

# Inhibition of lipase activities by basic polysaccharide

Takahiro Tsujita,<sup>1,\*</sup> Hiroe Takaichi,<sup>\*</sup> Takeshi Takaku,<sup>\*</sup> Toshiya Sawai,<sup>†</sup> Naoyuki Yoshida,<sup>†</sup> and Jun Hiraki<sup>†</sup>

Bioscience,<sup>\*</sup> Integrated Center for Sciences, Ehime University, Shitsukawa, Toon, Ehime 791-0295, Japan; and Research and Development Section,<sup>†</sup> Chisso Corporation, Kachidoki-3, Chuo-ku, Tokyo 104-8555, Japan

**Abstract** Basic polysaccharide strongly inhibited the hydrolysis of trioleoylglycerol (TO) emulsified with phosphatidylcholine and taurocholate by either pancreatic lipase or carboxylester lipase. DEAE-Sephadex dose-dependently inhibited the hydrolysis of TO by pancreatic lipase and carboxylester lipase; however, carboxymethyl-Sephadex and Sephadex G-50 did not inhibit the hydrolysis. Polydextrose (PD), a soluble polysaccharide, was a very weak inhibitor of pancreatic lipase. However, when a basic group, a DEAE group, was attached to PD, lipase inhibition by DEAE-PD was increased, and this was dependent on the substitution ratio of DEAE groups. The number of positive charges per PD molecule is important in lipase inhibition. Similar substitution effects were observed with other basic groups, such as piperidinoethyl and 3-triethylamino-2-hydroxypropyl. The natural basic polysaccharide, chitosan, also inhibited pancreatic lipase activity. Gel-filtration experiments suggested that DEAE-PD did not bind strongly to pancreatic lipase. The effect of DEAE-PD on TO hydrolysis by pancreatic lipase was studied using various emulsifiers: DEAE-PD (50  $\mu\text{g}/\text{ml}$ ) did not inhibit the hydrolysis of TO emulsified with arabic gum, phosphatidylserine, or phosphatidic acid. In vivo, oral administration of DEAE-PD to rats reduced the peak plasma triacylglycerol concentration and increased fecal lipid excretion. These results suggest that basic polysaccharide is able to suppress dietary fat absorption from the small intestine by inhibiting pancreatic lipase activity.—Tsujita, T., H. Takaichi, T. Takaku, T. Sawai, N. Yoshida, and J. Hiraki. **Inhibition of lipase activities by basic polysaccharide.** *J. Lipid Res.* 2007. 48: 358–365.

**Supplementary key words** polydextrose • pancreatic lipase • inhibitor

It is well known that dietary fat is not absorbed directly from the intestine unless it has been subjected to the action of lipases. The two main products formed by the hydrolysis of lipases are fatty acid and 2-monoacylglycerol. These lipolytic products are dispersed as mixed micelles containing bile salts and biliary phosphatidylcholine (PC) and carried to the site of fat absorption. Lipid absorption

takes place in the apical part of the plasma membrane of epithelial cells or enterocytes lining the gut. Dietary fiber affects lipid metabolism in a variable manner: certain dietary fibers inhibit gastrointestinal lipase reactions, such as pancreatic lipase. Lairon and colleagues (1, 2) reported that wheat bran and wheat germ inhibited pancreatic lipase and decreased intestinal absorption of dietary fats and cholesterol. We previously reported that citrus pectin strongly inhibited the activity of digestive lipases (3). These results suggest that some dietary fiber prevents the hydrolysis of dietary fat in the small intestine, reduces subsequent intestinal absorption of dietary fat, and enhances fat excretion in feces. The highly viscous dietary fibers delay the adsorption of cholesterol and triacylglycerol, and this is evaluated by the decrease in plasma lipid concentration or the increase in fecal lipid output. Ebihara and Schneeman (4) reported that viscose and soluble dietary fibers such as guar gum and konjac mannan might bind bile acids, reducing their recycling through the enterohepatic circulation and delaying the absorption of cholesterol and triacylglycerols. Vahouny et al. (5) reported that several dietary fibers bound fatty acids and monoolein from mixed micelles containing bile acids and PC. The dietary fibers have various binding powers; cellulose was reported to be weak and guar gum was reported to be strong. Ogata et al. (6) reported that polydextrose (PD) retarded the transport of trioleoylglycerol (TO) and cholesterol into the mesenteric lymph. Here, we report that dietary fiber becomes a strong inhibitor of lipase when a basic group is introduced. Experiments were undertaken to elucidate the mode of inhibition of pancreatic lipase by the basic dietary fiber.

Abbreviations: ABZ, aminobenzoyl; CM, carboxymethyl; PA, phosphatidic acid; PC, phosphatidylcholine; PD, polydextrose; PE, phosphatidylethanolamine; PIPE, piperidinoethyl; PS, phosphatidylserine; ST, cornstarch; TEAP, 3-triethylamino-2-hydroxypropyl; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; TO, trioleoylglycerol; <sup>14</sup>C-TO, glycerol tri[1-<sup>14</sup>C]oleate.

