Studies on the inhibition of pancreatic and microbial lipases by soybean proteins

Y. Gargouri,* R. Julien,* G. Pieroni, R. Verger,** and L. Sarda***

Laboratoire de Biochimie, Faculté St-Charles, Place V. Hugo, 13003 Marseille,* and Centre de Biochimie et de Biologie Moléculaire, Centre National de la Recherche Scientifique, 31 Avenue J. Aiguier, 13009 Marseille, France**

Abstract A protein, molecular weight 70,000 that inhibits pancreatic lipase has been isolated from soybean seeds. Inhibition is not reversed by colipase unless bile salts are added to the assay system. Inhibitory properties of the purified protein are very similar to those of serum albumin or α-lactoglobulin. It has been confirmed that, during intestinal lipolysis of dietary fats, bile salts play an essential role for the activation of the lipase-colipase system in the presence of inhibitory proteins. The purified soybean lipase inhibitory protein was shown to be highly surface-active and able to penetrate monomolecular films of various glycerides and phospholipids at high surface pressure. Inhibition of pancreatic lipase by proteins is related to their capacity to interact with lipids and to modify the quality of the substrate-water interface. The protein isolated from soybeans inhibits pancreatic and *Rh. delemar* lipase in contrast to the *Rh. arrhizus* enzyme.—**Gargouri, Y., R. Julien, G. Pieroni, R. Verger, and L. Sarda.** Studies on the inhibition of pancreatic and microbial lipases by soybean proteins. *J. Lipid Res.* 1984. **25:** 1214–1221.

Supplementary key words lipolysis • colipase • bile salts

Protein inhibitors of enzymes are widely distributed in plants. Among protease inhibitors, the Kunitz inhibitor and the Boweman-Birk inhibitor from soybean have been extensively studied (1, 2). They are polypeptides of 181 and 71 amino acid residues, respectively, which form well-characterized stable enzyme-inhibitor complexes with pancreatic trypsin on a one to one molar ratio. Studies on the structure-function relationships have allowed the identification of the trypsin inhibitory region in both inhibitors. Amylase inhibitors from plant sources have also been characterized. In particular, it has been recognized that the majority of wheat albumins were α-amylase inhibitors (3–6). From gel filtration experiments, evidence was obtained showing that salivary α-amylase binds to wheat albumins of the 24000 dalton group on a mole to mole ratio. In contrast, in the case of pancreatic amylase, the stoichiometry of the amylase-inhibitor complex could not be clearly demonstrated (7). No definite conclusion was attained regarding the biological function of protease and amylase protein inhibitors found in plant materials. Some of these proteins might act as inhibitors of endogenous enzymes and thereby play a repression role in catabolic reactions.

Ten years ago, Satouchi et al. (8, 9) reported the purification of a lipase protein inhibitor from soybean seeds. From kinetic studies it was suggested that inactivation of pancreatic or microbial lipases was caused, not by the direct interaction between enzyme and inhibitor, but rather between inhibitor and emulsified lipid substrate.

From studies by several groups, it is well documented that pancreatic lipase activity on short or long chain triacylglycerols is modulated by various effectors, some of which are of considerable physiological importance. In vitro studies have shown that bile salts are inhibitors of pancreatic lipase. Inhibition is counteracted specifically by colipase, a small protein found with lipase in the pancreatic secretion (10, 11). It has been hypothesized that the function of colipase is to anchor lipase to its lipid substrate in presence of bile salt. Ionic and nonionic detergents can also inhibit pancreatic lipase. However, with these synthetic surface-active substances, colipase failed to restore enzymatic activity unless bile salts were added to the assay system (12, 13). The same general observations were made in inhibition studies of pancreatic lipase by proteins such as serum albumin and β-lactoglobulin (14, 15). Thus, amphiphilic compounds such as synthetic detergents and proteins share the ability to inhibit pancreatic lipase activity on water-insoluble substrates. Because of the possible relevance of soybean proteins during fat digestion in animals, we have found it of interest to investigate further the inhibitory effect of soybean proteins on lipase activity. In this study, we report the isolation from soybean seeds of a protein that inhibits pancreatic lipase activity.

MATERIALS AND METHODS

Lipids

Bile salts and detergents were purchased from Sigma (St. Louis, MO) and used without further purification.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR

Sodium chenodeoxycholate, ursodeoxycholate, and dehydrocholate were prepared from the corresponding acids. Sodium dodecylsulfate was from Serva (Heidelberg, FRG). CHAPS (cholamidopropyldimethylammoniopropanesulfonate) and tributyrin (puriss) were from Fluka (Buchs, Switzerland). Trioctanoylglycerol was a product from Sigma and 1,2 didecanoylglycerol (dicaprin) was a gift from Dr. J. Rietsch (Marseille, France). Phosphatidylcholine was purified from egg lecithin (Sigma) and egg phosphatidylglycerol was obtained from Serdary Research Laboratories Inc. (London, Canada).

Enzyme sources

Pure horse lipase and colipase were prepared at the laboratory according to Rathelot et al. (16) and to Julien et al. (17), respectively. Lipase from *Rh. arrhizus* was purchased from Precibio (Paris, France) and lipase from *Rh. delemar* was a gift from Dr. A. Sugihara (Osaka, Japan).

