Citrus Pectin: Characterization and Inhibitory Effect on Fibroblast Growth Factor−Receptor Interaction

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This study was undertaken to characterize the pectin from four citrus species and to determine their in vitro inhibitory activities on the binding of fibroblast growth factor (FGF) to the FGF receptor (FGFR). Pectin from various parts of lemon, grapefruit, tangerine, and orange were isolated and characterized. Tangerine had the highest pectin content among the four citrus species. Segment membrane contained as much as or more pectin than flavedo/albedo. Anhydrogalacturonic content was highest in pectin from segment membrane of tangerine and flavedo/albedo of grapefruit. Lemon pectin contained the highest methoxyl content (MC), and grapefruit contained the largest proportion of lower molecular weight (<10000 Da) pectin. Tangerine contained the highest neutral sugar in both flavedo/albedo and segment membrane. The interdependency of heparin on factor−receptor interaction provides a means for identifying new antagonists of growth factor activity and thus for treatment of various diseases. These results showed that pectin significantly inhibited the binding of FGF-1 to FGFR1 in the presence of 0.1 μg/mL heparin. The pectin from the segment membrane of lemon was the most potent inhibitor. The inhibition activity was significantly correlated with sugar content, MC, and size of pectin. Kinetic studies revealed a competitive nature of pectin inhibition with the heparin, a crucial component of the FGF signal transduction process. The observation that the heparin-dependent biological activity of FGF signal transduction is antagonized by citrus pectin should be further investigated for the use of these pectins as anti-growth factor agents for potential health benefits.

Keywords: *Health benefits; citrus; sugars; molecular weight; heparin mimics*

INTRODUCTION

Pectin is a class of complex polysaccharides that function as a hydrating agent and cementing material for the cellulosic network (*1*, *2*). Although pectin occurs in a majority of plant tissues, it is most abundant in citrus fruits (*3*). Pectins are classified into three types depending upon their solubility: water-soluble, chelator-soluble, and alcohol-soluble (*4*). The structure of pectin is primarily composed of repeating units of anhydrogalacturonic acid (AGA) joined by α 1→4 glycosidic linkages creating a linear polymer. The linear regular structure is interrupted with the presence of neutral sugar side chains (*5*). Furthermore, the carboxyl groups of AGA may either remain as free acids, be esterified with methanol, or be neutralized with cations. The chemical structure of pectin varies from one fruit species to another and also during the different developmental stages of the fruit (*1*, *6*, *7*).

Commercial pectin is mostly derived from citrus (lime, lemon, grapefruit, and orange) and apple (*1*, *2*). Although traditionally pectin is used as a thickening agent in the food industry, such as in the production of jams and jellies (*2*), it has been shown to have potential beneficial effects in human health, such as a wound treatment ingredient, a hemostatic agent, and an immune complement activator (*1*, *8*, *9*). Dietary supplementation of pectin showed decreased levels of blood cholesterol (*10*) and reduced serum glucose in patients with diabetes mellitus (*11*). In addition, modified citrus pectin (MCP) has been shown to prevent spontaneous cancer metastasis (*12*, *13*) and inhibit cancer cell proliferation (*14*). Recent studies also suggest that the beneficial effects of pectin are closely related to its structural characteristics. For example, Briggs (*15*) suggested that pectin with less methoxyl content and lower molecular weight (<10000 kDa) is more efficient for cancer metastasis prevention, whereas pectin with greater methoxyl content and higher molecular weight is a better cholesterol lowering agent (*16*, *17*).

The fibroblast growth factor (FGF) signaling system

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10.1021/jf001020n CCC: $20.00
Published on Web 05/23/2001

is ubiquitous and a mediator of developmental processes in the embryo and homeostasis in the adult. Studies in our laboratory and by other investigators suggested that defects in this signal transduction system disturb the regulatory process and result in many diseases such as cancer, cardiovascular disease, and diabetes mellitus (*18*). Heparin, a sulfated polysaccharide, displays specificity for different fibroblast growth factor receptors (FGFRs) and FGFs (*19*, *20*) and acts as positive (stimulation) or negative (inhibition) regulators of FGF signaling (*21*). Several heparin-mimicking compounds, such as suramin (*22*), carrageenans (*23*), and pentosan polysulfate (*24*), compete with heparin for interaction with growth factor receptors and consequently inhibit FGF-dependent intracellular signal transduction. Pectin, although structurally similar to heparin (*25*) and an antagonist of heparin in the blood-clotting process (*1*, *26*), has not been studied for its effect on the FGF signal transduction system. Because heparin is an integrated part of the active FGF signaling complex, it would be interesting to know how pectin affects FGF and FGFR interaction and thus signal transduction.

