THE EFFECT OF SERUM PROTEINS ON THE ACTIVITY OF PANCREATIC LIPASE

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The whole serum, its protein fractions isolated electrophoretically, and the pure human and rabbit serum albumins inhibited hydrolysis of tributyrin by a glycerine extract of porcine pancreatic lipase or by human pancreatic juice. Out of the isolated fractions, an eluate corresponding to α-globulins had the strongest inhibitory effect. Under the given experimental conditions the inhibition by albumin depended on its concentration in the medium. Digestion of albumin by pepsin completely destroyed its inhibitory effect on the lipolytic reaction.

The effect of naturally occurring inhibitors of enzymic systems is an important problem. The early papers on this subject deal with the hydrolytic enzymes. Some reliable data were obtained by studying the action of natural inhibitors on proteolytic enzymes[1–6]. Similar studies[7–12] treated the effects of natural inhibitors and activators on the enzymic systems operating in the hydrolysis of fat. An important, but still obscure role in the hydrolysis of fat may be played by proteins[13–18]. The present paper demonstrates that the activity of pancreatic lipase may be affected by serum proteins.

EXPERIMENTAL

Chemicals. Tributyrin was freed from butyric acid in 20% NaOH, followed by extraction of sodium butyrate into water. Further used were freeze-dried human serum albumin (electrophoretic purity 90%, protein content 88·3%) and electrophoretically pure freeze-dried rabbit serum albumin. In order to compare the effects of the serum proteins we used a fraction of normal human serum, obtained by electrophoresis on paper in a barbitone buffer (pH 8·6, 250 V, 8 h). Some electrophoreograms were developed by bromophenol blue, the corresponding protein fractions were obtained from the remaining ones by elution with 0·9% NaCl. The protein contents in the eluates were determined according to Lowry and coworkers[19]. The amounts of the eluates used for the experimentals were such that the protein concentrations in the final reaction mixtures were 200 μg/ml. We also used a porcine serum obtained by centrifugation of fresh blood.

Extraction and determination of lipase. The experiments were carried out with a preparation of porcine pancreatic lipase (Swiss Ferment. Co.); 300 mg of the preparation were shaken for 1 h in 100 ml of 80% glycerine at 20°C. The lipase activity was determined by potentiometric titra-

tion[20] of the butyric acid released from tributyrin. The enzymic reaction was performed at 37°C and pH 7·1. The activity of the enzyme is given in μmol of butyric acid released from tributyrin or in % of activity (μ mol/min) related to a control, where the investigated compounds were absent.

RESULTS

In studying the effect of blood proteins on activity of pancreatic lipase we first used serum from fresh porcine blood. This markedly inibited hydrolysis of tributyrin (Fig. 1). The inhibitory effect was proportional to concentration of the serum added and was apparent even at a thousandfold dilution of the serum.

We investigated which constituent of the serum was responsible for the inhibition. From electropherogram of human sera we eluted the individual protein fractions and tested their effects on the lipolytic system (Fig. 2). With the given amounts

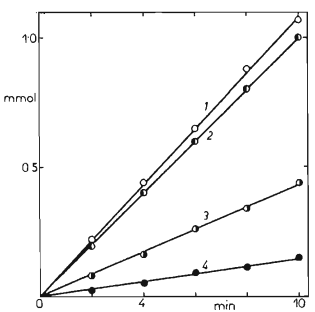

FIG. 1

Effect of Blood Serum on the Activity of Pancreatic Lipase

Total volume of the incubation medium 30 ml, pH 7·1, 37°C; mmol denotes the amount of butyric acid released from tributyrin, min time of incubation. *1* Control, *2* porcine blood serum added to a final dilution of 1 : 1000, *3* 1 : 100 and *4* 1 : 10.