<sup>†</sup>To whom correspondence should be addressed.

e-mail: tsujita@m.ehime-u.ac.jp

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## MATERIALS AND METHODS

### Reagents

Enzyme substrates and reagents used were obtained as follows. Taurocholate, TO, and colipase were from Sigma (St. Louis, MO). PCs (from soybean and egg) were from Nippon Shoji (Tokyo, Japan). Phosphatidylethanolamine (PE; from egg), phosphatidylserine (PS; from bovine brain), and phosphatidic acid (PA; from egg PC) were from Serdary Research Laboratories (London, Canada). PD was from Wako Pure Chemical Industries (Osaka, Japan). Sephadex G-50, DEAE-Sephadex, and carboxymethyl (CM)-Sephadex were from Amersham Biosciences KK (Tokyo, Japan). Chitosan was from Kimitsu Chemical Industries (Tokyo, Japan). Glycerol tri[1-<sup>14</sup>C]oleate (<sup>14</sup>C-TO; 1.85 MBq) was from GE Healthcare Bio-Sciences KK (Tokyo, Japan).

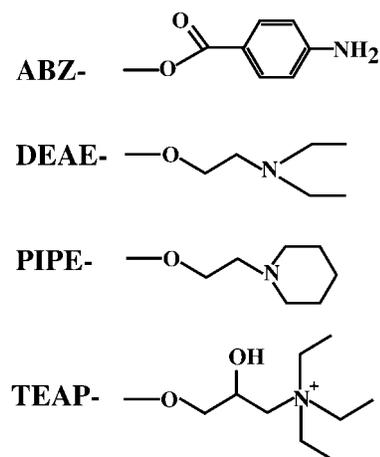
Pancreatic lipase was purified from rat pancreas by the procedure of Gidez (7) with some modifications. Activity during purification was monitored using the PC-TO emulsion described below under "Enzyme activity assays." The purified enzyme (3,200 U/mg protein, pH 6.8) gave a single band by SDS-PAGE, from which its molecular mass was estimated to be 49,000 Da. Carboxylester lipase was purified from porcine pancreas by the procedure of Rudd, Mizuno, and Brockman (8) with some modifications (9). The purified enzyme preparations were found to have specific activities of 700–800  $\mu\text{mol } p\text{-nitrophenol released/mg protein/min}$ , with *p*-nitrophenyl butyrate as the substrate.

### Preparation of basic polysaccharide

DEAE-PD and piperidinoethyl (PIPE)-PD were prepared by the procedure of Peterson and Sober (10) with some modifications. To prepare the basic PDs, which had various degrees of substitution of the basic group, the ratio of the basic group [diethylaminoethyl chloride hydrochloride for DEAE-PD and 1-(2-chloroethyl) piperidine hydrochloride for PIPE-PD] and PD in the reaction mixture was changed. Aminobenzoyl (ABZ)-PD was prepared as follows. PD (6 g) was dissolved in 20 ml of dimethylformamide at 40°C, and triethylamine (5 g) was added. A solution of *p*-nitrobenzyl chloride (13.52 g) in dimethylformamide (16 ml) was added dropwise to the mixture, and triethylamine (4.5 g) was then added in three portions (at 50 min, 3 h, and 5 h). Total reaction time was 6 h at 40°C.

The substitution degree of the basic group in basic PD was determined using <sup>1</sup>H nuclear magnetic resonance spectra [JNMGSX400 (400 MHz); JEOL, Ltd., Tokyo Japan]. The basic PD (4 mg) was dissolved in heavy water (800 mg), and the area ratios of the low magnetic field proton (2.4–5.5 ppm) and the high magnetic field proton (0.5–1.5 ppm) were calculated. The substitution degree of the basic group was expressed as the number of basic groups per glucose unit in PD.

3-Triethylamino-2-hydroxypropyl-cornstarch (TEAP-ST) was prepared as follows. Triethylglycidyl ammonium chloride was synthesized by incubation of triethylamine (20.2 g) with epichlorohydrin (18.5 g) in 100 ml of water at room temperature for 5 h. ST (8.1 g) and NaCl (1 g) were dissolved in water (25 ml), triethylglycidyl ammonium chloride (19.6 g) and 10% NaOH solution (2.8 ml) were then added, and the reaction mixture was stirred for 2.5 h at room temperature. The reaction mixture was then incubated for 68 h at 35°C. To prepare TEAP-ST with various degrees of substitution, the ratio of triethylglycidyl ammonium chloride and ST in the reaction mixture was changed. The substitution degree of the basic group in TEAP-ST was determined by the titration method. TEAP-ST (1.0 g) was resuspended in 1 mol/l HCl (50.0 ml), and the mixture was left overnight. Ten milliliters of the upper portion was titrated by 1 mol/l NaOH. **Figure 1** shows the chemical structures of the basic groups.



**Fig. 1.** Chemical structures of basic groups. ABZ, aminobenzoyl; PIPE, piperidinoethyl; TEAP, 3-triethylamino-2-hydroxypropyl.

### Enzyme activity assays

Pancreatic lipase activity was determined by measuring the rate of release of oleic acid from TO. A suspension of 90  $\mu\text{mol}$  of TO, 12.6  $\mu\text{mol}$  of soybean PC, and 9.45  $\mu\text{mol}$  of taurocholate in 9 ml of 100 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), pH 7.0, containing 0.1 M NaCl was sonicated for 5 min (TO-PC emulsion). The assay system was composed of the following components in a total volume of 200  $\mu\text{l}$ : 50  $\mu\text{l}$  of enzyme (0.998  $\mu\text{mol}$ ), 50  $\mu\text{l}$  of inhibitor solution, 0.25  $\mu\text{g}$  of colipase, 0.5  $\mu\text{mol}$  of TO, 0.053  $\mu\text{mol}$  of taurocholate, 0.07  $\mu\text{mol}$  of phospholipid, 10  $\mu\text{mol}$  of TES, and 20  $\mu\text{mol}$  of NaCl. Incubation was performed at pH 7.0 and 37°C for 30 min. The amount of oleic acid produced was determined by the method of Zapf et al. (11) with slight modification (12). Carboxylester lipase activity was determined using the same assay without colipase.

