Studies on the inhibition of pancreatic and carboxylester lipases by protamine

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Abstract The basic protein protamine strongly inhibited hydrolysis of triolein emulsified with soybean phosphatidylcholine (PC) by pancreatic and carboxylester lipases; 10 μg/ml protamine, about 1000 times lower than the concentration of bovine serum albumin for the same effect, inhibited triolein hydrolysis completely. This inhibition was not affected by the incubation pH or bile salt concentration. Two other basic proteins, histone and purothionin, also inhibited hydrolysis of triolein emulsified with soybean PC, but they did not inhibit triolein hydrolysis by gastric lipase. When gum arabic was used as an emulsifier instead of soybean PC, these basic proteins did not affect triolein hydrolysis by pancreatic or carboxylester lipases. The effects of protamine on triolein hydrolysis by pancreatic and carboxylester lipases was studied using various phospholipids as emulsifiers. Protamine (10 μg/ml) did not inhibit hydrolysis of triolein emulsified with dicaproyl PC (DCPC), phosphatidic acid (PA), or phosphatidylserine (PS) by pancreatic and carboxylester lipases. Conversely, protamine at high concentrations slightly stimulated hydrolysis of triolein emulsified with DCPC or PA. Hydrolysis of triolein-phosphatidylethanolamine (PE) emulsion was inhibited slightly by protamine. The profiles of protamine inhibition of triolein-phosphatidyl-N,N-dimethyl ethanolamine (PDME) and triolein-phosphatidyl-N-monomethyl ethanolamine (PMME) emulsions were intermediate between those of PC and PE emulsions. These results suggest that the phospholipid species, especially choline moieties and fatty acid chain length, affect the lipase inhibitory activity of protamine profoundly. In vivo, oral administration of protamine to rats reduced and delayed the peak plasma triacylglycerol concentration, but neither bovine serum albumin nor an amino acid mixture with an amino acid composition identical to protamine affected plasma triacylglycerol levels.—**Tsujita, T., Y. Matsuura, and H. Okuda.** Studies on the inhibition of pancreatic and carboxylester lipases by protamine. *J. Lipid Res.* 1996. **37:** 1481–1487.

Supplementary key words basic proteins • triacylglycerol • phosphatidylcholine • digestion

In mammals, dietary triacylglycerol digestion is mediated by three main enzymes, preduodenal (lingual or gastric), carboxylester, and pancreatic lipases. Under acidic conditions in the stomach, fat is hydrolyzed by preduodenal lipase(s), which leads to the hydrolysis of 10–30% of the dietary triacylglycerols to glycerols (mainly diacylglycerol) and free fatty acids (1). Carboxylester lipase has a broad substrate specificity, acting readily on triacylglycerols, diacylglycerols, monoacylglycerols, and cholesteryl esters (2) and it catalyzes the hydrolysis of water-soluble substrates such as methyl butyrate and *p*-nitrophenyl butyrate. Pancreatic lipase hydrolyzes triacylglycerols to 2-monoacylglycerols and free fatty acids. Typical substrates for these enzymes are long-chain triacylglycerols, which are separated from the aqueous medium by the surface phase. In contrast to their substrates, these lipases are water-soluble proteins. Thus, for catalysis, these enzymes must be adsorbed to (or penetrate) the lipid surfaces and, therefore, the quality of the surface of substrate lipids is an important factor for lipase activity. Enzymes are sometimes activated or denatured by surface adsorption (3). The amphiphilic proteins are also adsorbed to (or penetrate) the substrate lipid surfaces and affect lipase activity. Serum albumin has been shown to interact with the action of pancreatic lipase on emulsified substrates in two different ways. At low concentrations it protects this lipase from irreversible inactivation and at high concentrations it inhibits its catalytic activity by blocking the substrate surface (4). Gargouri et al. (5) reported that several proteins, including melittin, β-lactoglobulin A, serum albumin, ovalbumin, and myoglobin, inhibited pancreatic lipase activity and their inhibitory effects could be the result of lipase desorption from its substrate due to a change in interfacial quality. They stated

Abbreviations: PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleoyl phosphatidylcholine; DPPC, dipalmitoyl phosphatidylcholine; DCPC, docaproyl phosphatidylcholine; PE, phosphatidylethanolamine; POPE, 1-palmitoyl-2-oleoyl phosphatidylethanolamine; PDME, phosphatidyl-N,N-dimethyl-ethanolamine; PMME, phosphatidyl-N-monomethyl-ethanolamine; PA, phosphatidic acid; PS, phosphatidylserine.