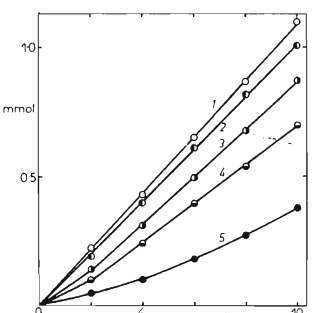

FIG. 2

Effect of Protein Fractions of Human Serum on the Activity of Pancreatic Lipase

Total volume of the incubation medium 30 ml, pH 7·1, 37°C. mmol denotes the amount of butyric acid released from tributyrin, min time of incubation. *1* Control, *2* with the addition of albumin, 200 μg . ml^{-1}; *3* γ-globulin, 200 μg ml^{-1}; *4* β-globulin, 200 μg ml^{-1}; *5* α-globulin, 200 μg . ml^{-1}.

of the individual proteins the isolated fractions exhibited different inhibitory effects. The strongest inhibition was attained with the eluate covering the region of α-globulins. The degree of inhibition gradually decreased in the order β-globulin, γ-globulin, albumin. The inhibitory effect of the albumin fraction, though the weakest, was still well apparent and reproducible.

In further work we used pure human and rabbit serum albumins. A number of papers[11-18] have shown that serum albumin markedly affects the course of lipolytic enzymic reactions, but its mode of action has not been elucidated. Fig. 3 shows that pure human albumin also inhibited (similarly to the serum) the cleavage of tributyrin in relation to its concentration. One mg of human albumin in 1 ml of the incubation mixture brought about an inhibition exceeding 50%. With the rabbit albumin the result was analogous. Further we investigated, whether this inhibition was caused by the protein or was a result of other factors. To decide this question we added, to the incubation medium, either native albumin, or albumin that had been treated with pepsin. As can be seen from Fig. 4, the albumin treated with pepsin

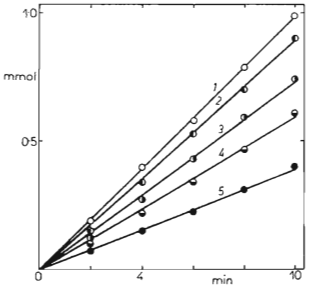

FIG. 3

Activity of Pancreatic Lipase in Relation to Increasing Concentration of Albumin

Total volume of the incubation medium 30 ml, pH 7·1, 37°C. mmol denotes the amount of butyric acid released from tributyrin, min the time of incubation. *1* Control, *2*—*5* with the additions of human albumin, 75, 150, 300 and 1000 μg ml^{-1} respectively.

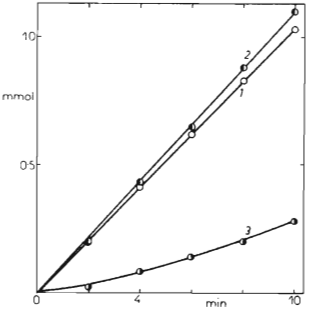

FIG. 4

Effect of Pepsin-Digested Albumin on Activity of Pancreatic Lipase

Total volume of incubation medium 30ml, pH 7·1, 37°C. mmol denotes the amount of butyric acid released from tributyrin, min the time of incubation. *1* Control, *2* pepsin — digested rabbit albumin added, 1000 μg ml^{-1}, *3* rabbit intact albumin added, 1000 μg ml^{-1}.

did not inhibit the hydrolysis of tributyrin (instead it rather catalyzed the reaction), whereas the intact albumin inhibited it considerably. The results of this and other experiments (Fig. 5), in which albumin was added at the beginning of the incubation or 8 min later, reveal that the inhibitor proper is a protein. We have also attempted to get some insight into the nature of the inhibition of lipase by albumin. Fig. 6 suggests that the inhibition is competitive. To verify our results we also used lipase isolated from fresh pancreatic juice of a patient. The conclusions from the experiments were the same as in the use of the standard pancreatic preparation.