In some experiments, pancreatic lipase activity against TO was determined using the assay described above except that 12.6  $\mu\text{mol}$  of soybean PC was replaced with other phospholipids or arabic gum (45 mg).

### Sephacryl S-200 gel filtration

Purified pancreatic lipase was incubated with TO-PC emulsion containing DEAE-PD. The incubation system was composed of the following components in a total volume of 3 ml: 0.5 ml of enzyme (7.66 nmol), 0.5 ml of TO-PC emulsion, 3  $\mu\text{g}$  of colipase, and 2.0 ml of TES buffer (0.1 M, pH 7.0) containing 0.1 M NaCl and DEAE-PD. After 10 min of incubation at 37°C, 3 ml of diethyl ether was placed onto the incubation mixture, which was then shaken for 10 s and centrifuged at 1,200 *g* for 5 min at 4°C. The upper ether phase was aspirated and discarded, and then ether extraction was repeated twice more. A 2 ml aliquot of the supernatant of the incubation mixture was applied to a Sephacryl S-200 HR column (2.6  $\times$  60 cm; GE Healthcare Bio-Sciences KK) equilibrated with 0.05 M phosphate buffer (pH 7.0) containing 0.1 M NaCl. Fractions of 5 ml were collected, and aliquots of the fractions were taken to measure lipase activity.

### Oral administration of DEAE-PD

A suspension of 6 ml of corn oil, 80 mg of cholic acid, and 2 ml of cholesteryl oleate in 6 ml of water was sonicated for 5 min. Male Wistar King rats, weighing 150–190 g, were starved overnight and divided into two groups, and 1 ml of corn oil emulsion was administered to each rat via a stomach tube. One group received this emulsion containing 0.8 ml of DEAE-PD (50 mg)

solution, and the control group received the emulsion containing 0.8 ml of water. After administration, blood samples were collected from the tail vein or artery into heparinized microcapillary tubes, at regular intervals, and centrifuged immediately at 10,000 rpm for 5 min. One week later, the sample and control groups were changed over and the experiment was repeated. Plasma triacylglycerol concentrations were determined using the Triglyceride E-Test (Wako Pure Chemical Industries).

### Estimation of fecal radioactive lipid excretion

A suspension of 6 ml of corn oil, 80 mg of cholic acid, 2 mg of cholesteryl oleate, and  $^{14}\text{C}$ -TO (0.74 MBq) in 6 ml of water was sonicated for 5 min. Male Wistar King rats, weighing 190–200 g, were starved overnight and divided into two groups, and 1 ml of corn oil emulsion containing  $^{14}\text{C}$ -TO (40.4 kBq) was administered to each rat via a stomach tube. One group received this emulsion containing 0.8 ml of DEAE-PD (50 mg) solution, and the control group received the emulsion containing 0.8 ml of water. After administration, fecal samples were collected from each group for 48 h. Fecal lipid was extracted by the method of Bligh and Dyer (13) with slight modifications as described previously (14). The lower chloroform phase was withdrawn, and radioactivity in this phase was measured. The fecal radioactive lipid excretion was estimated as follows: excretion ratio (%) = (fecal radioactivity)/(admitted radioactivity)  $\times$  100.

## RESULTS

The hydrolytic activity of rat pancreatic lipase toward TO emulsified with soybean PC and taurocholate was determined in the presence of increasing concentrations of Sephadex G-50, DEAE-Sephadex, and CM-Sephadex

(Fig. 2A). DEAE-Sephadex dose-dependently inhibited TO hydrolysis by pancreatic lipase, but up to 10 mg/ml Sephadex G-50 and CM-Sephadex did not cause inhibition. Identical results were observed using carboxylester lipase (Fig. 2B). Lingual lipase activity was not inhibited by up to 10 mg/ml DEAE-Sephadex (data not shown). A soluble polysaccharide, PD, was a weak inhibitor of pancreatic lipase: up to 10 mg/ml PD did not inhibit TO hydrolysis by pancreatic lipase (Fig. 3A). A basic PD, DEAE-PD, dose-dependently inhibited TO hydrolysis by pancreatic lipase. The 50% inhibition concentration of DEAE-PD was increased as the degree of substitution decreased: 50% inhibition concentration was 1.44, 16.9, 618, and >1,000  $\mu\text{g}/\text{ml}$  when the substitution degree was 1.09, 0.18, 0.079, and 0.048, respectively. Similar results were observed with carboxylester lipase (Fig. 3B). The 50% inhibition concentration of DEAE-PD for TO hydrolysis by carboxylester lipase was 2.47, 20.5, 938, and >1,000  $\mu\text{g}/\text{ml}$  when the substitution degree was 1.09, 0.18, 0.079, and 0.048, respectively. Lingual lipase activity was not inhibited by up to 10 mg/ml DEAE-PD (substitution degree 1.09) (data not shown). Amylase and  $\alpha$ -glycosidase (maltase and sucrose) activities were also not inhibited by up to 10 mg/ml DEAE-PD (substitution degree 1.09). PIPE-PD also dose-dependently inhibited the hydrolytic activity of pancreatic lipase, and the 50% inhibition concentration was increased when the degree of substitution was decreased (Fig. 4A). The 50% inhibition concentration by PIPE-PD for TO hydrolysis by pancreatic lipase was 0.725, 1.78, 15.5, and 183  $\mu\text{g}/\text{ml}$  when the substitution degree was 1.50, 0.632, 0.221, and 0.126, respectively. ABZ-PD also dose-

