

In Vitro Inhibition of Digestive Enzymes by Indigestible Polysaccharides

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ABSTRACT

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The influence of indigestible polysaccharides on the activities of digestive enzymes was studied. Trypsin was considerably inhibited after in vitro incubation with xylan, apple pectin, or buckwheat hemicellulose. Xylan, sodium alginate, and yeast mannan exerted a pronounced in vitro inhibitory effect on the activity of α -chymotrypsin. Apple pectin and carboxymethyl cellulose sodium salt had potent inhibitory activity against

α -amylase, and apple pectin alone towards pepsin. Agar-agar caused a significant loss of steapsin activity. Kinetic analyses showed that pectin and xylan were parabolic noncompetitive inhibitors against trypsin. Pectin was also shown to combine easily with the substrate protein under slightly alkaline conditions. These results showed that the inhibition of trypsin activity by pectin and xylan resulted from their binding with the substrates.

Interest in dietary fiber and its physiological effects is growing rapidly. Primary interest has focused on the beneficial effects in the prevention of certain gastrointestinal and vascular diseases (Ory and Mod 1981, Roth and Mehlman 1978). Although some of these beneficial effects are not unequivocally accepted, dietary fiber preparations and fiber-enriched foods are available, and their consumption by the health-conscious public is increasing. In view of this development, information on possibly hazardous effects of dietary fiber is urgently needed. Possible interference with the digestion and absorption of essential nutrients is one area of uncertainty. Several studies with rats on high-fiber diets have shown a significant increase in fecal nitrogen excretion (Harmuth-Hoene and Schwerdtfeger 1979, Shah et al 1982, Viola et al 1970). Increasing the amount of fiber in the diet of humans has also been shown to reduce apparent digestibility of nitrogen (Kelsay et al 1978). The activities of digestive enzymes appear to be affected by dietary fiber, perhaps leading to an effect on digestion and absorption in the gastrointestinal tract. Schneeman (1978) has indicated that several dietary fiber sources have inhibitory capacities towards some digestive enzymes. Rats fed diets containing high levels of fiber have lower levels of intestinal proteolytic enzymes (Schneeman and Gallaher 1980). These studies suggest that dietary fiber is not innocuous in the alimentary tract, but may reduce the availability of dietary proteins.

The present study was conducted to study in vitro inhibitory activities of various indigestible polysaccharides, major components of dietary fiber, against digestive enzymes, and to clarify the mechanism of the inhibitions.

MATERIALS AND METHODS

Materials

Ten different types of indigestible polysaccharides were examined. Agar-agar, cellulose powder (approximately 99% pure), carboxymethyl cellulose (CMC) sodium salt, sodium alginate (500 cp), xylan, inulin, and yeast mannan were purchased from Nakarai Chemicals Co. (Japan), and two pectins (from apple and lemon) from Wako Chemicals Co. (Japan). Water-soluble hemicellulose from buckwheat flour was prepared by the modified procedure of Cartaño and Juliano (1970) (Ikeda et al 1980). Digestive enzymes used were obtained from Sigma Chemicals Co.: trypsin ([EC 3.4.21.4], $2 \times$ cryst., from bovine pancreas, 12,000 BAEE units per milligram of protein); α -chymotrypsin ([EC 3.4.21.1], $3 \times$ cryst., from bovine pancreas, 49 BTEE units per milligram of protein); pepsin ([EC 3.4.23.1], $2 \times$ cryst., from hog stomach mucosa, 3,050 units per milligram of protein); steapsin ([EC 3.1.1.3], from porcine pancreas, 53 units per milligram of protein); and α -amylase ([EC 3.2.1.1], from *Bacillus subtilis*, 73 units per milligram of solids). The substrates for enzymes were obtained from the following companies: N^{α} -benzoyl-D, L-arginine *p*-nitroanilide (BAPNA), from Boehringer Mannheim Co.; benzoyl-L-tyrosine *p*-

nitroanilide (BTpNA), from Nakarai Chemicals Co.; Hammarstein's casein, from E. Merck AG; and hemoglobin, from Sigma Chemicals Co. Sephadex G-100 was a product of Pharmacia Fine Chemicals. All other chemicals used were of analytical grade.

