1
Ponkan	9.0	3.0	18.7	4.8	2.7	5.3	35.8	15.7	5.1
Hybrid (Tangor-based)									
Dekopon	6.3	1.8	15.5	7.7	2.0	5.4	44.0	15.6	1.8
Kiyomi	8.8	1.2	11.1	7.1	2.5	5.3	39.2	19.6	5.2
Hybrid (Mandarin-based)									
Nanka	11.0	2.6	14.9	8.3	1.9	5.0	36.6	14.2	5.6
Hybrid (Tangelo-based)									
Sweet Spring	8.1	2.2	21.7	7.6	1.4	5.1	42.6	10.5	0.8

*Unshiu pulp left after juice production. The pulp was the gift of Ehime-Inryo Co.
Precise species of the unshiu was not known.
The pulp consisted of peel, albedo and segmemt membrane.

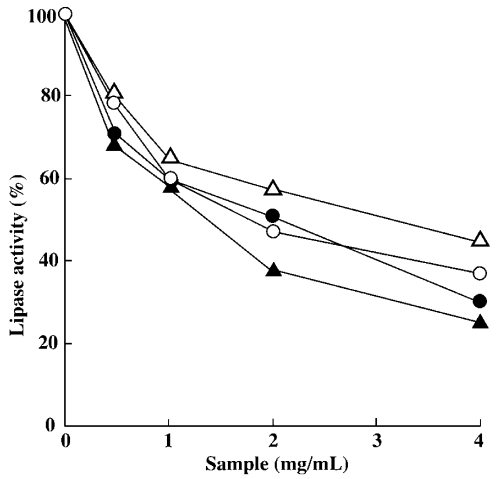

Fig. 1. Effect of segment membrane suspensions on pancreatic lipase activity. The activity is expressed as the percentage of activity obtained in the absence of segment membrane suspensions. (○) Ehime-nakate-unshiu, (●) Miyauchi-iyo, (△) Tosa-buntan and (▲) Amanatsu.

Table 2. Yields of the water extracts and insoluble residues from the segment membranes of citrus fruits. The water extract was separated into two fractions by ultrafiltration using a membrane with a molecular weight cut-off of 300,000.

Sample	Water extract (%)		Residue (%)
	M.W. >300,000	M.W. <300,000	
Ehime-nakate-unshiu	7.3	51.6	40.6
Miyauchi-iyo	6.4	40.6	48.3
Tosa-buntan	6.0	30.4	50.2
Amanatsu	4.8	33.4	48.0

M.W.: molecular weight.

Unshiu pulp (consisting of segment membrane, peel and albedo), demonstrated that the glucose content of Unshiu pulp was higher (52.5%) than that of Ushiu segment membrane (39.8%). This suggests that Unshiu pulp contains more cellulose and that the segment membrane is a better source for pectin preparation. The membranes with high glucose content, for example

Table 3. Glycosyl compositions of samples from fractionation of water extracts of segment membranes of citrus fruits. The fractions were separated by ultrafiltration using a membrane with molecular weight cut-off of 300,000.

Sample	Glycosyl composition (mol %)								
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Gal A	Glc A
Ehime-nakate-unshiu									
M.W. >300,000	9.8	2.9	8.6	1.4	1.0	3.1	11.6	44.1	17.6
M.W. <300,000	2.4	2.6	19.1	7.1	2.1	8.2	57.2	0.8	0.4
Miyauchi-iyo									
M.W. >300,000	7.6	1.3	4.1	0.5	1.8	2.0	10.6	56.0	16.1
M.W. <300,000	2.2	2.3	15.9	4.8	2.3	15.3	55.4	1.6	0.2
Tosa-buntan									
M.W. >300,000	3.9	0.7	4.4	2.3	1.4	1.2	15.2	53.6	17.4
M.W. <300,000	1.4	1.6	15.5	21.2	2.5	10.8	43.8	2.9	0.4
Amanatsu									
M.W. >300,000	4.9	2.2	9.6	3.5	3.0	6.8	15.6	41.3	13.1
M.W. <300,000	2.1	3.2	21.1	9.5	2.8	15.2	44.1	1.3	0.5

M.W.: molecular weight.