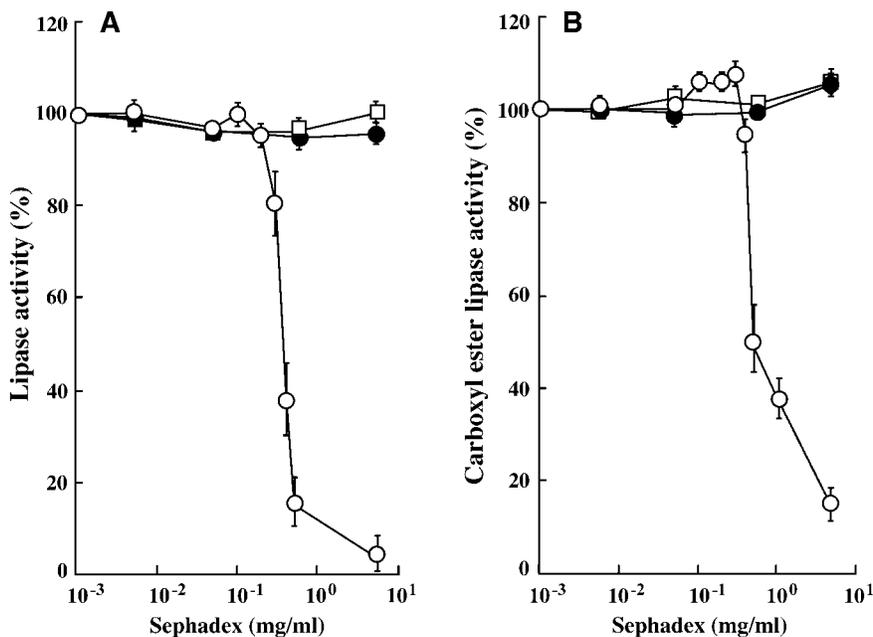
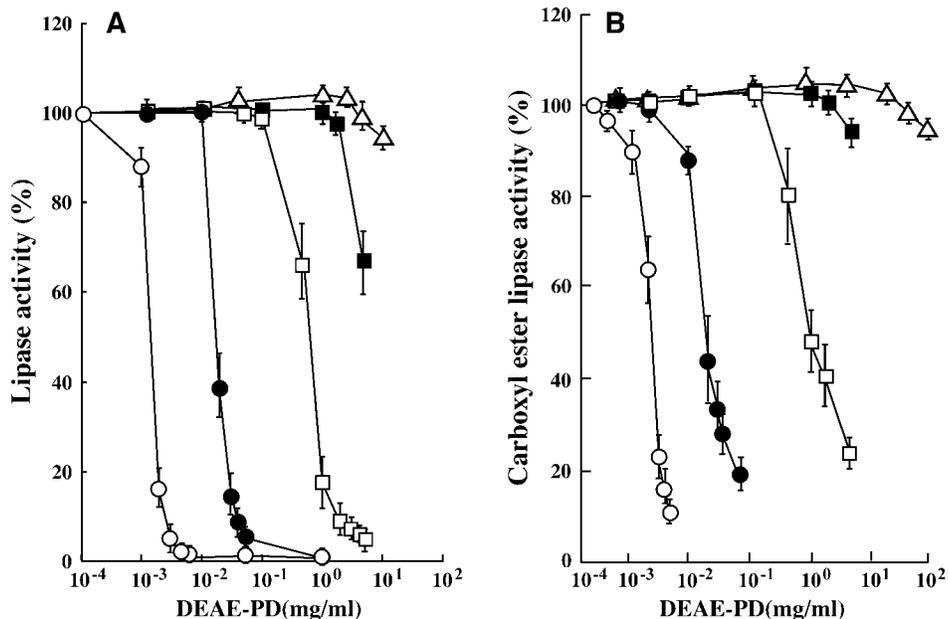


Fig. 2. Effects of Sephadex on the rate of reaction of pancreatic lipase (A) and carboxylester lipase (B) activity. Trioleoylglycerol (TO) emulsified with phosphatidylcholine (PC) was used as the substrate. Open squares, Sephadex G-50; open circles, DEAE-Sephadex; closed circles, carboxylmethyl (CM)-Sephadex. The results are expressed as means  $\pm$  SEM of four experiments.