Analytical methods

Protein concentration was routinely determined by absorbance at 280 nm. Protein content of samples of purified soybean lipase protein inhibitor was estimated by the colorimetric method of Lowry et al. (18) with bovine serum albumin as standard.

Quantitative amino acid analysis and N-terminal determination were performed as described previously (17). Analytical slab gel electrophoresis was carried out on 7.5% (w/v) polyacrylamide gels at pH 8.2, according to Laemmli (19) in Tris–glycine buffer with 1% sodium dodecylsulfate. The molecular weight of the lipase protein inhibitor was estimated under the same conditions with reference proteins (Pharmacia electrophoresis LMW calibration kit).

Assay of lipase activity

Lipase activity was assayed potentiometrically at pH 8 and 25°C with emulsified tributyrin as substrate. Assay conditions reported previously (20) were slightly modified as follows: 0.250 ml of tributyrin was added to 19 ml of 1 mM Tris-HCl buffer, pH 8, containing 4 mM $CaCl_2$ and 10 mM NaCl. Hydrolysis was measured during 3 min after addition of the enzyme. Activity was calculated from the slope of the curve and expressed as enyzme units. One enzyme unit corresponds to the release of one micromole of acid under assay conditions. In some cases, activity was also determined with triolein emulsified in gum arabic, under conditions previously reported (21).

Determination of lipase inhibitory activity

To determine the inhibitory activity of soybean protein samples, an aliquot of the solution was added to the tributyrin assay 4 min prior to lipase (10 enzyme units) and remaining activity was measured. The inhibitory activity of the soybean protein was expressed as inhibitor units. One inhibitor unit was arbitrarily defined as the amount of protein inhibitor that decreases horse pancreatic lipase activity to 50% of the initial value.

Surface pressure techniques

Before each experiment, the trough was cleaned with ethanol, rinsed several times with tap water, and finally with distilled water prepared from alkaline $KMnO_4$ in an all-glass apparatus. The aqueous subphase was composed of 10 mM Tris-HCl buffer, pH 8, containing 0.1 M NaCl, 21 mM $CaCl_2$, and 1 mM EDTA. Residual surface-active impurities were removed by sweeping and suction of the surface. The lipid was spread from chloroform solution with a syringe and several minutes were allowed to pass for solvent evaporation. Then, the lipid film was adjusted to the appropriate pressure. The content of the trough was stirred at a constant rate of 250 rpm. with a magnetic stirrer. In all cases, the surface pressure was measured with a thin platinum Wilhelmy plate (perimeter 3.94 cm) attached to a Beckman R-11C electromicrobalance (model LM 600).

Changes in surface pressure ($\Delta\pi$) during adsorption of the soybean inhibitory protein to the air-water interface or lipid-water interface was studied in a cylindrical trough drilled in a Teflon block (volume, 50 ml; area, 30.2 cm^2) (22).

Kinetic studies of the inhibitory activity of the soybean protein were carried out either in a cylindrical trough (see above) or in a special "zero order" trough (23). The reaction compartment contained 230 ml of solution with a total surface of 123 cm^2. The aqueous phase of the reaction compartment was kept at a constant temperature of 25°C by circulating water in an immersed glass coil.

Interfacial tension measurements

Tension at the tributyrin-water and the triolein-water interfaces in the presence of protein was determined with a Dognon-Abribat Tensiometer as described previously (13). Tris-HCl buffer, 1 mM, pH 8, was used. The concentration of the protein solution injected in the aqueous phase was 10 mg per ml. The volume of the aqueous phase was 50 ml. The surface pressure (π) is related to interfacial tension (γ) by the simple formula, $\pi = \gamma_0 - \gamma$, γ_0 being the interfacial tension of the pure air-water interface.

Isolation of a lipase inhibitory protein from soybeans

The procedure was adapted from that previously described by Satouchi and Matsushita (9). It includes a

Downloaded from www.jlr.org by guest, on June 18, 2012

delipidation step in order to eliminate endogenous lipids to which proteins might adsorb.