This study was conducted to determine the variation of pectin content and its chemical composition in representative citrus species. The effect of pectin from different citrus species on the FGF signal transduction system was investigated, and the relation between the structure of pectin and its effect on the FGF signaling system was also explored.

MATERIALS AND METHODS

Fruit Samples. Fruits from four representative citrus species, Meyer lemon (*C. meyeri* Y. Tan.), Dancy tangerine (*C. reticulata* Blanco), Marrs orange (*C. sinensis* L. Osb), and Marsh White grapefruit (*C. paradisi* Macf.) grown on sour orange rootstock (*C. aurantium* L.), were harvested in February 1999 at the South Farm of the Texas A&M University—Kingsville Citrus Center, Weslaco, TX. All four citrus species received similar cultural practices including fertilizer and irrigation.

Materials and Chemicals. Standard dextrans, blue dextran, arabinose, galactose, glucose, mannose, rhamnose, xylose, and heparin were obtained from Sigma Chemical Co. (St. Louis, MO). Native bovine FGF-1 was purchased from Upstate Biotechnology (Lake Placid, NY).

Pectin Extraction. The citrus fruits were separated into two parts: peel (flavedo/albedo) and segment membrane (without juice vesicles, core, and seed). To maintain the uniformity, we have combined flavedo and albedo together in the four citrus species because it was very difficult to separate flavedo from albedo in tangerines. Pectin was extracted from the two parts with the classical hot acid procedure as reported by Rouse (*6*) Briefly, the dried tissues were powdered and mixed with deionized water (1:40), and concentrated HCl was added to give a pH of 1.6 ± 0.5. The mixture was continuously stirred and maintained at 95 °C for 30 min. Iso-propanol and ethanol were applied to precipitate and purify the final pectin.

Analysis of Pectin Chemical Composition. The AGA content was determined according to the modified *m*-hydroxydiphenyl assay (*27*).

The spectrophotometer method described by Wood and Siddiqui (*28*) was used to determine the extent of methoxylation, expressed as methoxyl content (MC), which is the percentage of total pectin content (*29*). In this method, formaldehyde, the oxidation product of methanol, was measured in a Beckman DU 2400 spectrophotometer at 412 nm. The theoretical upper limit of MC (16.32%) was based on the molecular weight of a methoxylated galacturonic acid unit (*30*).

The lower molecular weight proportion of pectin was analyzed with fast protein liquid chromatography (FPLC). The pectin was dissolved in the mobile phase (0.3 M NaCl in 50 mM phosphate buffer) at 60 °C. Traces of insoluble materials were removed by centrifugation at 13000*g* followed by filtration through a 0.2 μm nucleopore filter. A 50 μL sample was injected into a Superdex 200HR 10/30 column connected to a computer-controlled FPLC system (Pharmacia Co., Peapack, NJ) at the flow rate of 1 mL/min. The peak was monitored with an on-line UV detector. Standard dextran (MW 10000) was used to determine the lower molecular weight portion. Exclusion and inclusion volumes of the column were determined using blue dextran (MW 2000000) and sucrose (MW 342), respectively.

The neutral sugars were determined as alditol acetates by gas chromatography (*31*). Pectin samples (20 mg) were hydrolyzed with trifluoroacetic acid (2 N) for 1 h at 121 °C. The hydrolysates were dried under a stream of air at 45 °C, reduced with sodium borohydride (10 mg) in ammonia (1.5 N, 0.2 mL), and acetylated with 3 mL of acetic anhydride for 30 min at room temperature in the presence of 1-methylimidazole (0.45 mL) as catalyst. Deionized water (5 mL) was added before the alditol acetate derivatives of the sugars were separated from the aqueous phase by extracting twice with dichloromethane (3 mL each). A 2 μL sample was injected onto the column (capillary fused silica, 30 m $\times$ 0.32 mm i.d., 0.2 μm film thickness, SP 2330, Supelco Co., Bellefonte, PA). A Hewlett-Packard (HP) model GC-5890 gas chromatograph system consisted of a series II injector, an oven, a flame ionization detector, and an HP 3390 series II integrator. The oven, injector, and detector temperatures were 240, 270, and 270 °C, respectively. The split ratio of 1:20 and the carrier gas (nitrogen) pressure of 70.0 kPa were used.