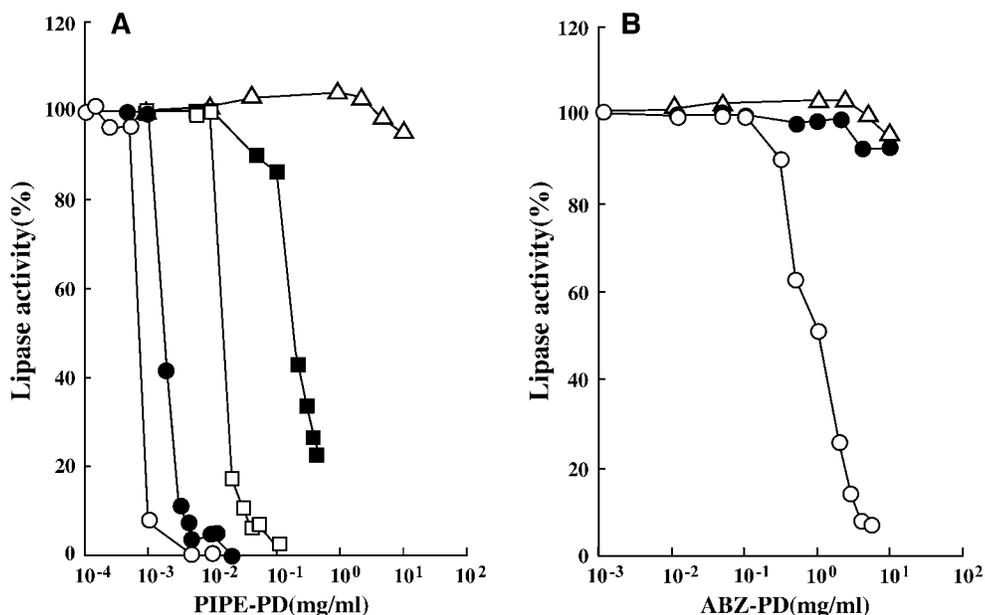


**Fig. 3.** Effects of DEAE-polydextrose (PD) at various substitution degrees on pancreatic lipase (A) and carboxylester lipase (B) activity. TO emulsified with PC was used as the substrate. The substitution degrees were 1.09 (open circles), 0.18 (closed circles), 0.079 (open squares), 0.048 (closed squares), and 0 (open triangles; original PD). Substitution degree is expressed as the number of basic groups per glucose unit. The results are expressed as means  $\pm$  SEM of four experiments.

independently inhibited the hydrolytic activity of pancreatic lipase (Fig. 4B). Inhibition by ABZ-PD was weak compared with that by DEAE-PD or PIPE-PD; the 50% inhibition concentration by ABZ-PD was 1.05 mg/ml when the substitution

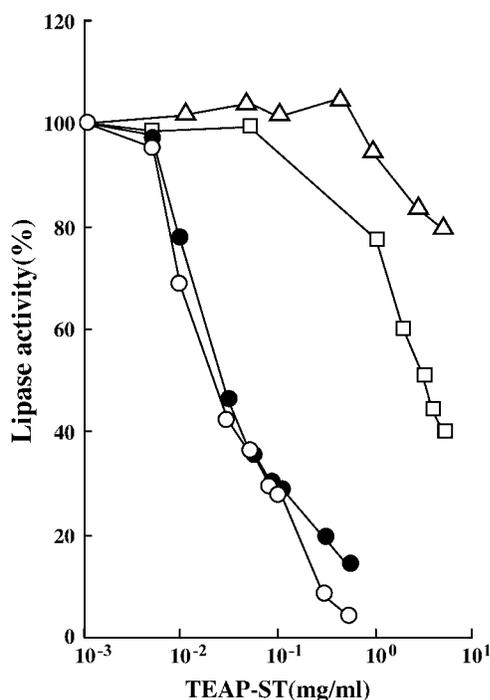
degree was 0.59, and up to 10 mg/ml ABZ-PD at substitution degree 0.16 did not inhibit pancreatic lipase activity.

ST was a weak inhibitor of pancreatic lipase: up to 1 mg/ml ST did not inhibit TO hydrolysis by pancreatic lipase; how-

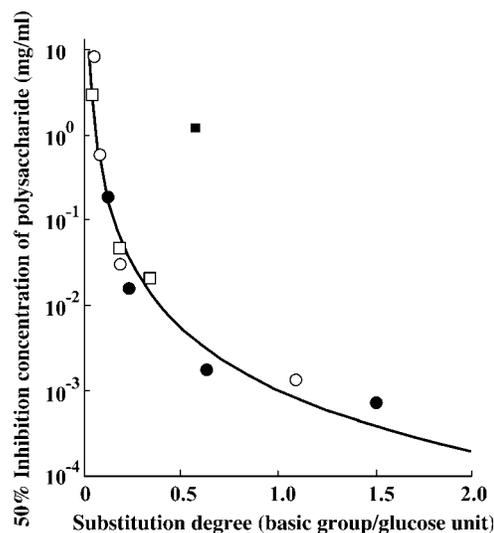


**Fig. 4.** Effects of PIPE-PD or ABZ-PD at various substitution degrees on pancreatic lipase activity. TO emulsified with PC was used as the substrate. The substitution degrees of PIPE-PD (A) were 1.50 (open circles), 0.63 (closed circles), 0.22 (open squares), 0.13 (open squares), and 0 (open triangles; original PD), and the substitution degrees of ABZ-PD (B) were 0.59 (open circles), 0.16 (closed circles), and 0 (open squares; original PD). Substitution degree is expressed as the number of basic groups per glucose unit. The results shown are averages of three experiments.