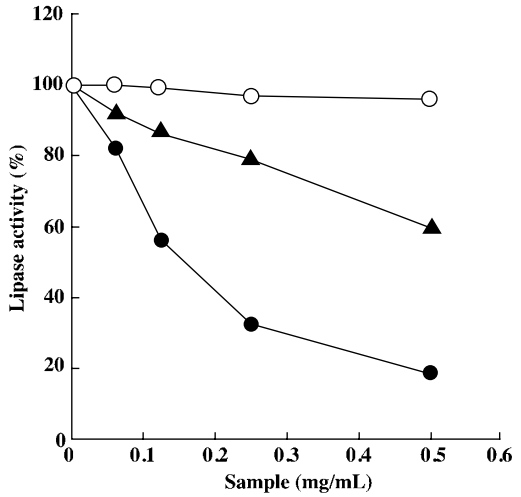

Fig. 2. Effect of the water extract of the segment membrane (Unshiu), and its fractions on pancreatic lipase activity. Each fraction was obtained from the water extract by ultrafiltration. (▲) water extract, (●) M.W. >300,000 and (○) M.W. <300,000.

Table 4. IC_{50} values of water extracts of segment membranes and their fractions.

Sample	Fractions	IC_{50} (mg/mL)
Ehime-nakate-unshiu	Water extract	>0.5
	M.W. >300,000	0.15
	M.W. <300,000	n.d.
Miyauchi-iyo	Water extract	>0.5
	M.W. >300,000	0.13
	M.W. <300,000	n.d.
Tosa-buntan	Water extract	>0.5
	M.W. >300,000	0.10
	M.W. <300,000	n.d.
Amanatsu	Water extract	>0.5
	M.W. >300,000	0.20
	M.W. <300,000	n.d.

IC_{50} refers to the concentration needed to halve pancreatic lipase activity.
Each water extract was separated by ultrafiltration (M.W. cut-off: 300,000).
"n.d." means "not determined." The inhibitory activity of these samples was extremely weak.

Hyuganatsu and Shikuwasha, were not suitable for pectin preparation. Furthermore, the difficulties in preparing the membranes from the fruits of Allen Eureka, Tachibana and Ponkan, meant such species were not used in further research. Thus, the membranes of Unshiu, Tosa-buntan, Miyauchi-iyo and Amanatsu were employed as the samples for pectin preparation.

These segment membrane suspensions inhibited pancreatic lipase activity dose-dependently at concentrations of 1–4 mg/mL in the assay system using trioleoylglycerol emulsified with PC. At 2 mg/mL, these preparations inhibited the enzyme by approximately 50% (Fig. 1). The effect was similar to that of commercial citrus pectin (ca. 2.0 mg/mL of the commercial pectin was necessary to attain the same effect) (*6*). For identification of the inhibitory material, the segment membranes were treated with water and the glycosyl compositions of the extracts were compared with their lipase inhibition activity.

Segment membranes were treated overnight with water at room temperature. Nearly half of each sample was extracted and the rest remained as insoluble residue (40.6–50.2%: Table 2). These extracts were further separated into large molecular weight (M.W. >300,000) and small molecular weight (M.W. <300,000) fractions. The majority of the water extracts separated into the small molecular weight fractions (30.4–51.6%: % of original sample), while the yields of the large molecular weight fractions were low (4.8–7.3%: % of original sample). The glycosyl compositions of these fractions were analyzed to identify their constituent polysaccharides (Table 3). The composi-

Table 5. Glycosyl compositions of fractions from water extraction of segment membranes of Unshiu. Fractions were eluted from an anion-exchange column with 0.01 M phosphate buffer containing 0 and 0.5 M NaCl.

Salt concentration in the buffer	Glycosyl composition (mol %)								
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Gal A	Glc A
0 M NaCl	0.6	trace	55.4	8.3	1.8	11.2	18.1	3.9	0.7
0.5 M NaCl	1.0	1.5	3.5	0.2	1.2	1.5	0.0	91.2	0.0

The amount of the eluate of 1.0 M NaCl step was so little that neither its inhibitory effect nor glycosyl composition was determined.

tions of the small molecular weight fractions were remarkably different from those of the large molecular ones. The small molecular weight fractions were composed mainly of arabinose (15.5–21.1%), xylose (4.8–21.2%), galactose (10.8–15.3%) and glucose (43.8–57.2%), which demonstrate that their major components were not pectin, but neutral polysaccharides. In contrast, with the exception of rhamnose (3.9–9.8%) and arabinose (4.1–9.6%), the large molecular weight fractions contained exclusively the acidic sugars, galacturonic acid (41.3–56.0%) and glucuronic acid (13.1–17.6%). Pectin, whose backbone is composed mainly of galacturonic acid residues, consists of three types of acidic polysacchrides, namely, homogalacturonan, rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II) (*22*). RG-I and RG-II are looked upon as ramified regions of pectin. Rhamnose is also another component of the backbone of RG-I in addition to galacturonic acid. Furthermore, arabinose is a main element of the side chains of pectin, while glucuronic acid is also found in RG-II. These glycosyl compositions indicate the large molecular weight fractions to be pectin.