Determination of In Vitro Inhibitory Activity Towards Digestive Enzymes

The digestive enzymes examined were trypsin, α -chymotrypsin, pepsin, α -amylase, and steapsin. Enzyme reactions were performed in the following buffer solutions: trypsin or α -chymotrypsin, in 58 mM Tris-HCl buffer (pH 7.6); pepsin, in 0.13M HCl-KCl buffer (pH 1.6); α -amylase, in 20 mM sodium phosphate buffer (pH 6.9) containing 10 mM NaCl; and steapsin, 0.11M Tris-HCl buffer (pH 7.6) containing 0.5 mM deoxycholate and 1 mM calcium acetate. The hydrolytic activity of trypsin with BAPNA was determined according to the procedure of Erlanger et al (1961), and its proteolytic activity with casein and hemoglobin as substrates according to the procedure of Laskowski (1955). The activities of α -chymotrypsin and pepsin were assayed by the procedures described by Rick (1974) and Anson (1939), respectively. α -Amylase activity was measured colorimetrically with 3,5-dinitrosalicylic acid (Rick and Stegbauer 1974). The activity of steapsin against olive oil as substrate was determined colorimetrically with diethylthiocarbamate (Näher 1974). Trypsin and α -chymotrypsin were dissolved in 10^{-3} M HCl at 48 μ g/ml and 100 μ g/ml, respectively. Other enzymes were dissolved in their respective buffer solutions at the following concentration: pepsin, 100 μ g/ml; α -amylase, 25 μ g/ml; and steapsin, 50 μ g/ml. Indigestible polysaccharides were dissolved or suspended at 0-5 mg/0.5 ml in each buffer solution used in the enzyme reaction. The inhibitory activity of indigestible polysaccharides against enzymes was determined by the same procedure used in the detection of proteinaceous protease inhibitors described previously (Ikeda and Kusano 1978): 0.5 ml of the fiber-containing solution or suspension was preincubated at 37°C for 10 min with both 0.5 ml of enzyme solutions and 1.0 ml of their respective buffers, and the remaining activity was then determined. The control mixture was prepared by replacing the fiber solution or suspension with an appropriate buffer solution. Galacturonic acid and glucuronic acid, principal constituents of certain indigestible polysaccharides including pectin and alginic acid, were also examined for their inhibitory activities against digestive enzymes.

Determination of Total Uronic Acid

Total uronic acid was measured by the carbazole-sulfuric acid method (Dische 1950).

RESULTS

In Vitro Effects of Indigestible Polysaccharides on the Activities of Digestive Enzymes

All the polysaccharides examined here, except for inulin, exhibited inhibitory activity against trypsin (Fig. 1). It is noticeable

that galacturonic acid, a major component of pectin, shows an inhibitory activity against the enzyme, whereas glucuronic acid exhibits less or substantially no inhibitory activity.

As with trypsin, all the polysaccharides examined, especially xylan, sodium alginate, and yeast mannan, exhibited a pronounced inhibitory effect on the activity of α -chymotrypsin (Table I). Both pectins (from apple and lemon) showed relatively high inhibitory activity towards pepsin. Apple pectin and CMC sodium salt exhibited significant inhibitory capacity against α -amylase. Agar-agar was found to be an inhibitor against steapsin. Several polysaccharides were shown to accelerate steapsin activity (Table I).