ever, 20% inhibition was observed at 10 mg/ml (Fig. 5). When the basic group TEAP was bound to ST, pancreatic lipase activity was strongly inhibited. The 50% inhibition concentration by TEAP-ST increased when the substitution degree decreased; the 50% inhibition concentration was 20.9, 25.2, and 3,190  $\mu\text{g}/\text{ml}$  when the substitution degree was 0.33, 0.15 and 0.03, respectively. The 50% inhibition concentration of basic polysaccharide calculated from Figs. 3–5 was replotted against the substitution degree (Fig. 6). The 50% inhibition concentration was decreased exponentially against the substitution degree except for ABZ-PD. Chitosan, a natural basic polysaccharide, dose-dependently inhibited TO hydrolysis by pancreatic lipase (Fig. 7). Low molecular mass chitosan, obtained from partial degradation of chitosan, also dose-dependently inhibited pancreatic lipase activity. Pancreatic lipase was more strongly inhibited by 46 kDa chitosan (average molecular mass of 46 kDa) than by original chitosan. However, inhibition by 10 kDa chitosan (average molecular mass of 10 kDa) was weaker than that by original chitosan. Up to 10 mg/ml glucosamine, a component of chitosan, did not inhibit pancreatic lipase activity. Other natural basic polyamines, such as spermine and spermidine, also dose-dependently inhibited TO hydrolysis by pancreatic lipase (data not shown); the 50% inhibition con-



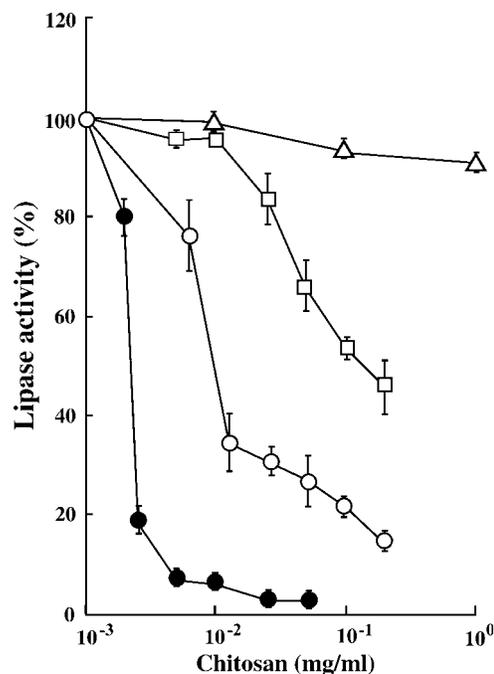
**Fig. 5.** Effects of 3-triethylamino-2-hydroxypropyl-cornstarch (TEAP-ST) at various substitution degrees on pancreatic lipase activity. TO emulsified with PC was used as the substrate. The substitution degrees of TEAP-ST were 0.33 (open circles), 0.15 (closed circles), 0.03 (open squares), and 0 (open triangles; original ST). Substitution degree is expressed as the number of basic groups per glucose unit. The results shown are averages of three experiments.



**Fig. 6.** Effects of substitution degree on 50% inhibition concentration of polysaccharide. The 50% inhibition concentration of polysaccharide was replotted against substitution degrees of DEAE-PD (open circles), PIPE-PD (closed circles), TEAP-ST (open squares), and ABZ-PD (closed squares).

centrations of spermine and spermidine were 0.20 and 0.64 mg/ml, respectively.

The effect of DEAE-PD on TO hydrolysis by pancreatic lipase was studied using various phospholipids as emulsifiers (Table 1). DEAE-PD (50  $\mu\text{g}/\text{ml}$ ) strongly inhibited



**Fig. 7.** Effects of chitosan on pancreatic lipase activity. TO emulsified with PC was used as the substrate. Open circles, chitosan; closed circles, 46 kDa chitosan; open squares, 10 kDa chitosan; open triangles, glucosamine. The results are expressed as means  $\pm$  SEM of four experiments.

TABLE 1. Effect of DEAE-PD on TO-hydrolyzing activity of pancreatic lipase

Emulsifier	Activity	
	Control	DEAE-PD
	$\mu\text{mol}/\text{mg}/\text{min}$	
Soya phosphatidylcholine	417.9 $\pm$ 16.5	13.8 $\pm$ 2.4
Egg phosphatidylcholine	384.9 $\pm$ 4.8	6.8 $\pm$ 0.98
Egg phosphatidylethanolamine	193.6 $\pm$ 17.0	0.0 $\pm$ 0.0
Brain phosphatidylserine	96.5 $\pm$ 11.3	241.2 $\pm$ 18.3
Egg phosphatidic acid	112.3 $\pm$ 9.7	109.2 $\pm$ 8.4
Arabic gum	236.2 $\pm$ 5.9	237.8 $\pm$ 5.1

PD, polydextrose; TO, trioleoylglycerol. TO was emulsified with phospholipids or arabic gum, as described in Materials and Methods, with or without DEAE-PD (50  $\mu\text{g}/\text{ml}$ ). Results are means  $\pm$  SEM (n = 4).

the hydrolysis of TO-PC or TO-PE emulsion but did not inhibit the hydrolysis of TO-PS, TO-PA, or TO-arabic gum emulsions. Pancreatic lipase was incubated with TO-PC emulsion containing DEAE-PD and applied to a Sephacryl S-200 column. Lipase activity was eluted in a single peak, and the molecular mass was estimated as 45,000 Da. The total lipase activity of the peak fraction,  $\sim 97\%$  compared with that of pancreatic lipase alone, was not changed by incubation with DEAE-PD (data not shown).