Fibroblast Growth Factor Binding Assay. In this study, the FGF binding assay established in our laboratory (*32*) was essentially followed, and fibroblast growth factor-1 (FGF-1) and FGF receptor 1 (FGFR1) were used. Heparan sulfate deficient insect Sf9 cells were cultured and maintained in TNM-FH medium supplemented with 5% fetal bovine serum (FBS) in a 27 °C incubator. The Sf9 cells (6×10^5) harvested from a previous culture were transferred to six-well plates (9.6 cm^2) and introduced with 40 μL of conditioned medium from cells infected by baculovirus bearing FGFR1β in 2 mL of TNM-FH medium supplemented with 5% FBS at 27 °C for 60 h. Native bovine FGF-1 was iodinated to achieve the specific activities of $\sim 3 \times 10^5$ cpm/ng with $Na^{125}I$ using the Chloramine T method (*33*). Fresh pectin samples from four citrus species were prepared in 1$\times$ phosphate-buffered saline (PBS) at 60 °C. Infected Sf9 cells (5×10^4) were harvested, centrifuged at 1000*g* for 3 min, and washed with and incubated in binding buffer [1$\times$ PBS, 10 mM $MgCl_2$, 1 mg/mL bovine serum albumin (BSA)] for 30 min at room temperature either in the presence of 0.1 μg/mL heparin with or without increasing concentration of pectin solution or in the presence of an increasing concentration of heparin solution with 30 μg/mL pectin. The cells were then incubated with [^{125}I]FGF-1 [4 ng/mL, reduced with 10 mM dithiothreitol (DTT)] for another 30 min at room temperature. Later, the cells were washed with 1$\times$ PBS three times, and the remaining radioactivity was determined by γ-counter. The results were expressed as the remaining specific binding. Two experiments were performed with different preparations of radiolabeled FGF.

Also, after FGF binding to FGFR expressed on the surface of Sf-9 cells, the [^{125}I]FGF—FGFR complex was washed twice with 1$\times$ PBS and then chemically cross-linked by incubating the complex with 0.025 mM disuccinimidyl suberate (DSS) for 15 min at room temperature. Cells were centrifuged, and the pellet was washed with 1$\times$ PBS, mixed with sample buffer (100 mM Tris-HCl, 4% SDS, 10% glycerol, 0.05% bromophenol blue), and boiled for 5 min. The samples were separated on a 7.5% sodium dodecyl sulfate—polyacrylamide gel. Then the gel was stained for proteins with 0.25% Coomassie blue and destained with 10% acetic acid/45% methanol, dried, and finally autoradiographed by exposure to a Kodak X-omat film at −80 °C for 3 days.

Statistical Analysis. A completely randomized design was used. Three trees of each citrus species were randomly selected, and 18 fruits of uniform size were collected. Statistical

Table 1. Pectin Content and Chemical Composition in the Four Citrus Species[a,b]

citrus species	flavedo/ albedo	segment membrane
pectin content (% of fresh wt fruit component)		
Meyer lemon	1.91 ± 0.11 d	3.74 ± 0.06 b
Marsh White grapefruit	2.99 ± 0.02 c	3.62 ± 0.05 b
Dancy tangerine	5.29 ± 0.04 a	5.10 ± 0.25 a
Marrs orange	3.68 ± 0.10 b	3.69 ± 0.03 b
anhydrogalacturonic acid content (AGA, % of pectin wt)		
Meyer lemon	75.90 ± 4.29 ab	78.68 ± 1.31 a
Marsh White grapefruit	78.91 ± 1.87 a	75.41 ± 0.88 b
Dancy tangerine	67.29 ± 3.63 b	79.02 ± 0.21 a
Marrs orange	71.34 ± 3.33 ab	77.52 ± 0.31 ab
methoxyl content (% of pectin wt)		
Meyer lemon	4.34 ± 0.10 b	6.04 ± 0.04 a
Marsh White grapefruit	4.82 ± 0.07 a	5.73 ± 0.04 b
Dancy tangerine	4.66 ± 0.04 a	5.40 ± 0.06 c
Marrs orange	4.78 ± 0.06 a	4.39 ± 0.07 d
lower molecular weight portion (% of fraction volume)		
Meyer lemon	52.16 ± 2.11 b	19.20 ± 0.75 c
Marsh White grapefruit	78.18 ± 1.45 a	71.64 ± 3.72 a
Dancy tangerine	75.82 ± 2.85 a	66.74 ± 2.15 a
Marrs orange	34.24 ± 2.68 c	37.67 ± 1.45 b
total neutral sugar content (% of pectin wt)		
Meyer lemon	98.15 ± 3.89 b	53.89 ± 4.31 b
Marsh White grapefruit	73.35 ± 4.86 c	56.41 ± 2.36 ab
Dancy tangerine	133.46 ± 3.24 a	64.10 ± 3.77 a
Marrs orange	128.84 ± 5.58 a	63.18 ± 0.53 ab

[a] Data are given as the means of six fruits with three replications ± standard error. [b] Values for a variable followed by the same letter in a column are not significantly different at $P = 0.05$.

analysis of pectin chemical composition was performed with least-squares design (LSD) procedure. Statistical analysis was performed to determine the correlation between the inhibitory activity of pectin on FGF−receptor interaction and pectin structure (*34*).