Seed coats were removed, and the meal obtained from 100 g of ground cotyledons was extracted with ice-cold acetone. The defatted meal was stored at −20°C until used. Protein was extracted at 4°C from 15 g of soybean meal suspended in 100 ml of 10 mM Tris-HCl buffer, pH 8 (standard buffer). After 15 min under gentle stirring, insoluble material was removed by centrifugation. The resulting supernatant was used for protein fractionation. The protein extract (90 ml) containing a total amount of 1200 mg of protein representing 15% of the protein content of the seeds was saturated to 0.8 ammonium sulfate at 4°C and left for 30 min. Precipitated proteins were separated by centrifugation and dissolved in standard buffer. This fraction was used for the isolation of a pancreatic lipase inhibitory protein. The protein sample obtained after ammonium sulfate fractionation was filtered through a column (5 × 100 cm) of Sephacryl S-200 (Pharmacia, Uppsala, Sweden) in standard buffer. Fractions eluted from the column between the first and the second void volumes contained inhibitory activity. Fractions corresponding to 1.15 to 1.50 void volumes were pooled and used for further protein purification. Analysis of pooled fractions by gel electrophoresis in the presence of sodium dodecylsulfate, revealed the presence of three major proteins and the absence of the Kunitz inhibitor of trypsin. The protein solution, which contained about 80% of the inhibitory activity of the aqueous extract of soybean meal, was placed on a column (1.6 × 25 cm) of DEAE-Trisacryl (IBL, Villeneuve-la-Garenne, France) equilibrated in standard buffer. Unadsorbed proteins were eluted from the column by passage of 180 ml of standard buffer. Elution of adsorbed proteins was performed with a linear salt gradient. As observed from the elution diagram shown in **Fig. 1,** the inhibitory activity recovered from the column distributed in three peaks. Sodium dodecylsulfate gel electrophoresis of pooled fractions corresponding to peak I showed one major and several minor protein bands. Peak I was dialyzed twice against distilled water and lyophilized. Purification of the major protein of this sample was completed by gel filtration through a column (2.5 × 100 cm) of Sephacryl S-200 in 100 mM NaCl, 10 mM Tris-HCl buffer, pH 8. Protein-containing fractions were dialyzed twice against distilled water and lyophilized. About 20 mg of purified lipase inhibitory protein was obtained from 15 g of soybean meal. This preparation, which contained one protein as shown by electrophoresis in the presence of sodium dodecyl sulfate, had a specific activity of 13 inhibitor units per mg, a value sevenfold higher than that found for the crude protein extract from soybean meal.

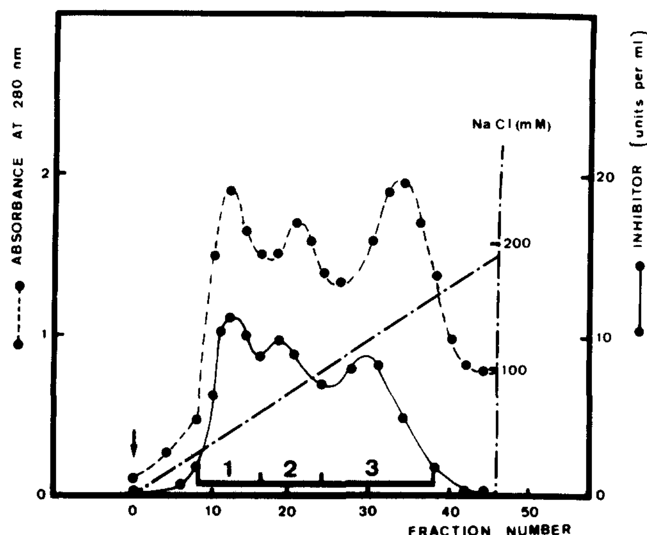

Fig. 1. Chromatography on DEAE-Trisacryl of a protein fraction from soybean seeds. The protein fraction was obtained from ammonium sulfate fractionation and gel filtration on Sephacryl S-200. The column (1.6 × 25 cm) was equilibrated in 10 mM Tris-HCl buffer, pH 8. Unadsorbed proteins were washed out through the column with the same buffer and adsorbed proteins were eluted with a linear NaCl gradient (2 × 200 ml) from 0 to 0.2 M at a flow rate of 30 ml per hr. Fractions of 8 ml were collected. Fractions corresponding to peak I were pooled for further purification. (-----), Absorbance at 280 nM; (——), lipase inhibitory activity. The arrow indicates the start of the gradient.

Gel filtration studies

Gel filtration was used to estimate the molecular weight of the protein inhibitor of lipase isolated from soya. The column (2.5 cm × 100 cm) of Sephacryl S-200 (Pharmacia) equilibrated in 100 mM Tris-HCl buffer, pH 8.0, with 100 mM NaCl was calibrated with bovine serum albumin (M_r: 69,000), ovalbumin (M_r: 45,000), trypsin inhibitor (STI) (M_r: 20,100), and cytochrome C (M_r: 12,400). The molecular weight of the protein inhibitor of lipase was derived from the linear correlation found between elution volumes and log of the molecular weight of the proteins.

Gel filtration was also used to investigate the interaction of horse pancreatic lipase (M_r: 47,000) with the protein inhibitor isolated from soya. A column of Sephacryl S-200 was equilibrated with the same buffer as that used in standard lipase assay (1 mM Tris-HCl buffer, pH 8.0, 10 mM NaCl, and 4 mM $CaCl_2$). One ml containing 5 mg of horse lipase and 12 mg of protein inhibitor in buffer was placed on the column and elution was performed with the same Tris buffer. The protein content of each fraction (2 ml) was determined spectrophotometrically at 280 nm. Lipase activity was measured as described above except that 4 mM sodium deoxycholate and colipase in excess were added to the assay system. Under these conditions, lipase activity could be detected in the presence of protein inhibitor.

Downloaded from www.jlr.org by guest, on June 18, 2012

RESULTS

Properties and stability of the protein-inhibiting lipase activity isolated from soybeans

The protein inhibitor of lipase (PIL) has a molecular weight of about 70,000 as determined by sodium dodecylsulfate polyacrylamide gel electrophoresis and gel filtration. A single N-terminal residue of alanine was identified by the dansylation method. The amino acid composition is given in **Table 1.** The molecular weight calculated from the amino acid composition is similar to that found by other methods. The average hydrophobicity index estimated according to Tanford (24) is 1.8 kcal · $mole^{-1}$, a value which reflects the high content of the protein in nonpolar residues.