Figure 8A shows the time course of the plasma triacylglycerol concentration when corn oil emulsion with or without DEAE-PD was orally administered to rats. Two hours after DEAE-PD administration, the plasma triacylglycerol concentration decreased significantly compared with that of the control. Fecal radioactive lipid excretion was also determined when corn oil emulsion containing  $^{14}\text{C}$ -TO with or without DEAE-PD was orally administered

to rats (Fig. 8B). Fecal lipid excretion was increased significantly ( $\sim 4.5$ -fold) by DEAE-PD administration.

## DISCUSSION

Pancreatic lipase is a key enzyme in dietary triacylglycerol absorption, hydrolyzing triacylglycerols to 2-monoacylglycerol and fatty acids. Typical substrates for this enzyme are long-chain triacylglycerols, which are separated from the aqueous medium by the surface phase. Thus, for catalysis to occur, lipase must be adsorbed on the substrate lipid surface (oil-water interface), and the nature of the surface of the substrate is an important factor for lipase activity. Enzymes are sometimes activated or denatured by surface adsorption. Previously, we demonstrated that the basic peptides, protamine and polylysine, strongly inhibited the hydrolysis of TO emulsified with PC (14–16). Inhibition by the basic peptides has the characteristics of a reversible reaction; it does not bind strongly to lipase, but it may interact with the substrate emulsion (16). Inhibition was dependent on how the substrate was presented to the lipases: it occurred strongly with TO-PC and TO-PE emulsions but not with TO-PS, TO-PA, and TO-arabic gum emulsions (16). The distinction between the emulsifiers is that PC and PE are zwitterionic, whereas the others are anionic. Common to the emulsion systems in these assays was taurocholate, an anionic bile salt. The basic peptide may form a surface-active complex with taurocholate, which binds to TO-PC emulsion particles, thereby retarding lipase adsorption to the emulsion particles and triacylglycerol hydrolysis both in vivo and in vitro (15, 16). Inhibition by basic polysaccharides, such as DEAE-PD, was similar to that by basic peptides, and they share the fol-

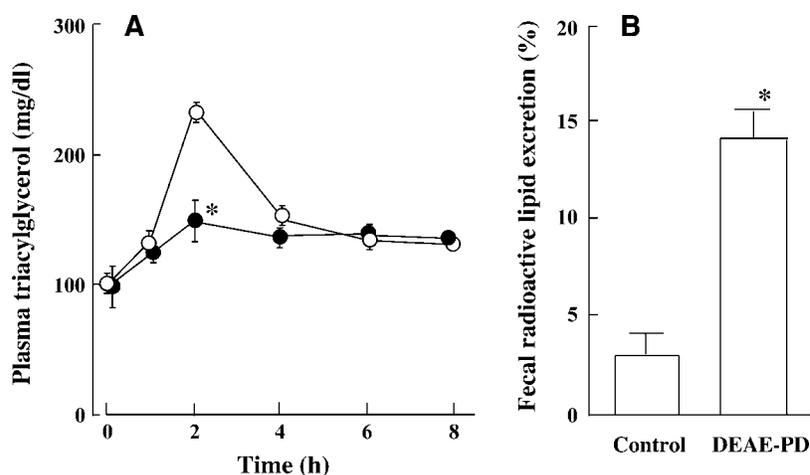


Fig. 8. Effects of DEAE-PD on rat plasma triacylglycerol (A) and fecal radioactive lipid excretion (B) after oral administration of lipid emulsion. A: Lipid emulsion (1 ml/rat) and water (0.8 ml/rat) were administered to the control group (open circles), and lipid emulsion (1 ml/rat) and DEAE-PD (50 mg/0.8 ml/rat) were administered to the DEAE-PD group (closed circles). B: Lipid emulsion (1 ml/rat) containing glycerol tri[ $^{14}\text{C}$ ]oleate ( $^{14}\text{C}$ -TO; 40.4 kBq) and water (0.8 ml/rat) were administered to the control group, and lipid emulsion (1 ml/rat) containing  $^{14}\text{C}$ -TO and DEAE-PD (50 mg/0.8 ml/rat) were administered to the DEAE-PD group. The results are expressed as means  $\pm$  SEM of four experiments. \*  $P < 0.05$  (compared with the corresponding control).

lowing characteristics: *a*) they are biopolymers; *b*) they have many positive charges; *c*) they do not bind strongly to pancreatic lipase; *d*) inhibition occurs strongly with TO-PC and TO-PE emulsions but not with TO-PS, TO-PA, and TO-arabic gum emulsions (Table 1); *e*) they do not inhibit the hydrolyzing activity of lipase toward a monomelic substrate such as *p*-nitrophenyl butyrate or 4-methylumbelliferyl oleate (data not shown); and *f*) they do not inhibit TO hydrolysis by lingual lipase. Therefore, DEAE-PD may bind to TO-PC emulsion particles and retard lipase adsorption to the emulsion particles and triacylglycerol hydrolysis (Fig. 3).