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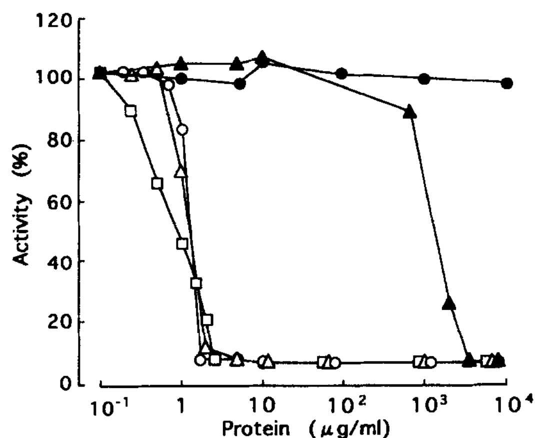

Fig. 1. Effects of increasing concentrations of basic proteins on the rate of hydrolysis of triolein emulsified with soybean PC by pancreatic lipase (1.25 μg/ml). Experiments were performed in the presence of protamine (○), purothionin (□), histone (△), bovine serum albumin (▲), and an amino acid mixture with an amino acid composition identical to protamine (●).

that further investigations into lipase inhibition by proteins should be carried out using the monolayer technique and concluded that interfacial inhibition by proteins was related to the respective penetration fluxes of the enzyme and inhibitor (6).

The experiments presented in this report were undertaken to elucidate the mode of inhibition of pancreatic and carboxylester lipases by protamine.

MATERIALS AND METHODS

Materials

The enzyme substrates and reagents used were obtained as follows. Triolein, cholesterol oleate, taurocholic acid, pancreatic lipase (Type VI-S, from porcine pancreas), and colipase were from Sigma (St. Louis, MO). Phosphatidylcholine (PC, from soybean) and phosphatidic acid (PA, from soybean PC) were from Niphon Shoji (Tokyo, Japan). Phosphatidylethanolamine (PE, from egg), phosphatidylserine (PS, from bovine brain), PC (from egg), and PA (from egg PC) were from Serdary Research Laboratories (London, Canada). Phosphatidyl-N,N-dimethyl-ethanolamine (PDME, from egg PC), phosphatidyl-N-monomethyl-ethanolame (PMME, from egg PC), and synthetic phospholipids were from Avanti Polar Lipids Inc. (Alabster, AL). Histone (from calf thymus) was from Funakoshi Kogyo Co. Ltd. (Tokyo, Japan). Protamine (average molecular weight 3000–4000, from herring sperm) was a gift from Dr. Hideo Kato (Hiroshima Women's College, Hiroshima, Japan). Purothionin was purified from wheat flour (7). Bovine serum albumin was from Wako Pure Chemical Industries (Osaka, Japan) and was extracted by the method of Chen to remove free fatty acid (8).

Enzyme assay

Lipase activity was determined by measuring the rate of release of oleic acid from triolein. A suspension of 90 μmol triolein, 12.6 μmol phospholipid, and 9.45 μmol taurocholic acid in 9 ml 0.1 M TES, pH 7.0, containing 0.1 M NaCl was sonicated for 5 min. The assay system comprised the following components in a total volume of 200 μl: 50 μl enzyme solution, 50 μl inhibitor solution, 0.5 μmol triolein, 0.053 μmol taurocholic acid, 0.07 μmol phospholipid, 20 μmol TES, and 20 μmol NaCl. Incubation was carried out 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. (9) with a slight