RESULTS AND DISCUSSION

In an effort to investigate the potential biological activity of citrus pectin, we isolated pectins from four commercially cultivated citrus species and determined their chemical composition and structure. The biological activity was judged by the binding capacity of FGF to FGFR in the presence of pectin.

Pectin Content. The pectin contents (determined by the pectin extraction rate) of flavedo/albedo and segment membrane in lemon, grapefruit, tangerine, and orange are summarized in Table 1. On a fresh weight basis (*3*), the pectin content in flavedo/albedo varied significantly among the four citrus species. Dancy tangerine had the highest pectin content followed by Marrs orange and Marsh White grapefruit. In segment membrane, Dancy tangerine also contained the most pectin, which was significantly higher than the contents of the other three species. Results presented in Table 1 indicate that the segment membrane contains as much or more pectin, based on the percent fresh weight of the fruit component, than the flavedo/albedo. These results are consistent with values of pectin reported from other sources in the past (*35*, *36*). Previous studies have shown that consumption of pectin may have direct health benefits (*10*, *12*−*14*). Although further studies on the absorption of pectin by the cells is needed, our results seems to suggest that the consumption of citrus fruits along with the segment membrane, the edible part, is expected to result in higher intake of pectin and may have enhanced health benefits.

Chemical Composition of Pectin. *Anhydrogalacturonic Acid Content.* Our results show significant differences in the AGA contents among the four citrus species (Table 1). In general, the AGA content in segment membrane is similar to that in flavedo/albedo among the four citrus species, except for tangerines. In most of the previous studies, the AGA content was used as the measurement of pectin content (*3*). Because pectin consists of neutral sugars in addition to AGA, the results on AGA are reported as percent of pectin rather than fresh weight. The observed indirect relationship between AGA and pectin content (Table 1) may be due to the highly heterogeneous structure of pectin (*6*, *31*).

Methoxyl Content (MC). In flavedo/albedo, the pectin from Meyer lemon had a significantly lower content of methoxylated esters compared to the other three species (Table 1). However, in segment membrane, Meyer lemon contained the highest MC. In addition, the MC of pectin from segment membrane varied significantly among the four citrus species in contrast to the pectin from flavedo/albedo. Our MC values are consistent with results of previous authors (*37*). A direct relationship between the MC and the cholesterol-lowering effect of pectin is well documented (*17*). Results of this study also show a significant correlation between the MC of pectin and its ability to antagonize FGF signal transduction activity (described below).

Molecular Weight. Because of the extreme structural heterogeneity of pectin, it is difficult to accurately determine the molecular weight of pectin (*38*). This experiment was, therefore, designed just to find the percent of pectin with molecular weight <10 kDa (LMW pectin). Furthermore, only LMW pectin is suggested to be effective in the prevention of prostate cancer metastasis (*13*). Our results show that Marsh White grapefruit and Dancy tangerine, in both flavedo/albedo and segment membrane, contained significantly higher percentages of LMW pectin than Meyer lemon and Marrs orange. Similarly, the lowest percentage of LMW pectin was found in the segment membrane of lemon and the flavedo/albedo of orange (Table 1).

Total Neutral Sugar. In general, the total sugar content of pectin in flavedo/albedo was significantly higher than in segment membrane (Table 1, statistical data not presented). Pectin from Dancy tangerine contained the highest sugar content in both flavedo/albedo and segment membrane among the four species. Because the neutral sugar content influences the conformation of the pectin molecule (*2*, *38*), it is possible that the variation in the neutral sugar content will affect the biological function of pectin as well.

Individual Neutral Sugars. Six pectin neutral sugars including rhamnose, arabinose, xylose, mannose, galactose, and glucose were investigated (*6*, *32*). The content and relative ratio of individual sugars varied among the citrus species studied (Table 2). Also, in both flavedo/albedo and segment membrane, galactose was the most abundant neutral sugar (>45%) in all four citrus species (Table 2). The galactose content, therefore, appeared to be responsible for the variation of total neutral sugar content. Xylose and mannose were present in trace amounts in all species. In flavedo/albedo, the relative ratio of rhamnose varied significantly; nevertheless, the rhamnose contents were similar among the four species (Table 2). Because the presence of rhamnose interrupts the regularity of the linear structure of the AGA