Inhibitory effects of water extracts of segment membranes on pancreatic lipase activity

The inhibition of pancreatic lipase activity produced by addition of the water extracts or the large and small molecular weight fractions prepared from the segment membranes were measured. The Unshiu extract and its fractions inhibited the activity in a concentration-dependent manner, though the effect of the small molecular weight fraction was weak (Fig. 2). The water extract of Unshiu reduced the activity to ca. 60% at a concentration of 0.5 mg/mL. The inhibitory effect was a little stronger than that of segment membrane suspensions. While the small molecular weight fraction scarcely showed any inhibitory effect, the large molecular weight fraction inhibited the activity strongly (by ca. 80% at a concentration of 0.5 mg/mL). The similar inhibitory effects were observed for other species, namely Miyauchi-iyo, Tosa-buntan and Amanatsu. These inhibitory effects were remarkably higher than that of other dietary fibers such as fine wheat bran (*15*). To allow comparison of the inhibitory effects of pectins of these species, the sample concentrations (mg/mL) needed to halve pancreatic lipase activity (IC_{50}) were determined (Table 4). The effects of both water extracts and low molecular weight fractions were too weak to allow determination of the value (up to addition of sample to 0.5 mg/mL). Of the high molecular weight fractions, Tosa-buntan was most effective (0.1 mg/mL), followed by Miyauchi-iyo (0.13 mg/mL), Unshiu (0.15 mg/mL) and Amanatsu (0.2 mg/mL). In terms of galacturonic acid content, Tosa-buntan and Miyauchi-iyo contained the highest levels (53.6 and 56.0% respectively) while Unshiu and Amanatsu contained lower levels (44.1 and 41.3% respectively). These results suggest that purity of pectin and/or galacturonic acid content has an effect on inhibitory activity. Though the galacturonic acid content of Miyauchi-iyo was slightly higher than that of Tosa-buntan, the inhibitory activity of the former was a little weaker. Factors other than purity of pectin such as viscosity and/or degree of esterification may play a role in the inhibitory effect. Further experiments are needed to confirm this possibility.

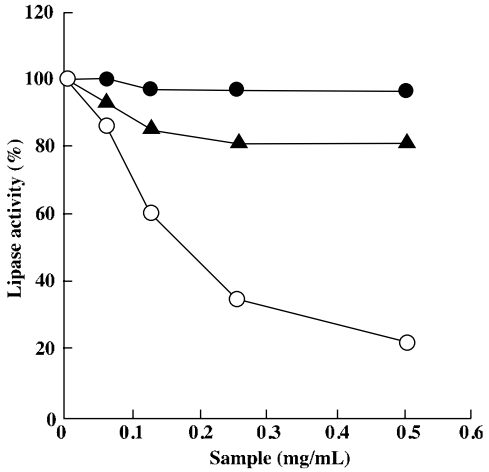

Fig. 3. Effect of the fractions separated by anion-exchange chromatography (DEAE-Cellulose) and water-soluble fraction of commercial citrus pectin on pancreatic lipase activity. Fraction I (●): elution with phosphate buffer without NaCl (0 M NaCl), Fraction II (○): elution with buffer containing 0.5 M NaCl, Commercial pectin (▲): water-soluble fraction of commercial citrus pectin (Wako Pure Chemical Industries, Ltd.).

Given this observation, pectin was purified from a water extract (Unshiu) by anion-exchange chromatography and its inhibitory effect on pancreatic lipase activity measured. The polysaccharides adsorbed to the

Table 6. Glycosyl compositions of commercial citrus pectin and its water-soluble fraction.