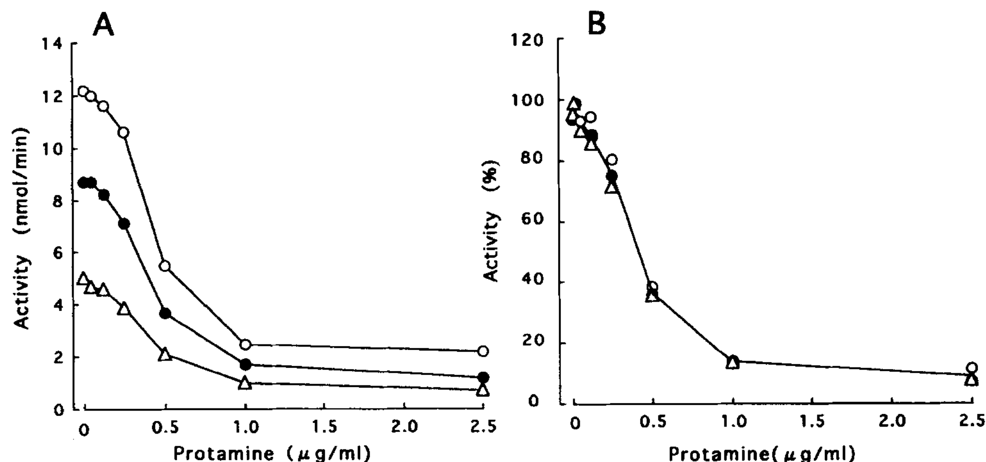

Fig. 2. Inhibition of pancreatic lipase at various concentrations by increasing concentrations of protamine. Triolein emulsified with soybean PC was used as the substrate. (A) The lipase concentrations were 1.25 (○), 0.625 (●), and 0.313 (△) μg/ml. (B) The experimental values shown in Fig. 2A replotted as percentages of the remaining enzyme activity.

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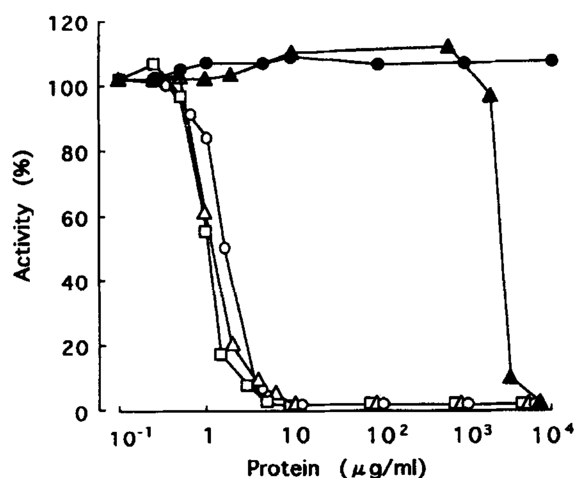

Fig. 3. Effects of increasing concentrations of basic proteins on the rate of hydrolysis of triolein emulsified with soybean PC by carboxylester lipase (10 μg/ml). Experiments were performed in the presence of protamine (○), purothionin (□), histone (△), bovine serum albumin (▲), and an amino acid mixture with an amino acid composition identical to protamine (●).

modification (10). The incubation mixtures were added to 3-ml aliquots of a 1:1 (v/v) mixture of chloroform and heptane containing 2% (v/v) methanol and extracted by shaking the tubes horizontally for 10 min in a shaker. The mixture was centrifuged (2000 *g*, 10 min), the upper aqueous phase was removed by suction, 1 ml copper reagent was added to the lower organic phase, the tubes were shaken for 10 min, the mixture was centrifuged (2000 *g*, 10 min), and 1 ml upper organic phase, which contained the copper salts of the extracted oleic acid, was treated with 1 ml 0.1% (w/v) bathocuproine containing 0.05% (w/v) 3-*tert*-butyl-4-hydroxyanisol. Calorimetric determination at 480 nm was performed.