Table 2. Individual Sugar Content[a] and Relative Ratio[b] of Pectin in Four Citrus Species[c,d]

citrus species	rhamnose	arabinose	xylose	mannose	galactose	glucose
			Flavedo/Albedo			
Meyer lemon	12.72 ± 0.65 a	16.89 ± 0.53 a	3.04 ± 0.37 a	5.13 ± 0.22 a	45.27 ± 1.66 b	15.10 ± 0.81 b
	(12.96 ± 0.65 B)	(17.22 ± 0.26 A)	(3.08 ± 0.27 A)	(5.23 ± 0.55 A)	(46.14 ± 0.35 C)	(15.37 ± 0.34 B)
Marsh White grapefruit	13.49 ± 1.11 a	8.92 ± 0.32 c	2.29 ± 0.12 b	2.75 ± 0.22 c	31.81 ± 2.84 b	8.08 ± 0.34 c
	(18.35 ± 0.32 A)	(12.21 ± 0.45 BC)	(3.13 ± 0.13 B)	(3.75 ± 0.05 C)	(51.49 ± 0.46 B)	(11.06 ± 0.31 C)
Dancy tangerine	12.84 ± 0.27 a	17.40 ± 0.11 a	2.15 ± 0.09 b	4.06 ± 0.16 b	69.98 ± 1.87 a	27.02 ± 0.82 a
	(9.63 ± 0.09 D)	(13.05 ± 0.25 B)	(1.61 ± 0.03 C)	(3.04 ± 0.05 D)	(52.43 ± 0.18 B)	(20.24 ± 0.31 A)
Marrs orange	14.59 ± 0.64 a	15.06 ± 0.88 b	3.24 ± 0.16 a	5.55 ± 0.19 b	76.36 ± 4.12 a	14.03 ± 0.64 b
	(11.32 ± 0.14 C)	(11.67 ± 0.18 C)	(2.52 ± 0.13 B)	(4.32 ± 0.05 B)	(59.22 ± 0.60 A)	(10.94 ± 0.77 C)
			Segment Membrane			
Meyer lemon	11.47 ± 0.82 ab	8.64 ± 0.34 c	2.12 ± 0.09 a	2.03 ± 0.05 b	24.63 ± 1.05 b	5.00 ± 2.50 a
	(21.36 ± 1.13 AB)	(16.15 ± 0.76 AB)	(3.97 ± 0.26 A)	(3.81 ± 0.28 A)	(46.03 ± 0.03 B)	(8.69 ± 0.16 A)
Marsh White grapefruit	12.88 ± 0.68 ab	6.96 ± 0.19 d	2.04 ± 0.05 a	2.25 ± 0.18 ab	28.00 ± 2.01 b	4.27 ± 0.76 a
	(23.02 ± 2.26 A)	(12.36 ± 0.22 C)	(3.63 ± 0.06 AB)	(4.00 ± 0.29 A)	(49.52 ± 1.54 B)	(7.48 ± 1.09 A)
Dancy tangerine	10.78 ± 0.54 b	9.67 ± 0.22 b	1.91 ± 0.02 a	1.35 ± 0.15 c	37.62 ± 2.54 a	2.77 ± 0.53 a
	(16.84 ± 0.43 B)	(15.15 ± 0.54 B)	(3.00 ± 0.20 B)	(2.15 ± 0.18 B)	(58.64 ± 0.45 A)	(4.27 ± 0.65 A)
Marrs orange	13.14 ± 0.69 a	10.80 ± 0.30 a	2.13 ± 0.21 a	2.74 ± 0.34 a	29.64 ± 1.16 b	4.74 ± 0.45 a
	(20.81 ± 1.22 AB)	(17.08 ± 0.44 A)	(3.36 ± 0.31 AB)	(4.34 ± 0.55 A)	(46.90 ± 1.55 B)	(7.52 ± 0.74 A)

[a] Values in the top row indicate sugar content (mg/g of pectin). [b] Values in parentheses indicate relative ratio (percent of total sugar). [c] Data are given as the means of six fruits with three replications ± standard error. [d] Means in a column followed by the same letter are not significantly different at $P = 0.05$.

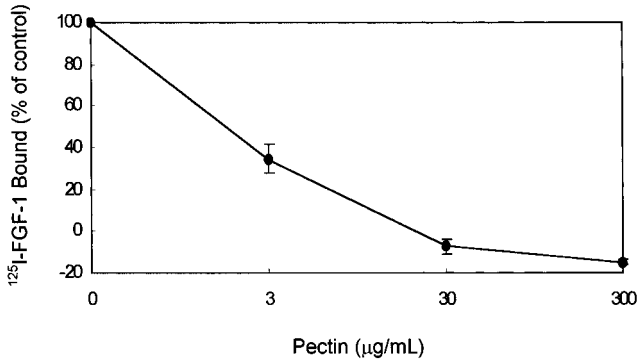

Figure 1. Effect of pectin concentration on FGF-1−FGFR1 binding. Sf9 cells expressing FGFR1 were incubated with [^{125}I]-FGF-1 in the presence of 0.1 μg/mL heparin with increasing concentrations of lemon (segment membrane) pectin. Vertical bars indicate standard error.