The inhibitory activity of the protein was stable in the pH range from 3 to 10 at room temperature for 18 hr. Activity was abolished in 5 min at temperature above 50°C in 10 mM Tris-HCl buffer, pH 8, or by treatment at 30°C for 10 min with trypsin in the same buffer (trypsin to protein ratio: 0.1 per cent w/w). Properties of the purified protein are comparable to those of the protein inhibitor previously characterized by Satouchi et al. (8, 9).

Inhibition of lipase activity

Effect of protein concentration. The inhibitory activity of a crude extract of soybean meal was tested on horse pancreatic lipase by using tributyrin or triolein as substrate. Results are shown in **Fig. 2.** The effect of increasing concentrations of purified soybean inhibitory protein on pancreatic lipase activity is presented in **Fig. 3a.** Hydrolysis of tributyrin was inhibited at protein concentration similar to that found in studies with β-lactoglobulin (14). Enzyme activity was not restored by colipase unless bile salt was present in the assay (Fig. 3b). Sodium deoxycholate, chenodeoxycholate, ursodeoxycholate, and cholate appear as potent activators of the lipase-colipase system inactivated by the soybean protein inhibitor. In contrast, no reactivation was obtained with bile salt analogs as CHAPS or sodium dehydrocholate.

TABLE 1. Amino acid composition of a protein inhibiting pancreatic lipase isolated from soybeans

	Number of Residues	
Residues	Experimental	Nearest Integral
Ala	37.3	37
Arg	31.0	31
Asx	61.8	62
Cys	5.4	5
Glx	79.7	80
Gly	49.3	49
His	17.3	17
Ile	34.4	34
Leu	41.1	41
Lys	38.4	38
Met	9.1	9
Phe	19.6	20
Pro	28.9	29
Ser	46.3	46
Thr	36.1	36
Trp	10.9	11
Tyr	25.3	25
Val	38.2	38
Total number of residues		608

Total weight of residues: 68,582.

Effect of enzyme concentration. The inhibitory activity of the purified soybean protein was determined with three different amounts of lipase in the tributyrin assay. Results are shown in **Fig. 4a and b.** It was found that, at any given protein inhibitor concentration in the assay, the level of inactivation of lipase was independent of enzyme concentration. At inhibitor concentration higher than 0.3 M, no stimulation of tributyrin hydrolysis was observed when the concentration of lipase was increased.

Effect of the amount of substrate. Studies of the influence of the amount of tributyrin on the inactivation of pancreatic lipase by soybean protein showed that the protein concentration needed to obtain 50% inactivation was linearly related to the amount of emulsified substrate in the assay (data not shown).

Effect on various lipases. Lipase from *Rh. delemar* was fully inhibited by the purified protein, while activity of the enzyme from *Rh. arrhizus* was not affected. These inhibition studies were performed in the same protein concentration range as previously used with pancreatic lipase (see above). No reactivation by bile salt and colipase was observed in the case of *Rh. delemar* lipase in contrast to pancreatic lipase.

Surface properties of the soybean inhibitory protein

The effect of increasing concentration of soybean protein on the lipid-water interfacial tension was studied with tributyrin and with triolein. Results are shown in **Fig. 5.** It appears from the curves of Fig. 5a and b that the protein is more surface-active than bovine serum albumin.

The variation of the surface pressure with time during adsorption of the soybean protein at the air-water interface was measured at 25°C at a protein concentration of 0.3 μM in the aqueous phase. The surface pressure reached a steady value of 25 dynes cm^{-1}, 15 min after injection of the protein into the stirred aqueous phase. This value is similar to that measured with bee venom melittin (24 dynes cm^{-1}) (25) and higher than the increase in surface pressure found with snake venom cardiotoxins (10 dynes cm^{-1}) (22).

Downloaded from www.jlr.org by guest, on June 18, 2012

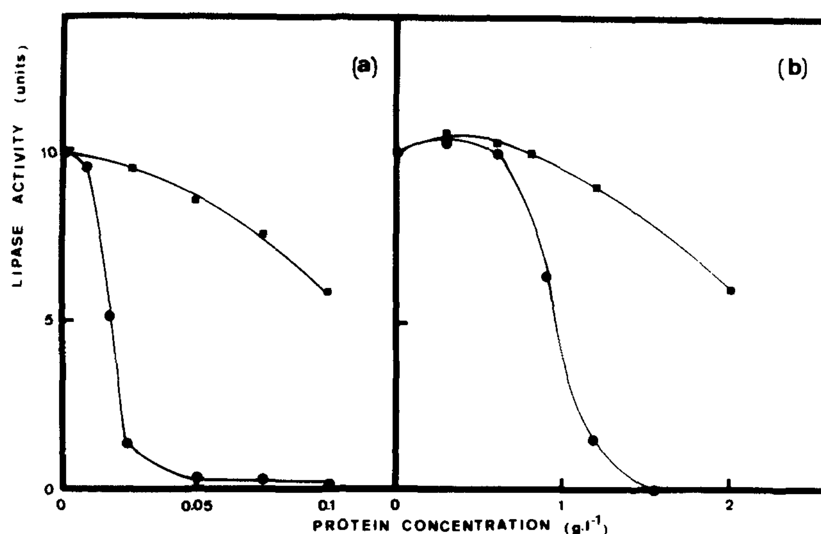

Fig. 2. Inhibition of pancreatic lipase activity by a soybean crude extract. Lipase activity was determined in the absence of colipase with tributyrin (a) and triolein (b) as substrate in the presence of increasing concentrations of protein of an aqueous extract of soybean meal (●). Same experiments with bovine serum albumin (■) are reported for the purpose of comparison.