Lipase activity was also determined using gum arabic as an emulsifier; 45 mg gum arabic, instead of phospholipid, was used and the enzyme activity was assayed as described above. Lingual lipase activity was assayed in 0.1 M citrate-potassium diphosphate buffer, pH 5.4, using soybean PC as an emulsifier. Cholesterol esterase activity was determined by measuring the rate of oleic acid release from cholesteryl oleate as follows. A suspension of 90 μmol cholesteryl oleate, 12.6 μmol soybean PC, and 9.45 μmol taurocholic acid in 9 ml 0.1 M TES, pH 7.0, containing 0.1 M NaCl was sonicated for 5 min. The assay system comprised the following components in a total volume of 200 μl: 50 μl enzyme solution, 50 μl inhibitor solution, 0.5 μmol cholesteryl oleate, 0.053 μmol taurocholic acid, 0.07 μmol soybean PC, 20 μmol TES, and 20 μmol NaCl. Incubation was carried out at pH 7.0 and 37°C for 30 min and the amount of oleic acid produced was determined by the method described above. The rate of tributyrin hydrolysis was measured using a pH-stat by titration of liberated butyric acid with 0.02 N NaOH, as described previously (11).

Enzyme preparations

Carboxylester lipase was purified from porcine pancreas as described previously (12). The purified enzyme preparations were found to have specific activities of 700–800 μmol *p*-nitrophenol released/mg protein per min with *p*-nitrophenyl butyrate as the substrate.

A lingual lipase fraction was prepared from rat tongues. The entire lingual serous glandular region was homogenized in cold 25 mM potassium phosphate buffer, pH 6.3, containing 0.9% NaCl. The homogenate was centrifuged at 100,000 *g* for 60 min (13) and the supernatant, which was used as the enzyme solution, was stored at -80°C.

Oral administration of protamine

A suspension of 6 ml corn oil, 80 mg cholic acid, and 2 mg cholesteryl oleate in 6 ml water was sonicated for 5 min. Male Wistar King rats, weighing 150–190 g, were starved overnight, then divided into two groups and 1 ml corn oil suspension was administered to each rat via a stomach tube. One group received this suspension containing 0.8 ml required protein (100 mg) solution and the control group received the suspension containing 0.8 ml water. After protein 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 triglyceride concentrations were determined using the Triglyceride E-Test (Wako Pure Chemical Industries).

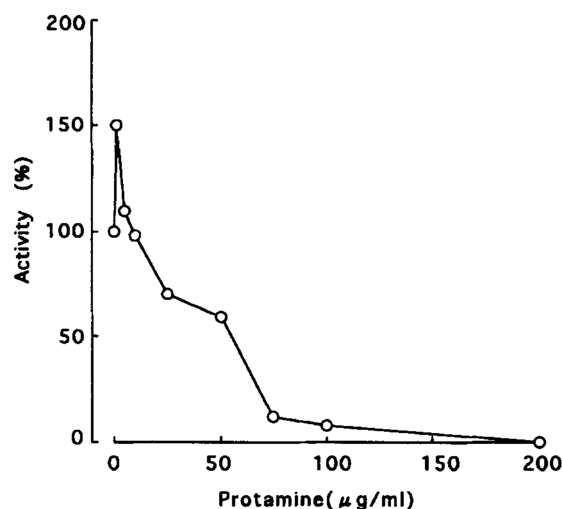

Fig. 4. Effects of increasing concentrations of protamine on the rate of hydrolysis of cholesterol-oleate emulsified with soybean PC by pancreatic carboxylester lipase (10 μg/ml).

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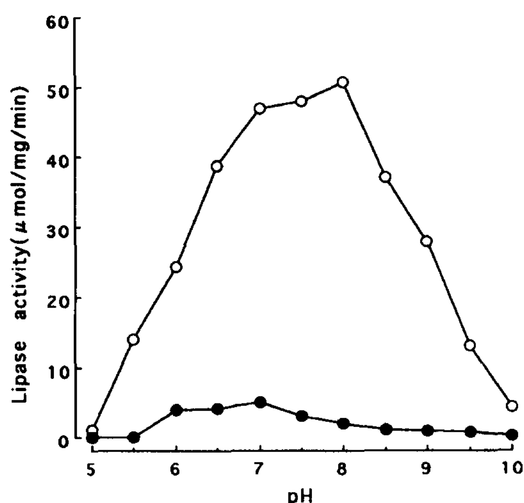

Fig. 5. Effects of pH on the rate of hydrolysis of triolein emulsified with soybean PC by pancreatic lipase. The enzyme activities were determined in the presence (●) and absence (○) of protamine (5 μg/ml).