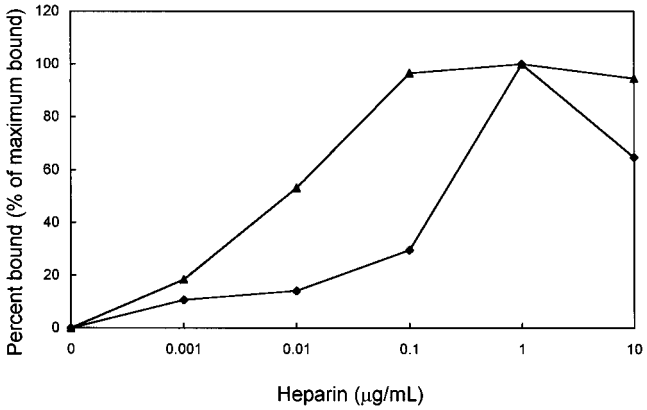

Figure 2. Inhibitory effect of pectin on FGF-1−FGFR1 binding in the presence of various concentrations of heparin. Sf9 cells expressing FGFR1 were incubated with [^{125}I]FGF-1 in the presence of increasing amounts of heparin with 30 μg/mL pectin from segment membrane of lemon (diamonds) or without pectin (triangles).

backbone, the percentage of rhamnose may play an important role in the structure and function of pectin (*2*).

In Vitro Effect of Pectin on FGF Signaling System. In this study, we have used the FGF signal transduction system as an in vitro model to explore the physiological role and mechanism of pectin in disease prevention. The effects of pectin were investigated by examining its ability to modulate binding of the radiolabeled FGF to its corresponding receptor. For the first time, our results demonstrated that citrus pectin inhibited the FGF-1 binding to FGFR1 by competing with heparin. Figure 1 clearly demonstrates that pectin prevented the binding of FGF-1 to FGFR1 in a dose-dependent manner. Lemon pectin (30 μg/mL, segment membrane) pectin completely inhibited the binding of FGF-1 to FGFR1 with an IC_{50} value of <3 μg/mL in the presence of 0.1 μg/mL heparin. The inhibitory effect of pectin on FGF-1−FGFR1 binding can be rescued by increasing the concentration of heparin; the optimal rescue occurred at heparin concentrations from 0.1 to 1 μg/mL when 30 μg/mL pectin was present (Figure 2). At the same time, a higher concentration (10 μg/mL) of heparin sequestrated FGF away from FGFR (Figure 2). Autoradiograph results of the FGF inhibition by pectin (Figure 3) were also consistent with the kinetic data presented in Figures 1 and 2. It is evident from Figure

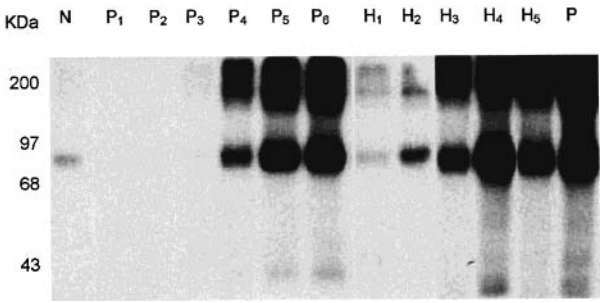

Figure 3. Inhibitory effect of pectin (from segment membrane of lemon) viewed by FGF−FGFR cross-linking and autoradiograpy. Lanes P_1−P_6 contained 0.1 μg/mL heparin with various concentrations of pectin (P_1, P_2, P_3, P_4, P_5, and P_6 contained 600, 300, 60, 30, 6, and 3 μg/mL pectin, respectively). Lanes H_1−H_5 contained 30 μg/mL pectin with various concentrations of heparin (H_1, H_2, H_3, H_4, and H_5 contained 0.001, 0.01, 0.1, 1, and 10 μg/mL heparin, respectively). Lanes N and P represent negative and positive controls, respectively. Details of the experiment are given in the text.

3 that the intensities of the bands (lanes P_1−P_6, Figure 3) were significantly decreased in the presence of pectin, reflecting the inhibition of FGF-1 binding to FGFR1.