Sample	Glycosyl composition (mol %)								
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Gal A	Glc A
Commercial citrus pectin	2.7	0.1	2.4	0.3	0.2	7.1	44.7	41.7	0.8
Water-soluble fraction	2.4	0.3	2.1	0.3	0.4	4.2	37.7	52.6	trace

Commercial citrus pectin was purchased from Wako Pure Chemical Industries, Ltd.
Water-soluble fraction was prepared by water extraction of above-mentioned pectin at room temperature for 3 h.

anion-exchange column were eluted stepwise with buffer containing 0 M NaCl, 0.5 M NaCl and 1.0 M NaCl. The eluate with 0 M NaCl merely contained galacturonic acid (3.9%), which showed that its main constituents were neutral polysaccharides. The amount of the eluate obtained from the 1.0 M NaCl step was so little that neither its inhibitory effect nor glycosyl composition was determined. The eluate from the 0.5 M NaCl step, which was composed almost only of galacturonic acid (91.2%), was nearly pure homogalacturonan. The other pectin components, RG-I and RG-II, were possibly lost during dialysis (molecular weight cut-off: 300,000). The inhibitory effect of the 0 M NaCl and 0.5 M NaCl eluates on pancreatic lipase activity were determined (Fig. 3). The eluate (0 M NaCl), which merely contained pectin, hardly inhibited the activity, while the 0.5 M NaCl eluate, which was almost pure homogalacturonan (i.e. the linear region of pectin), showed ca. 80% inhibition upon addition to 0.5 mg/mL. The result shows clearly that the inhibitory effect is due to pectin. The inhibitory effect (IC_{50}: 0.16 mg/mL) was, however, very similar to the effect of the large molecular weight fraction (IC_{50}: 0.15 mg/mL). Therefore, purification of linear regions of pectin was concluded not to contribute to remarkable improvement in the inhibitory effect, though such regions play a role in the effect. The ramified regions such as RG-I and RG-II may enhance the inhibitory effect. Their role will be discussed in later papers.

Comparison of inhibitory effect of commercial pectin with that of pectin from segment membrane

Though commercial citrus pectin (M.W. of ca. 750,000) showed only a weak inhibitory effect on pancreatic lipase activity (IC_{50}: 2.0 mg/mL), it is inexpensive. Fine wheat bran also inhibited the activity (*15*). To adopt pectin extracted from segment membranes as a source of functional foods, the pectin must be a more attractive inhibitor of pancreatic lipase activity than both commercial citrus pectin and other dietary fibers. Therefore, pectin from segment membrane must inhibit the activity significantly. Thus, the inhibitory effects of the water-soluble fraction of commercial citrus pectin and fine wheat bran (*15*) were compared with that of pectin from segment membranes. About 84.1% of commercial citrus pectin was extracted with water at room temperature. Commercial citrus pectin contained glucose (44.7%) and galacturonic acid (41.7%) as its main constituent sugars, which indicates that it is contaminated with neutral polysaccharides (Table 6). The water-soluble fraction of commercial citrus pectin contains more galacturonic acid (52.6%), which was almost equal to the amount in the high molecular weight fraction of Tosa-buntan, the most effective pectin preparation. The water-soluble fraction from the commercial product, however, reduced pancreatic lipase activity only slightly (to 80%) upon its addition to 0.5 mg/mL. This level of inhibition was almost equal to the effect of the original unfractionated commercial citrus pectin (added as powder). Thus, water extraction did not improve the effect of commercial citrus pectin. Commercial citrus pectin is prepared by boiling orange peels in acidic solution (*17*). Most of the acid-labile regions of pectin, particularly the ramified region, are partially degraded under such harsh conditions. Thus, commercial citrus pectin is not only contaminated with neutral polysaccharides, but is structurally altered such that its inhibitory effect is reduced. Regarding inhibitory effect on pancreatic lipase activity, IC_{50} of fine wheat bran, one of the effective inhibitors of the activity, was ca. 5 mg/mL (*15*), while the values of pectins from citrus segment membranes were 0.1 to 0.2 mg/mL. These pectins are 25 to 50 times more effective than fine wheat bran. These results suggest that pectin, which can inhibit pancreatic lipase activity, is not easily prepared from commercial citrus pectin and that citrus segment membrane is a promising source for functional foods. Regrettably, pectin concentration in the water extracts was so insufficient that its purification is necessary. If such pectin as in the crude water-extract of segment membrane is purified, its dosage to animals will not only inhibit lipase activity, but also lower total cholesterol and LDL concentration remarkably. Water extraction at room temperature, which is a simple process, however, is not an ideal method to isolate pectin effectively. Almost structurally intact pectin is reportedly prepared by treatment with chelating agents or diluted alkaline solutions at room temperature (*22*). These extraction procedures are fitting for isolation of intact homogalacturonan and ramified pectin containing both RG-I and RG-II. Though these pectin preparations were used in structural analyses, the effects on lipase activity of such intact pectin have not yet been reported. Their effect on pancreatic lipase activity will be reported in later papers.

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