RESULTS

The hydrolytic activity of porcine pancreatic lipase toward triolein emulsified with soybean PC was determined in the presence of increasing concentrations of various proteins (**Fig. 1**). Protamine inhibited triolein hydrolysis strongly; at 10 μg/ml, it inhibited triolein hydrolysis completely, and this concentration was about 1000 times lower than that of bovine serum albumin required to produce the same effect. When the solution of protamine was injected to the triolein emulsion after 5 min hydrolysis by pancreatic lipase, similar inhibitory effect of protamine was still observed (data not shown). Two other basic proteins, histone and purothionin, also inhibited triolein hydrolysis to the same degree. An amino acid mixture (up to 10 mg/ml) with an amino acid composition identical to protamine did not affect triolein hydrolysis. The enzyme activity in the presence of 5 μg/ml protamine was not restored by adding up to 3 μmol/ml taurocholic acid, and colipase (up to 25 μg/ml) also failed to restore the enzyme activity (data not shown). The inhibitory action of protamine was determined using three different concentrations of pancreatic lipase (**Fig. 2**). The extent of lipase inactivation by each protamine concentration tested was independent of the enzyme concentration (Fig. 2,B). Half-inhibition relative protamine concentration values were inversely proportional to the lipase concentration: 0.81 μg protamine/μg lipase, 1.70 μg protamine/μg lipase, and 3.20 μg protamine/μg lipase at 1.25 μg lipase, 0.625 μg lipase, and 0.313 μg lipase, respectively.

Similar effects of the three basic proteins on the hydrolysis of triolein emulsified with soybean PC by carboxylester lipase were observed (**Fig. 3**). At 10 μg/ml, protamine, purothionin, and histone completely inhibited triolein hydrolysis by carboxylester lipase. Bovine serum albumin (up to 1 mg/ml) did not affect the enzyme activity, but at 10 mg/ml, it inhibited activity completely. The amino acid mixture (up to 10 mg/ml) with an amino acid composition identical to protamine did not affect the activity. The concentration of protamine required to inhibit the cholesteryl oleate-hydrolytic activity of carboxylester lipase was about 10 times higher than that required to inhibit its triolein-hydrolytic activity (**Fig. 4**). At a low concentration (1 μg/ml), protamine stimulated the former enzyme activity (about 50%), and at 100 μg/ml, it inhibited it completely.

The triolein-hydrolytic activity of lingual lipase was not affected by protamine, purothionin, or histone (up to 1 mg/ml, data not shown). As lingual lipase activity was determined at pH 5.4, the effect of pH on pancreatic lipase activity was determined (**Fig. 5**). The optimum pH for the triolein-hydrolytic activity of pancreatic lipase was between pH 7.0 and 8.0, and protamine (5 μg/ml) inhibited this activity at all pH values between 5.0 and 10.0.

When triolein was emulsified with gum arabic instead of soybean PC, the triolein-hydrolytic activities of pan-

TABLE 1. Effects of basic proteins on the triolein-hydrolytic activity of pancreatic lipase

Proteins (10 μg/ml)	Triolein-Hydrolysis	
	Soybean PC	Gum Arabic
	μmol/mg/min	
None	47.0 ± 1.6	18.2 ± 1.30
Protamine	5.8 ± 0.5	18.5 ± 0.23
Purothionin	4.9 ± 0.5	18.1 ± 0.15
Histone	4.5 ± 0.2	17.6 ± 0.57

Triolein was emulsified with soybean PC or gum arabic, as described in Materials and Methods, and the triolein-hydrolytic activity by pancreatic lipase in the presence of 10 μg/ml each basic proteins was measured.