The inhibitory activity of pectin isolated from different

Table 3. Inhibitory Activities of Citrus Pectins on FGF Signal Transduction[a,b]

citrus species	flavedo/albedo	segment membrane
Meyer lemon	31.14 ± 6.95 a	−7.10 ± 3.31 c
Marsh White grapefruit	38.25 ± 7.12 a	34.75 ± 0.95 a
Dancy tangerine	37.07 ± 6.20 a	17.83 ± 5.33 b
Marrs orange	36.64 ± 4.70 a	18.50 ± 5.02 b

[a] Data are given as the means of three replications ± standard error. Values represent FGF-1 bound (percent of control). An amount of 30 μg/mL of pectin from different citrus species was used. [b] Means in a column followed by the same letter are not significantly different at P = 0.05.

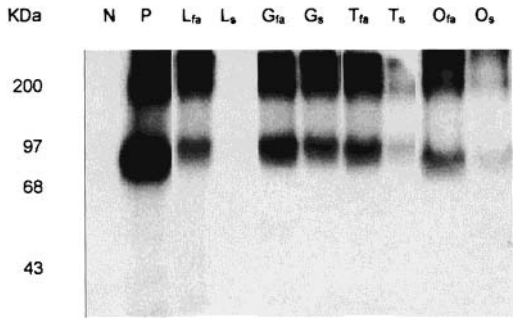

Figure 4. Inhibitory effect of pectin from various citrus species viewed by FGF−FGFR cross-linking and autoradiograpy. Samples in all lanes contained 0.1 μg/mL heparin and 30 μg/mL pectin from different sources (L, Meyer lemon; T, Dancy tangerine; O, Marrs orange; G, Marsh White grapefruit). The pectins from the flavedo/albedo and segment membrane were designated fa and s, respectively. Lanes N and P represent negative and positive controls, respectively. Details of the experiment are given in the text.

parts of four commercially cultivated citrus species was also investigated on the FGF signal transduction system. Interestingly, our results clearly show that pectin from all four species strongly but differentially inhibited the binding of FGF-1 to FGFR1 (Table 3). In general, the pectin from segment membrane showed a stronger inhibitory effect compared to pectin from flavedo/albedo. Whereas the inhibitory activities of pectins from segment membrane showed significant variation, no significant difference was observed in the pectin from flavedo/albedo in all citrus species. In addition, pectin from the segment membrane of lemon appears to have the highest inhibitory activity followed by those from tangerine, orange, and grapefruit. The inhibitory activities of pectin of the four citrus species were further confirmed by autoradiography (Figure 4).

The inhibitory effect of pectin could be explained on the basis of our working conformation model (*18*): a specific heparin chain must interact simultaneously with FGF and FGFR for the conformational activation of the FGF−FGFR signaling complex. Although pectin is seldom sulfated (*1*), like other nonsulfated polysaccharides, pectin may bind to FGF−FGFR by ionic interaction and/or hydrogen bond through carboxyl and hydroxyl groups, respectively. This interaction may only occur directly with FGF-1 or FGFR1, but not both at the same location on a pectin chain, thus exert inhibitory activity. It is shown that heparin-derived unsulfated di- or trisaccharide interferes with FGF activity (*18*). This interaction is possible due to the fact that heparin and pectin share certain structural characteristics such as monosaccharide composition (*25*) and possibly some other substitute groups.

Our studies may thus explain the mechanism of certain biological activities of pectin such as the cholesterol-lowering effect. We have recently reported the involvement of FGF signal transduction system in cholesterol metabolism: FGFR deficiency caused a significant elevation of the excreted bile acid pools (*39*). The observed inhibitory effects of pectin on the FGF−FGFR interaction in this study may indicate the possible involvement of pectin in cholesterol metabolism. It is therefore tempting to speculate that pectin could exert a cholesterol-lowering effect by interfering with FGF−FGFR interaction.

Table 4. Correlation between the Structure of Pectin and Inhibitory Effects on FGF Signal Transduction[a]

	AGA	MC	TNS	LMW	INH
AGA		0.2785	−0.6537*	−0.0752	−0.2666
MC			−0.5559*	−0.0447	−0.4148*
TNS				0.0625	0.4248*
LMW					0.5463*
INH					

[a] Significant correlation at $P = 0.05$ (value is the Pearson correlation oefficient). TNS, total neutral sugar; INH, inhibitory activity of pectin in the FGF signal transduction system.

Our data, for the first time, show that pectin from lemon is the most effective agent inhibiting the FGF−FGFR interaction compared to pectin from the other three citrus species (Table 3 and Figure 4). On the basis of these results of the in vitro inhibitory effect of pectin on FGF−FGFR interaction, lemon may provide the best beneficial effect in preventing diseases. However, these findings need further studies before any conclusions on the beneficiary effects of pectin from lemon or any other source are drawn.