In Vitro Inhibition of Trypsin and α -Chymotrypsin by Pectin and Xylan

Pectin and xylan were found to inhibit the activity of trypsin, not only with the synthetic chromogenic substrate but also with protein substrates (hemoglobin and casein) (Table II). On the other hand, these polysaccharides exhibited potent inhibitory activity against

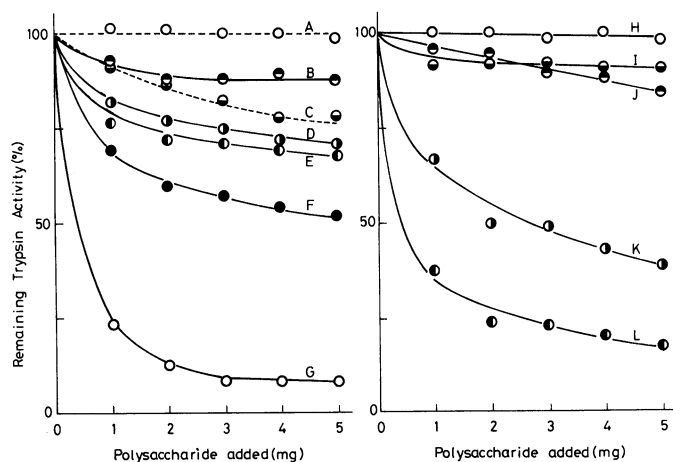


Fig. 1. Effects of various indigestible polysaccharides on the activity of trypsin. The reaction mixture contained 24 μ g of trypsin, 0–5 mg of the polysaccharides, 1.74 mg of BApNA, and 0.1 μ mol of Ca^{2+} in a total volume of 3.0 ml. The enzyme reaction was performed at 35°C for 15 min and stopped by the addition of 1.0 ml of 20% acetic acid. A, glucuronic acid; B, carboxymethyl-cellulose sodium salt; C, galacturonic acid; D, lemon pectin; E, sodium alginate; F, apple pectin; G, buckwheat water-soluble hemicellulose; H, inulin; I, cellulose powder; J, yeast mannan; K, agar-agar; and L, xylan.

TABLE I
Effects of Various Indigestible Polysaccharides^a
on the Activities of Digestive Enzymes

Substrate	Remaining Enzyme Activity (%)					
	α -Chymo-		Pepsin	α -Amylase		Steapsin
	Trypsin	trypsin		Soluble	Olive	
	BApNA	BTpNA	Casein	Starch	Oil	
Pectin						
Lemon	71.9	74.7	67.0	64.8	100	
Apple	52.4	72.8	42.6	50.7	>100	
Sodium alginate	68.5	54.4	>100	67.5	>100	
Yeast mannan	84.9	56.0	86.5	77.4	>100	
Carboxymethyl cellulose sodium salt	88.2	57.7	74.3	57.1	93.1	
Agar-agar	38.3	59.5	84.0	80.2	52.8	
Inulin	98.0	66.9	88.3	86.7	97.7	
Xylan	23.0	46.5	82.8	76.7	73.6	
Cellulose	90.6	57.1	81.1	78.9	>100	
Galacturonic acid	78.2	48.5	90.7	63.4	100	
Glucuronic acid	99.2	53.9	81.7	100	100	

^a Five milligrams of the indicated polysaccharides or uronic acids was added in each reaction mixture, and their inhibitory activities against the enzymes were assayed.

α -chymotrypsin in synthetic chromogenic substrate, but not in protein substrate.

The inhibition of trypsin activity by pectin and xylan was estimated with a wide range of the substrate concentration. Lineweaver-Burk plots showed that these polysaccharides were parabolic noncompetitive inhibitors against the enzyme (Fig. 2).

In Vitro Binding of Pectin Towards Hemoglobin

Figure 3 shows the gel filtration chromatographic pattern of hemoglobin incubated with pectin at pH 7.6. Hemoglobin emerged together with pectin, indicating that hemoglobin was able to combine with the polysaccharide under the assay conditions for trypsin and α -chymotrypsin activity.

DISCUSSION

Several indigestible polysaccharides were shown to possess significant inhibitory in vitro activity towards the digestive enzymes (Fig. 1, Table I). Pectins (from apple and lemon) and xylan were potent inhibitors against trypsin and α -chymotrypsin under the conditions of our test. The galacturonic acid content of the two pectins from apple and lemon constituted 67.2 and 59.6% (dry weight), respectively. In comparison to lemon pectin, the higher inhibitory activity of apple pectin appears to be ascribed to the constituent sugars other than galacturonic acid.