TABLE 2. Effects of basic proteins on the triolein-hydrolytic activity of carboxylester lipase

Proteins (10 μg/ml)	Triolein-Hydrolysis	
	Soybean PC	Gum Arabic
	μmol/mg/min	
None	5.56 ± 0.10	2.29 ± 0.04
Protamine	0.26 ± 0.00	2.01 ± 0.08
Purothionin	0.75 ± 0.00	2.03 ± 0.09
Histone	0.13 ± 0.01	2.20 ± 0.04

Triolein was emulsified with soybean PC or gum arabic, as described in Materials and Methods, and the triolein-hydrolyzing activity of carboxylester lipase in the presence of 10 μg/ml each basic proteins was measured.

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TABLE 3. Effects of phospholipids on the triolein-hydrolytic activity of pancreatic lipase

Phospholipids	Activity	
	Control	Protamine (10 μg/ml)
	μmol/mg/min	
Soybean PC	46.1 ± 0.24	5.8 ± 0.23
Egg PC	46.3 ± 0.39	3.7 ± 0.76
POPC	37.5 ± 0.21	5.0 ± 0.33
DPPC	34.9 ± 0.59	0.7 ± 0.30
DCPC	29.6 ± 0.51	24.0 ± 1.1
PDME	40.3 ± 0.28	9.8 ± 1.2
PMME	33.0 ± 0.34	11.3 ± 0.98
Egg PE	37.7 ± 0.69	29.0 ± 0.23
POPE	37.0 ± 0.37	19.7 ± 1.40
Egg PA	37.0 ± 0.35	38.5 ± 0.32
Soya PA	33.4 ± 0.76	31.2 ± 0.37
Bovine brain PS	21.4 ± 0.13	20.9 ± 0.35

Triolein was emulsified with phospholipids, as described in Materials and Methods, and the triolein-hydrolytic activity was measured in the presence and absence of protamine (10 μg/ml).

creatic and carboxylester lipases were reduced to about 40% (**Table 1** and **Table 2**). The three basic proteins (protamine, purothionin and histone, at 10 μg/ml) did not inhibit hydrolysis of triolein emulsified with gum arabic, whereas they completely inhibited hydrolysis of triolein emulsified with soybean PC.

Protamine (up to 1 mg/ml) did not affect the tributyrin-hydrolytic activities of pancreatic and carboxylester lipases (data not shown), neither did it affect the *p*-nitrophenyl butyrate-hydrolytic activity of the latter (data not shown).

The effect of protamine on triolein hydrolysis by pancreatic lipase was studied using various phospholipids as emulsifiers (**Table 3**). Protamine strongly inhibited hydrolysis of triolein-PC emulsion, but when the short chain fatty acyl PC, DCPC was used as an emulsifier, it did not inhibit the hydrolytic activity and at high concentrations, protamine stimulated triolein-DCPC emulsion hydrolysis slightly (**Fig. 6**). Protamine (10 μg/ml) did not inhibit hydrolysis of triolein-PA or triolein-PS emulsions, but hydrolysis of triolein-PS emulsion was inhibited by about 80% by 100 μg/ml (data not shown) and hydrolysis of triolein-PE emulsion was inhibited by about 20–40% by 10 μg/ml protamine. Similar inhibitory effects of protamine on triolein hydrolysis by carboxylester lipase in various phospholipid emulsions were observed (**Table 4**). The profiles of protamine inhibiton of triolein-PDME and triolein-PMME emulsions were intermediate between those of PC and PE emulsions (Fig. 6).

Figure 7 shows the time course of the plasma triacylglycerol concentrations when corn oil suspension with or without various proteins was administered orally to rats. One and 2 h after protamine administration, the plasma triacylglycerol concentrations decreased significantly compared with controls, and increased after 6 h. Therefore, the peak plasma triacylglycerol concentration was reduced and delayed by protamine administration. Neither bovine serum albumin nor the amino acid mixture with an identical amino acid composition to protamine affected the plasma triacylglycerol levels. The lipoprotein lipase activities in post-heparin plasma were not affected significantly by oral administration of protamine (data not shown).