Further we investigated the participation of calcium in the cleavage of the substrate by pancreatic lipase, since calcium is still believed to activate this system[7,13–15,21]. At 0·02–20 mM Ca^{2+} in a medium containing 150 μg of albumin per ml calcium had an inhibitory effect. If the medium contained 1 mg of albumin per ml, then the same concentrations of calcium produced a higher activity of lipase than that in a control containing albumin only. However, this activity was still far short of that exhibited by a control without albumin (Table I).

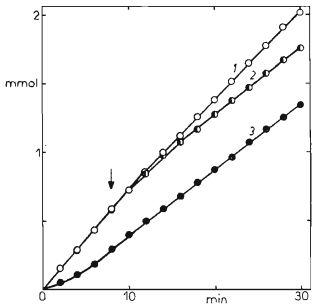

FIG. 5

Effect of Subsequent Addition of Albumin on the Activity of Pancreatic Lipase

Total volume of incubation medium 30 ml, pH 7·1, 37°C, mmol denotes the amount of butyric acid released from tributyrin. *1* Control, water added after 8 minutes' incubation, *2* with rabbit albumin, 600 μg ml^{-1}, added after 8 minutes' incubation, *3* with rabbit albumin, 600 μg ml^{-1}, added at the start of incubation.

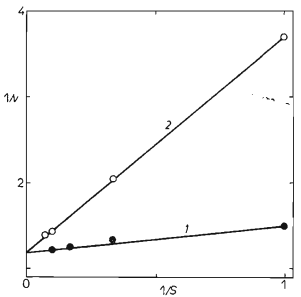

FIG. 6

Effect of Substrate Concentration on Inhibition of Pancreatic Lipase by Albumin

Total volume of incubation medium 30 ml, pH 7·1, 37°C. *S* denotes the concentration of tributyrin in ml 30^{-1}; v the reaction rate in mmol of butyric acid released from tributyrin . 10 min^{-1}. *1* Control, *2* with the addition of rabbit albumin, 300 μg ml^{-1}.

DISCUSSION

The chosen substrate of pancreatic lipase was tributyrin, which is frequently used for this purpose[22]. The action of lipase on substrates is not strictly specific[23]. Under the conditions we used emulsified tributyrin is rapidly hydrolysed by pancreatic lipase[24]. It was suggested in some papers[13-15] that lipase is strongly activated by albumin and calcium. However, inhibition by albumin under certain conditions has been observed too[11,16]. Our results also are inconsistent with the reported activation of pancreatic lipase by protein[14]. We observed that the enzyme, extracted from a pancreatic preparation or from human pancreatic juice, was inhibited by whole human serum, its protein fractions isolated electrophoretically, and by pure human or rabbit albumin (Figs 1–3, 5). A direct inhibitory effect of proteins can be inferred from the experiment with albumin, which after digestion by pepsin was no longer an inhibitor of the hydrolysis (Fig. 4). The other blood proteins were not exposed to this digestion, so that we cannot rule out that their complexes with other components participate in the inhibition.

The blood constituents also affect enzymic reactions of other types. This has been shown, *e.g.*, by Levy and Lepow[16] in a study of C-1-esterase and by Tolnay and Bagdy[25] in a study of pancreatic elastase. These serum inhibitors exhibit properties typical of proteins and in the case of pacreatic elastase their inhibitory effect was 1 000–2 000 fold stronger than that of the inorganic components of serum. The results of our experiments are consistent with these findings.

TABLE I

Inhibition of Pancreatic Lipase in Relation to Concentration of Ca^{2+} Ions

The total volume of the incubation medium 30 ml, pH 7·1, 37°C.

$CaCl_2$ added M	Addition of albumin µg/ml		
	0	150	1 000
	butyric acid released from tributyrin mmol . 10 min^{-1}		
0	1·053	0·953	0·335
$2 . 10^{-4}$	1·050	0·897	0·431
$2 . 10^{-3}$	1·072	0·827	0·533
$2 . 10^{-2}$	1·070	0·715	0·465

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