The in vitro inhibition of trypsin activity by pectin and xylan was

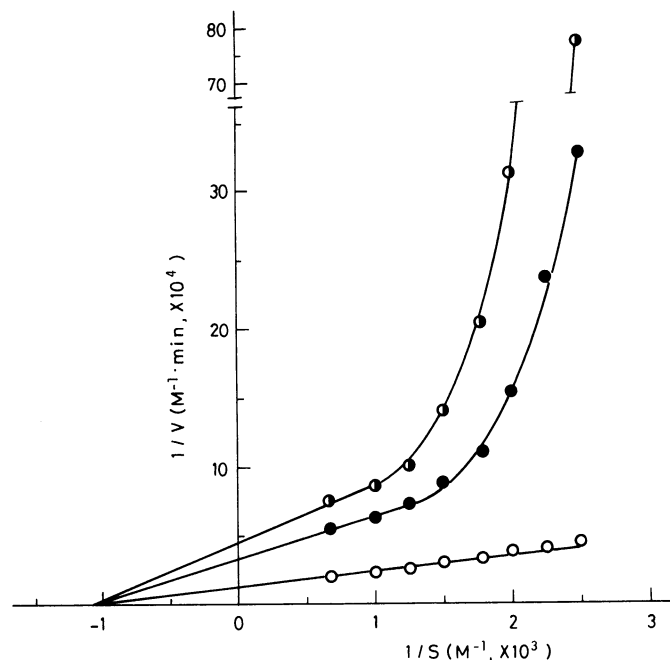


Fig. 2. Parabolic noncompetitive inhibition of trypsin activity by xylan and pectin. The control reaction mixture contained 24 μ g of trypsin, the indicated concentration of BApNA and 0.1 μ mol of Ca^{2+} in a total volume of 3.0 ml. The detailed conditions for inhibitory activity assay are essentially the same as described in Fig. 1. —○— = Without the polysaccharides; —●— = with pectin (20 mg); and —●— = with xylan (10 mg).

TABLE II
Effects of Pectin and Xylan on the Activities
of Trypsin and α -Chymotrypsin

Substrate	Trypsin (%)			α -Chymotrypsin (%)	
	BApNA	Hemoglobin	Casein	BTpNA	Casein
None	100	100	100	100	100
Pectin					
(from apple)	60.5	37.9	60.9	72.8	100
Xylan	23.7	53.4	73.6	46.5	100

found not only with the synthetic chromogenic substrate but also with protein substrates (Table II). Kinetic analyses showed that the inhibition of trypsin activity by these polysaccharides conformed with a parabolic noncompetitive type (Fig. 2). Kanaya et al (1976) have demonstrated that phytate from rice bran, as it combines with the substrate protein, renders the protein poorly available for pectic action, and that phytate is a parabolic competitive inhibitor against pepsin. A similar relationship was exhibited between carrageenin and pepsin (Houck et al 1960, Vaughan et al 1962). Hence, the observed trypsin inhibition by the indigestible polysaccharides may result from their binding with the substrate. Pectin combined with hemoglobin under slightly alkaline conditions (Fig. 3). Conversely, it is well known that calcium ion stabilizes the activity of trypsin (Walsh 1970). The inhibition of trypsin activity by pectin and xylan became significant as the concentration of Ca^{2+} in the enzymic reaction mixture was decreased, but became less significant with an increased concentration of Ca^{2+} . These results suggest that the inhibition of trypsin activity by the indigestible polysaccharides may result from their binding with the substrates. Added Ca^{2+} to the assay mixture appears to combine with the polysaccharides under the conditions employed, thus leading to a decrease in the binding capacity of the polysaccharides towards other cationic species, ie, the substrates.