DISCUSSION

The lipase reaction occurs at the substrate surface (lipid–water interface) and is dependent upon surface adsorption of the enzyme. Therefore, amphiphilic substances acting as emulsifiers would be expected to influence the lipase reaction rate. It is well known that bile salts and synthetic detergents behave as inhibitors of lipolysis (14, 15). Hydrophobic proteins, such as bovine serum albumin and β-lactoglobulin which are also amphiphilic, have been shown to inhibit lipase activity toward its triglyceride substrate by competing for the surface (4, 5), and therefore, the inhibition might be due to enzyme desorption from the substrate lipid particles surface. In this study, we demonstrated that protamine strongly inhibited hydrolysis of triolein emulsified with PC, and two other basic proteins, purothionin and

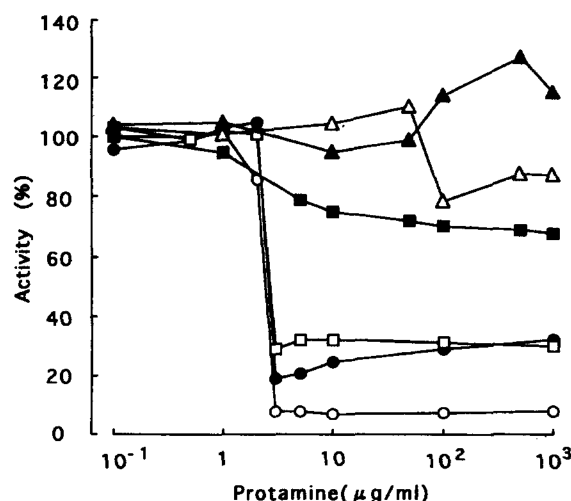

Fig. 6. Effects of increasing concentrations of protamine on the rate of hydrolysis of triolein emulsified with various phospholipids by pancreatic lipase (1.25 μg/ml). The triolein was emulsified with egg PC (○), PMME (●), PDME (□), egg PE (■), egg PA (△), and DCPC (▲).

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TABLE 4. Effects of phospholipids on the triolein-hydrolytic activity of carboxylester lipase

Phospholipids	Activity	
	Control	Protamine (10 μg/ml)
	μmol/mg/min	
Soybean PC	5.35 ± 0.11	0.057 ± 0.014
Egg PC	4.37 ± 0.10	0.51 ± 0.053
POPC	5.79 ± 0.02	0.033 ± 0.022
DPPC	2.44 ± 0.06	0.035 ± 0.011
DCPC	3.28 ± 0.12	2.45 ± 0.19
PDME	4.79 ± 0.13	0.43 ± 0.085
PMME	3.83 ± 0.06	0.078 ± 0.030
Egg PE	3.54 ± 0.06	0.36 ± 0.028
POPE	4.83 ± 0.14	1.08 ± 0.045
Egg PA	4.17 ± 0.12	3.85 ± 0.14
Soya PA	2.25 ± 0.14	2.86 ± 0.092
Bovine brain PS	1.56 ± 0.03	2.47 ± 0.038

Triolein was emulsified with phospholipids, as described in Materials and Methods, and the triolein-hydrolytic activity was measured in the presence and absence of protamine (10 μg/ml).

histone, also inhibited triolein hydrolysis. Protamine, purothionin, and histone contain large amounts of basic amino acid residues (arginine + lysine); about 76, 24, and 23%, respectively, of their total amino acid contents, and their isoelectric points are over pH 10. The responsible mechanism for the inhibition by these proteins seems unlikely to be the same as that by hydrophobic proteins in the light of the following observations: *a)* inhibition of lipase activity by hydrophobic proteins was reversed by adding bile salts in the presence of colipase (16), whereas inhibition by basic protein was not; *b)* hydrophobic proteins inhibited hydrolysis of triolein emulsified with gum arabic, whereas basic proteins did not; and *c)* the concentrations of basic protein that inhibited lipase activity were about 1000 times lower than that of the hydrophobic protein, bovine serum albumin (Figs. 1 and 3).