PD is a polysaccharide synthesized by random polymerization of glucose and sorbitol. PD is a water-soluble dietary fiber and is not digested or absorbed in the small intestine. A large portion of the ingested PD is excreted in the feces; therefore, it is used widely in many countries as a bulking agent and as a lower energy ingredient (4.2 kJ/g). PD itself is not an inhibitor of pancreatic lipase; however, when a basic group is introduced to PD, it becomes a strong inhibitor of pancreatic lipase. The inhibition power of basic PD was increased as the degree of substitution of basic groups was increased (Figs. 3, 4). The 50% inhibition concentration of the basic polysaccharide was decreased exponentially against the substitution degree (Fig. 6). These results suggest that the number of positive charges per backbone carbohydrate molecule is important in lipase inhibition. However, in a univalent positive charge, such as in ABZ-PD, lipase inhibition was weaker than in the basic carbohydrate with a divalent or trivalent positive charge, such as DEAE-PD, PIPE-PD, or TEAP-ST. Therefore, the 50% inhibition concentration of ABZ-PD deviated from the exponential line. The results shown in Fig. 6 also demonstrated that the species of backbone carbohydrate might not be important for the lipase inhibition, because the 50% inhibition concentrations of PD and ST derivatives were consistent with the same exponential line against the substitution degree.

Chitosan, a natural basic polysaccharide, strongly and dose-dependently inhibited TO hydrolysis by pancreatic lipase (Fig. 7). However, one of its components, glucosamine, did not inhibit pancreatic lipase. Similar results were observed for the basic peptide; basic peptide strongly inhibited the pancreatic lipase activity, but an amino acid mixture with an amino acid composition identical to that of basic peptide did not (15, 16). Previously, we reported that the high polymerization of polylysine was the most effective factor for lipase inhibition on a weight concentration basis (16). Water-soluble chitosan, with an average molecular mass of 46 kDa, was a more effective inhibitor than chitosan, with an average molecular mass of 10 kDa (Fig. 7). These results suggest that the extent of polymerization is also an important factor in lipase inhibition.

With high substitution degrees of 1.09 for DEAE-PD and 1.5 for PIPE-PD, the 50% inhibition concentrations were 1.44 and 0.725  $\mu\text{g}/\text{ml}$ , respectively. These values are similar to the 50% inhibition concentration of orlistat at 0.49  $\mu\text{g}/\text{ml}$  (14). Orlistat has been reported as a selective and potent inhibitor of lipase (17, 18). The inhibition of

orlistat is irreversible; it binds to the serine of the catalytic triad of pancreatic lipase, and a stoichiometric enzyme-inhibitor complex of an acyl-enzyme type is formed (19, 20). Previously, we named orlistat an “enzyme inhibitor” or “lipase inhibitor,” because it interacted directly with the enzyme (lipase) and inhibited lipase action. Alternatively, we named the basic peptide a “reaction inhibitor” or “lipolytic inhibitor,” because it may adsorb to the substrate surface and retard the lipolytic reaction (14). The lipase inhibition mechanism of basic PD is similar to that of basic peptide (14–16); PD does not bind strongly to lipase, and the inhibition by DEAE-PD is reversible and specific to the substrate emulsifier. Therefore, basic polysaccharide (DEAE-PD) is also a reaction inhibitor or lipolytic inhibitor.

Basic polysaccharide is a strong inhibitor of gastrointestinal lipase reactions on TO-PC emulsion. Because the major bile phospholipid is PC, constituting >90% of the total, basic polysaccharide might be an effective inhibitor in vivo: oral administration of DEAE-PD to rats reduced the peak plasma triacylglycerol concentration and increased fecal lipid excretion (Fig. 8). Therefore, basic polysaccharide might suppress dietary fat absorption by inhibiting fat digestion. Han, Kimura, and Okuda (21) reported that a basic polysaccharide, chitin-chitosan, prevented high-fat diet-induced obesity in mice. Further experiments are needed to confirm the effectiveness of basic polysaccharide in animal models. Furthermore, toxicity studies of basic polysaccharides such as DEAE-PD are necessary because the functional group, DEAE, has been attached to the parent molecule, PD. An acute toxicity test using Jcl:ICR mice has been performed: DEAE-PD (2 g/kg body weight) was administered to mice, and these were observed for 10 days. All mice were well, with no diarrhea, and body weight was not changed significantly for 10 days compared with control mice (water administration). Plasma glutamic oxaloacetic transaminase and glutamic pyruvic transaminase activities were not changed significantly (data not shown). Further toxicity studies, such as chronic toxicity tests, are needed for approval for food additive use.

Dietary fiber affects lipid metabolism in a variable manner, and some dietary fibers inhibit gastrointestinal lipase reactions, such as pancreatic lipase (1, 22–24). The effective concentration of these dietary fibers for lipase inhibition is 1–5% (10–50 mg/ml). When a basic group, such as DEAE, was attached to these dietary fibers, they became strong inhibitors of pancreatic lipase. The effective concentration of these basic dietary fibers for lipase inhibition was 1–5  $\mu\text{g}/\text{ml}$ , >10,000 times lower than the concentration of the original dietary fibers (Figs. 3–5). Basic dietary fibers are strong inhibitors of gastrointestinal lipase reactions and are reversible reaction inhibitors. In comparison with basic peptides (14–16), we conclude that the basic biopolymer is a strong inhibitor of gastrointestinal lipase reactions. The number of positive charges per molecule and the extent of polymerization are important factors in lipase inhibition by basic biopolymers. The basic biopolymer could be considered a potential new tool to reduce intestinal lipolysis. 

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