The surface pressure increase with protein concentration at the trioctanoylglycerol-water interface and the didecanoylglycerol-water interface at 25°C is shown in **Fig. 6a and b.** It is clear from the observed increase in surface pressure that the protein purified from soybean binds to monomolecular lipid films whose initial surface pressure are as high as 25 dynes cm^{-1}. Similar conclusions were reached from results of experiments performed with films of phosphatidylcholine and phosphatidylglycerol (data not shown). In contrast, no change in surface

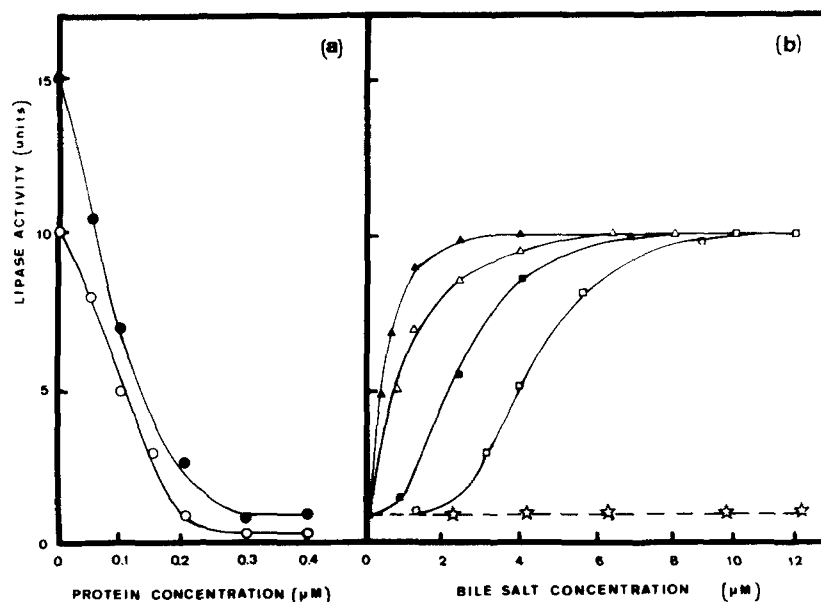

Fig. 3. Effect of a lipase-inhibitory protein isolated from soybeans on the hydrolysis of tributyrin by equine pancreatic lipase. (a) Experiments performed in the absence (○) and in the presence (●) of a fivefold excess (molar ratio) of colipase. (b) Reactivation of protein-inhibited lipolysis reaction by increasing amounts of various bile salts. Sodium deoxycholate (▲), chenodeoxycholate (△), ursodeoxycholate (■), and cholate (□) were added to the assay system containing lipase (2 nM), colipase (10 nM), and the soybean lipase inhibitory protein at the concentration of 0.4 μM (28 mg l^{-1}). Stars represent experiments performed with sodium dehydrocholate or CHAPS.

Downloaded from www.jlr.org by guest, on June 18, 2012

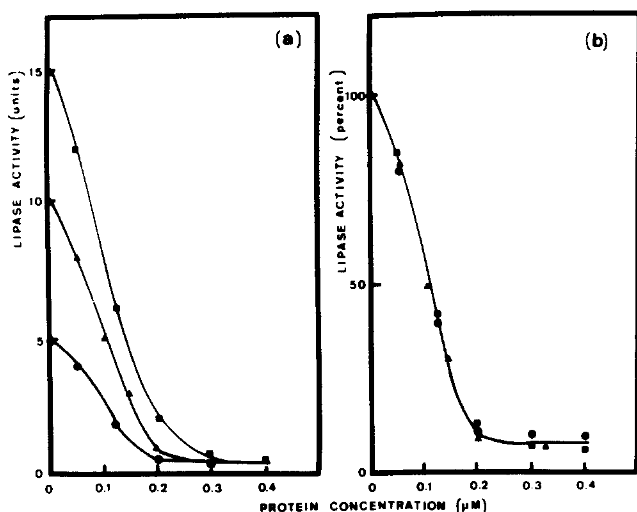

Fig. 4. Inhibition of pancreatic lipase by increasing concentration of soybean inhibitory protein at various lipase concentrations. The tributyrin assay system contains no colipase and no bile salts. (a), Experimental points obtained at lipase concentrations of 1 nM (●), 2 nM (▲), and 3 nM (■). (b), Experimental values as in Fig. 3a have been replotted as % of remaining lipase activity.

pressure was observed with films of sodium taurodeoxycholate at initial pressures of 18 or 27 dynes cm^{-1} (data not shown).