Evidence has accumulated indicating that dietary fiber exerts a considerable influence upon the activities of digestive enzymes, especially of proteolytic enzymes, in the gastrointestinal tract. A significant increase in fecal nitrogen was found in rats on diets containing large amounts of dietary fiber (Harmuth-Hoene and Schwerdtfeger 1979, Shah et al 1982, Viola et al 1970). Studies with

¹ Unpublished data.

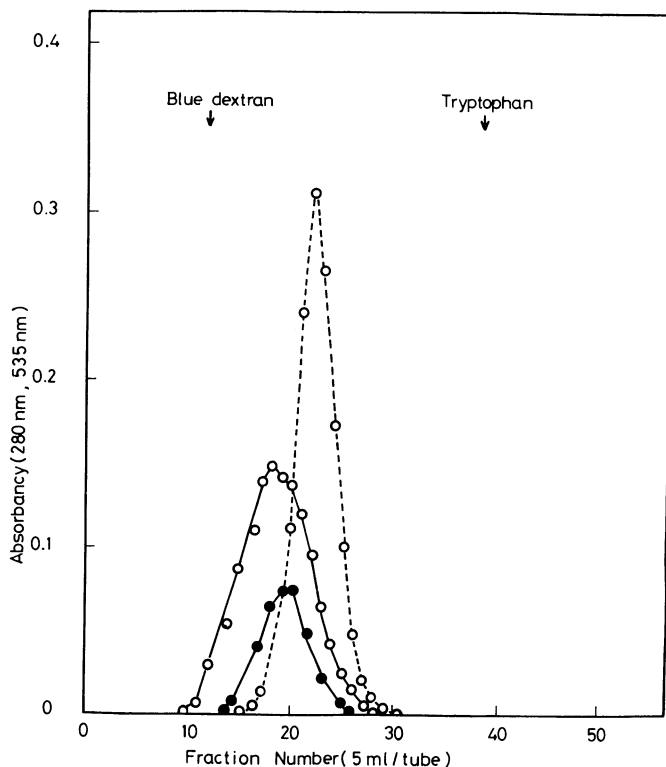


Fig. 3. Gel filtration patterns of hemoglobin incubated with or without pectin on a Sephadex G-100 column. Five milligrams of hemoglobin was dissolved in 3 ml of 0.1M Tris-HCl buffer (pH 7.6). Pectin solution (10 mg/3 ml of the above buffer) was then added to the protein solution. The mixture was incubated at 37°C for 30 min. Aliquots of the incubate were applied on the column (90 × 1.8φ cm), which was previously equilibrated with the same buffer. Protein was measured by monitoring at 280 nm, and pectin was determined by monitoring at 535 nm after incubation with carbazole. ---○--- = A₂₈₀ in case of hemoglobin alone; —○— = A₂₈₀ in case of hemoglobin incubated with pectin; —●— = A₅₃₅ in case of hemoglobin incubated with pectin.

human subjects demonstrated that high-fiber diets caused a significant decrease in apparent protein digestibility (Kelsay et al 1978). Several food gums have been shown to substantially lower the in vitro digestibility of casein (Acton et al 1982). The inhibitory effect of rice bran and alfalfa against trypsin has been reported (Schneeman 1978), but the inhibitory mechanism has not been elucidated.

The in vitro inhibition of proteolytic enzymes by indigestible polysaccharides reported here generally agrees with in vivo studies, which show decreases in apparent protein digestibility on high-fiber diets. However, the findings of the present study may not apply directly to a human situation, where there is a continual supply of new digestive enzymes entering the gastrointestinal tract. Within the pancreatic tissue of rats, however, the significant elevation of activities of digestive enzymes, including proteolytic enzymes, was found when wheat bran was added to the diets (Sheard and Schneeman 1980). Increased excretion of endogenous fecal nitrogen has been shown to occur when rats are fed fiber without protein (Shah et al 1982). Although the mechanism by which pancreatic enzyme adaptation to diet composition is not clearly understood, dietary fiber appears to affect the excretion of digestive enzymes, especially proteolytic enzymes, through its inhibitory capacity against the enzymes. Further characterization of dietary fiber may give more definite answers to these questions.

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