Lipase inhibition by protamine was specific to substrate species; it occurred only with triolein-PC emulsion, not triolein-PA or triolein-DCPC emulsions (Tables 3 and 4). The profiles of the inhibition of hydrolysis of triolein-PDME and triolein-PMME emulsions by protamine were intermediate between those of PC and PE emulsions (Fig. 6). Protamine did not affect hydrolysis of triolein-gum arabic emulsion (Tables 1 and 2), triolein-glycerol emulsion, or water-soluble substrates (data not shown). The extent of the inactivation of lipase activity by protamine was independent of lipase concentration (Fig. 2A and B). The effective relative concentrations of protamine to lipase were irreversibly proportional to the lipase concentration. These results suggest that protamine did not bind strongly to lipase, but may have interacted with the triolein-PC emulsion. The choline moieties of phospholipids may be important for the interaction of protamine with the substrate emulsion. Hydrophobicity of the emulsifier is also an important factor, because hydrolysis of triolein-DCPC emulsion was not affected by protamine.

Even when triolein-soybean PC emulsion was used as a substrate, lingual and *Pseudomonas fluorescens* lipase activities were not inhibited by protamine up to 1 mg/ml (data not shown). It is well known that protamine inhibits lipoprotein lipase activity, but not hepatic triglyceride lipase activity (17). The mechanism of the inhibition of lipoprotein lipase by protamine has been postulated to be that protamine either binds directly to the lipoprotein lipase or to heparin that functions as a cofactor. Pancreatic lipase is a member of the lipase gene family, which includes lipoprotein and hepatic triglyceride lipases (18), and it has a heparin-binding site and binds to heparin present in small intestinal membranes (19). However, the mechanism of inhibition of pancreatic lipase activity by protamine seems unlikely to be the

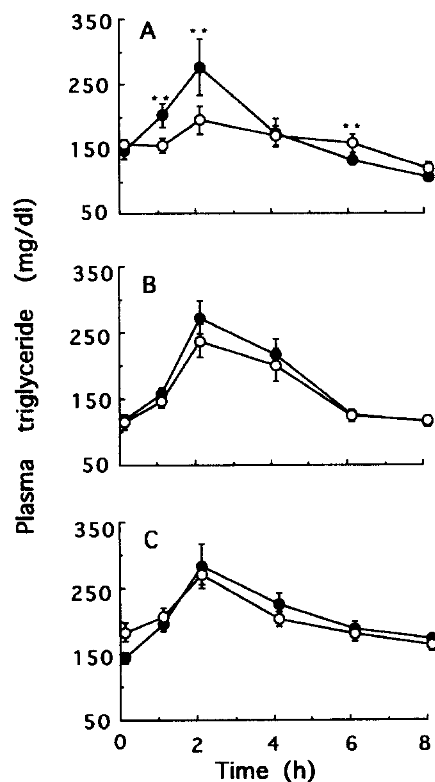

Fig. 7. Effects of proteins on rat plasma triacylglycerol levels after oral administration of lipid emulsion. Lipid emulsion alone (●) or lipid emulsion containing protein (○, A, protamine; B, bovine serum albumin; and C, amino acid mixture with an amino acid composition identical to protamine) were orally administered. The results are expressed as means ± SE of ten experiments. $^{**}P < 0.01$ (compared with the corresponding control, Newman-Keuls' range test).

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same as that of lipoprotein lipase, because heparin does not affect pancreatic lipase activity and protamine may not bind to pancreatic lipase directly (Fig. 2).

Dietary lipids are digested by three main enzymes, lingual, carboxylester, and pancreatic lipases which are water-soluble molecules and thus have access to the lipid only at the surface of the lipid droplets. The lipid surface area available for digestion is increased by emulsification. In vivo, the emulsifying agents are bile acids and phospholipids. High concentrations of the latter are present in bile and the major bile phospholipid is PC, comprising over 90% of the total. In the presence of PC and bile acids, therefore, the lipid surface area on which the digestive enzyme can work is large. In this study, we determined various lipase activities using triolein emulsified with taurocholic acid and PC, and discovered that basic proteins inhibited carboxylester and pancreatic lipase activities. Furthermore, an in vivo experiment in the rat showed that protamine, administered orally, reduced and delayed the peak plasma triacylglycerol concentrations (Fig. 7). These results suggest that basic proteins, such as protamine, are strong candidates for agents that inhibit lipid adsorption.

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