Effect of the soybean inhibitory protein on the kinetics of hydrolysis of dicaprin monolayers by various lipases

Kinetic inhibition studies of lipase activity were carried out with dicaprin films as substrate either at constant surface area of the film with the cylindrical Teflon trough (**Fig. 7a**) or a constant surface pressure using the "zero order" trough (Fig. 7b). The protein inhibitor (final concentration, 0.2 μM) was injected under the dicaprin film spread at the initial surface pressure of 35 dynes per cm. Upon injection of the protein inhibitor, no significant change in surface pressure was observed under these conditions. No further change in surface pressure was detected after horse pancreatic lipase (final concentration, 80 pM) was injected into the aqueous subphase. By contrast to pancreatic lipase, injection of *Rh. arrhizus* lipase caused a drop in surface pressure which indicated the hydrolysis of dicaprin (Fig. 7a). The kinetic study of the hydrolysis of a dicaprin film maintained at 35 dynes per cm in the "zero order" trough (Fig. 7b) showed that the rate of the reaction in the presence of horse lipase and protein inhibitor represented less than 5% of the control performed in the absence of inhibitor. Under the same conditions, a maximal rate of hydrolysis was obtained after the injection of *Rh. arrhizus* lipase and protein inhibitor (Fig. 7b).

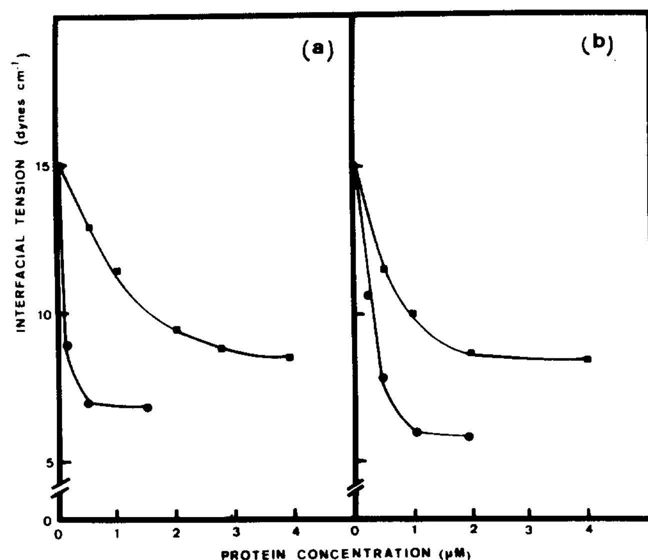

Fig. 5. Influence of the concentration of soybean lipase inhibitory protein on the interfacial tension. Experiments with the purified soybean protein (●) were performed at tributyrin-water (a) and triolein-water (b) interfaces. Results of experiments performed with serum albumin (■) are shown for the purpose of comparison.

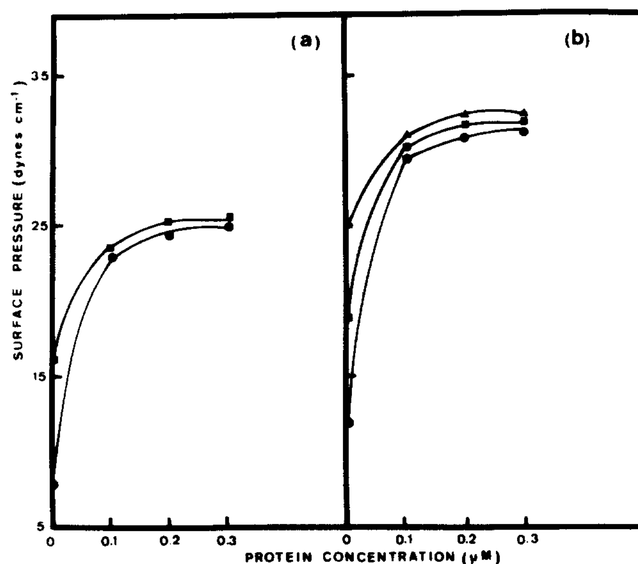

Fig. 6. Variation of the surface pressure of glyceride monomolecular films spread at the air-water interface as a function of increasing concentrations of soybean lipase inhibitory protein. (a) Experiments with trioctanoylglycerol films at initial pressures of 8 dynes cm^{-1} (●) and 16 dynes cm^{-1} (■). (b) Experiments with 1,2-didecanoylglycerol films at initial pressures of 12 dynes cm^{-1} (●), 19 dynes cm^{-1} (■), and 25 dynes cm^{-1} (▲).

Study of the interaction of horse pancreatic lipase with soybean protein inhibitor by gel filtration

A mixture of horse lipase and protein inhibitor was passed through a column of Sephacryl S-200. Proteins were eluted in separate symmetrical peaks at 1.76 and 1.4 void volumes, respectively. Positions of the peaks were identical to those found when horse lipase and protein inhibitor were separately applied to the column.