Structure—Function Relationship. Because the structure of pectin is closely related to its application in the food industry (*2*), a statistical analysis was performed to explore the correlation between the structure of pectins and their inhibitory activities on the FGF−FGFR interaction. Our analysis indicated that the inhibitory activity of pectin was significantly correlated with MC, total neutral sugar content, and percent of LMW pectin (Table 4). The correlation study data suggest that a higher inhibitory activity of pectin may be attained with higher MC, lower neutral sugar content, and lower proportion of LMW pectin. Higher neutral sugar content of pectin may prevent the interaction of pectin to the specific binding domain on pectin with FGF−FGFR.

Furthermore, we analyzed the relationship between inhibitory activities of pectins and their neutral sugar composition. Correlation data suggest that the inhibitory activity of pectin is significantly related to the galactose content (data not shown). Because galactose is the major neutral sugar constituent of pectin (Table 2), the variation in galactose content could be responsible for the observed correlation. In addition, the lower relative ratio of rhamnose in pectin seems to be related with the higher inhibitory activity (data not shown). This may be due to the fact that rhamnose plays a critical role in defining the three-dimensional structure of pectin (*2*), therefore enhancing its biological activity.

The FGF system is a ubiquitous mediator of development and homeostasis in the adult, intrinsic to tissues that are perturbed in diverse ways either positively or negatively (*18*). Chronic perturbation may underlie diverse pathologies that include developmental disorders and disease in adult tissues (*18*). The most common perturbation of the FGF complex occurs by disruption of the specific relationship between the FGF, FGFR

kinase, and pericellular matrix heparan sulfate. Thus, a wide variety of agents structurally similar to heparan sulfate are capable of mimicking it and impacting the FGFR complex. Our results serve to show that pectin can be a source of activity that impacts the assembly and activity of the complex in vitro (Figures 1−4 and Table 4*)*. Pectin is composed of heterogeneous polysaccharides. The hypothesis upon which our study is built is that within the bulk of the pectin molecule is a relatively specific bioactivity that impacts the FGF signaling system. The precise structural nature of the specific sequence motif within a chain of heparin/heparan sulfate involved in a specific combination of FGF and FGFR is unclear. Similar to pectin, heparin/heparan sulfate is ill-defined and extremely heterogeneous with respect to chain length, monosaccharide composition, and sulfation. However, there is increasing evidence that the active motif for the FGFR complex in heparin/heparan sulfate is rare and has a very specific structure (unpublished results). Thus, the active component within the bulk fraction called pectin that mimics this activity may also be rare and unique. It may be a minor component that is not precisely "pectin" that is hidden away in the preparation or a breakdown and modified fragment from a longer chain pectin molecule. The study by Pienta and co-workers (*13*) on the inhibition of metastasis in a rat prostate cancer by modified citrus pectin provides strong evidence that the modified citrus pectin (MW 10 kDa) is indeed absorbed by the cells. Although pectin is believed to be metabolized to short-chain fatty acid in intestine, we hypothesize that the candidates for bioactivity from pectin that have potential to impact in vivo action are twofold: (1) high molecular weight pectin with the active motif that acts at the gut−intestinal uptake interface, either directly or indirectly on the FGFR complex and/or (2) metabolic modification in the gut, gut−intestine interface, or circulatory system, either fragmentation or charge generation on the fragments. In addition, there is a different pattern of expression of FGF−FGFR in the colon between normal tissues and adenoma and malignancy (*40*). It is, therefore, not inconceivable that pectin may impact FGF signaling at least within the colon, which may not require a complete absorption of the pectin. However, further in vivo studies are needed to establish the specific role of pectin in preventing disease related to the FGF system.

ABBREVIATIONS USED

AGA, anhydrogalacturonic acid; BSA, bovine serum albumin; FBS, fetal bovine serum; FGF-1, fibroblast growth factor 1; FGFR1, fibroblast growth factor receptor 1; LMW, lower molecular weight; MC, methoxyl content; MCP, modified citrus pectin; PBS, phosphate-buffered saline.

ACKNOWLEDGMENT

We thank Dr. Fen Wang and Dr. Mikio Kan for providing FGFR1 complementary DNA and iodized FGF-1, respectively. We also thank Dr. Marshall Fishman, Dr. Arland Hothkiss, Dr. Nancy Turner, and Jessica Sanchez for critical comments and reviewing the manuscript.

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Received for review August 15, 2000. Revised manuscript received January 4, 2001. Accepted April 16, 2001.

JF001020N