ASBMB JOURNAL OF LIPID RESEARCH JLR
Downloaded from www.jlr.org by guest, on June 18, 2012

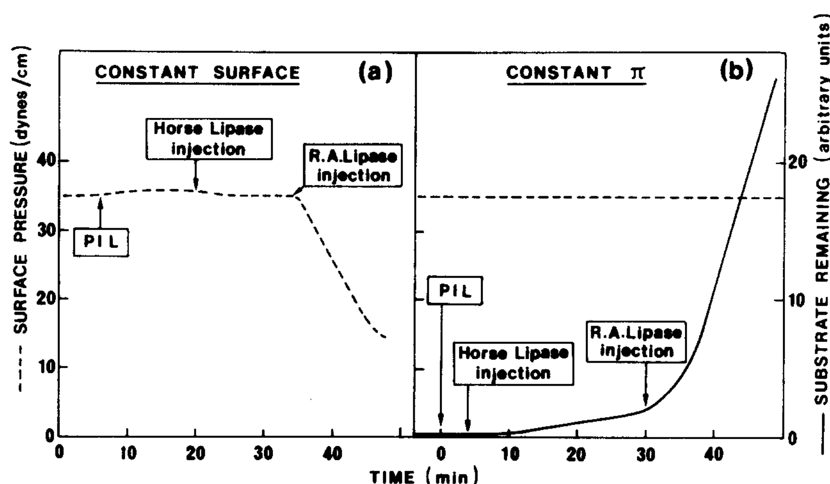

Fig. 7. Kinetic study of the hydrolysis of dicaprin film by various lipases at (a) constant surface area in a cylindrical Teflon trough (volume, 50 ml; surface, 30.2 cm^2) and (b) constant surface pressure in a "zero order" trough. (For details see Materials and Methods). Panels (a) and (b): the arrows indicate the successive injections of protein inhibitor (PIL) at a final concentration of 0.2 μM, horse lipase at a final concentration of 80 pM, and *Rh. arrhizus* lipase (R.A.) at a final concentration of 40 pM into the aqueous subphase. Buffer: 10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 21 mM $CaCl_2$, 1 mM EDTA.

DISCUSSION

Lipases and phospholipases represent a particular class of esterases that catalyze the hydrolysis of ester bonds in lipid molecules that form multimolecular aggregates such as micelles, vesicles, membranes, or emulsified particles. The reaction occurs at a lipid-water interface and is critically dependent upon the binding of the enzyme to the interface. In this respect, all amphiphilic substances acting as emulsifiers are expected to influence the rate of the interfacial reaction. It has been shown that bile salts or synthetic detergents added to preformed emulsions of short or long chain triacylglycerols behave as strong inhibitors of lipolysis (12, 13). Inhibition is due to enzyme desorption from the surface of the particles. It is not yet known whether desorption results from direct interaction between the enzyme and amphiphilic molecules or from modification of the properties of the substrate by adsorbed amphiphile. The activity of bile salt-inhibited pancreatic lipase is specifically restored by colipase. However, this cofactor fails to restore the activity of detergent-inhibited lipase activity unless naturally occurring bile salt is added to the lipolysis system.

There is evidence, from in vitro studies, that proteins can also affect pancreatic lipase activity on emulsified triacylglycerols. Ten years ago, Satouchi et al. (8, 9) reported that protein inhibitors of pancreatic lipase are present in soybean meal. More recently, Borgstrom and Erlanson (14) and Blackbert et al. (15) showed that pancreatic lipase activity is inhibited by hydrophobic proteins such as serum albumin or β-lactoglobulin in absence of colipase and bile salt.

Because of the nutritional relevance of the inhibitory activity of some dietary proteins on pancreatic lipase, we have isolated a lipase-inhibiting protein from soybean meal and studied its influence on lipase activity in comparison with serum albumin and β-lactoglobulin previously investigated.

Pancreatic lipase activity on tributyrin or triolein is inhibited by a crude aqueous extract from soybean meal. We have isolated a protein of about 70,000 molecular weight. This protein-inhibits pancreatic lipase in the same protein concentration range as β-lactoglobulin. Activity is not restored by colipase unless bile salt is present in the assay system. Only naturally occurring bile salts are activators of pancreatic lipase and colipase. Bile salt analogs such as sodium dehydrocholate or CHAPS cannot restore lipase activity.

The lipase-inhibiting protein isolated from soybean is highly surface-active. It decreases interfacial tension at the tributyrin-water and triolein-water interfaces at protein concentration in the micromolar range. It also adsorbs to monomolecular films of glyceride or phospholipid spread at the air-water interface at surface pressure as high as 25 dynes cm^{-1}.

It is found, as in the case of lipase inhibition by bile salt or synthetic detergent (13), that the level of inhibition of the enzyme was independent of the amount of lipase in the assay. No interaction between pancreatic lipase and soybean inhibitory protein could be observed in gel filtration studies. It appears then unlikely that lipase

Downloaded from www.jlr.org by guest, on June 18, 2012
ASBMB JOURNAL OF LIPID RESEARCH JLR

inhibition by plant proteins, or proteins from other sources, results from the formation of a well-characterized soluble enzyme-inhibitor complex as in the case of proteases.

Lipases from fungi showed different sensitivity to inhibition by the soybean protein. Lipase from *Rh. delemar* is strongly inhibited while activity from the *Rh. arrhizus* enzyme remained unchanged under the same experimental conditions. This finding might reflect differences in binding capacities of these lipases to the protein-modified lipid substrate.

The influence of dietary proteins on the hydrolysis of fat in the intestinal lumen remains difficult to evaluate. It is reasonable to think that surface-active proteins interacting with lipids might affect in vivo lipolysis of triacylglycerols, in particular at low bile salt concentration. Further studies are certainly necessary to evaluate their possible influence upon intestinal fat absorption.

The authors would like to thank Dr. A. G. Bois for helpful discussions. They gratefully acknowledge the excellent technical assistance of Mrs. F. Ferrato and Mr. J. Vernoni and they express their appreciation to Mrs. Charpentier for typing the manuscript.

Manuscript received 10 April 1984.

REFERENCES

1. Kassell, B. 1970. Trypsin and chymotrypsin inhibitors from soybeans. *Methods Enzymol.* **19:** 853–862.
2. Birk, Y. 1976. Trypsin and chymotrypsin inhibitors from soybeans. *Methods Enzymol.* **45:** 700–707.
3. Silano, V., F. Pocciari, and D. D. Kasarda. 1973. Physical characterization of α-amylase inhibitors from wheat. *Biochim. Biophys. Acta.* **317:** 139–148.
4. O'Donnell, M. D., and K. F. McGeeney. 1976. Purification and properties of an α-amylase inhibitor from wheat. *Biochim. Biophys. Acta.* **422:** 159–169.
5. O'Conner, C. M., and K. F. McGeeney. 1981. Isolation and characterization of four inhibitors from wheat flour which display differential inhibition specificities for human salivary and human pancreatic α-amylases. *Biochim. Biophys. Acta.* **658:** 387–396.
6. Orlando, A. R., P. Ade, D. Di Maggio, C. Fanelli, and L. Vitozzi. 1983. The purification of a novel amylase from *Bacillus subtilis* and its inhibition by wheat proteins. *Biochem. J.* **209:** 561–564.
7. O'Conner, C. M., and K. F. McGeeney. 1981. Interaction of human α-amylases with inhibitors from wheat flour. *Biochim. Biophys. Acta.* **658:** 397–405.
8. Satouchi, K., T. Mori, and S. Matsushita. 1974. Characterization of inhibitor protein for lipase in soybean seeds. *Agr. Biol. Chem.* **38:** 97–101.
9. Satouchi, K., and S. Matsushita. 1976. Purification and properties of a lipase-inhibiting protein from soybean cotyledons. *Agr. Biol. Chem.* **40:** 889–897.
10. Semeriva, M., and P. Desnuelle. 1976. Pancreatic lipase and colipase. An example of heterogeneous biocatalysis. *Horiz. Biochem. Biophys.* **2:** 32–59.
11. Borgström, B., C. Erlanson-Albertsson, and T. Wieloch. 1979. Pancreatic colipase: chemistry and physiology. *J. Lipid Res.* **20:** 805–816.
12. Borgstöm, B., and C. Erlanson. 1973. Pancreatic lipase and colipase. Interactions and effects of bile salts and other detergents. *Eur. J. Biochem.* **37:** 60–68.
13. Gargouri, Y., R. Julien, A. G. Bois, R. Verger, and L. Sarda. 1983. Studies on the detergent inhibition of pancreatic lipase activity. *J. Lipid Res.* **24:** 1336–1342.
14. Borgstöm, B., and C. Erlanson. 1978. Interaction of serum albumin and other proteins with porcine pancreatic lipase. *Gastroenterology.* **75:** 382–386.
15. Blackberg, L., O. Hernell, G. Bengtsson, and T. Olivecrona. 1979. Colipase enhances hydrolysis of dietary triglycerides in the absence of bile salts. *J. Clin. Invest.* **64:** 1303–1308.
16. Rathelot, J., R. Julien, I. Bosc-Bierne, Y. Gargouri, P. Canioni and L. Sarda. 1981. Horse pancreatic lipase. Interaction with colipase from various species. *Biochimie.* **63:** 227–234.
17. Julien, R., G. Bechis, J. Grégoire, J. Rathelot, H. Rochat, and L. Sarda. 1980. Evidence for the existence of two isocolipases in horse pancreas. *Biochem. Biophys. Res. Commun.* **95:** 1245–1252.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
19. Laemmli, V. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature.* **227:** 680–685.
20. Canioni, P., R. Julien, J. Rathelot, and L. Sarda. 1976. Inhibition of sheep pancreatic lipase activity against emulsified tributyrin by non-ionic detergents. *Biochimie.* **58:** 751–753.
21. Rathelot, J., R. Julien, P. Canioni, C. Coeroli, and L. Sarda. 1975. Studies of the effect of bile salt and colipase on enzymatic lipolysis. Improved method for the determination of pancreatic lipase and colipase. *Biochimie.* **57:** 1117–1122.
22. Bougis, P., H. Rochat, G. Pieroni, and R. Verger. 1981. Penetration of phospholipid monolayer by cardiotoxins. *Biochemistry.* **20:** 4915–4920.
23. Verger, R., and G. H. De Haas. 1973. Enzyme reactions in a membrane model. 1. A new technique to study enzyme reactions in monolayers. *Chem. Phys. Lipids.* **10:** 127–136.
24. Tanford, C. 1962. Contribution of hydrophobic interactions to the stability of the globular conformation of proteins. *J. Am. Chem. Soc.* **84:** 4240–4247.
25. Sessa, G., G. H. Freer, G. Colacicco, and G. Weissmann. 1969. Interaction of a lytic peptide, melittin, with lipid membrane systems. *J. Biol. Chem.* **244:** 3575–3582.

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH JLR