A FACTOR OF PROTEIN CHARACTER IN RAT LIVER INHIBITING LIPASE ACTIVITY OF RAT PANCREAS

R.MACHOVICH, J.CSILLAG and Anikó NÁRAY
Institute of Medical Chemistry, Semmelweis University Medical School, Budapest, Hungary

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1. Introduction

We have observed that a component of rat liver homogenate inhibits the lipase (EC 3.1.1.3) activity of rat pancreas extracts (fig. 1). In order to characterize the nature of this inhibition, the inhibitory agent has been partially purified and its effect on the activity of lipase has been studied.

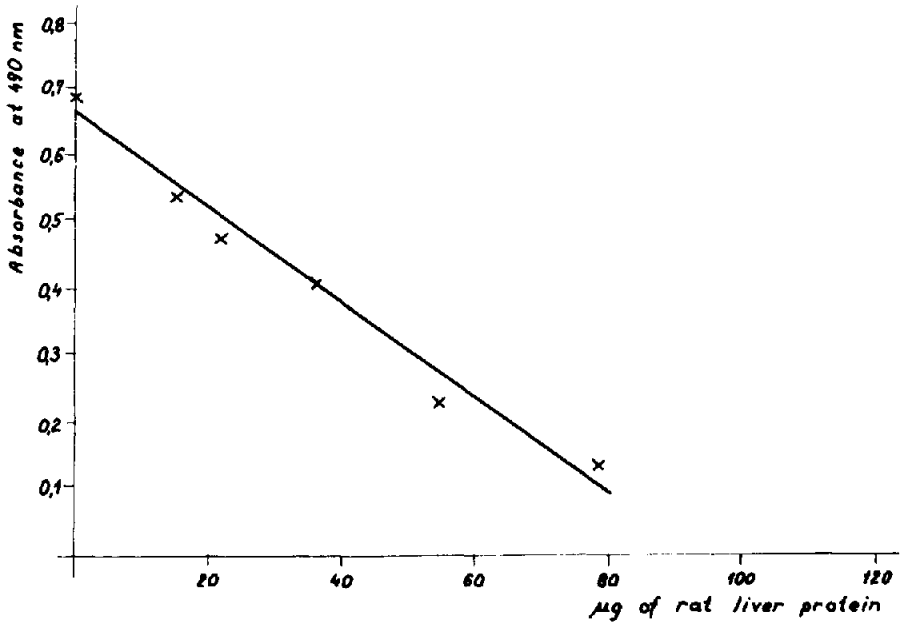

Fig. 1. Inhibition of lipase activity. The activity of lipase was assayed according to the method of Meyer-Bertenrath [1]. The standard assay mixture consisted of the following components: 500 µg fluorescein-di-laurylate dissolved in 0.1 ml of the ethyleneglycol monomethylether, 3.9 ml of 0.1 M tris-HCl buffer, pH 8.0, 50 µg of rat pancreas protein partially purified, µg of rat liver protein as indicated on the graph and water in a total volume of 5.0 ml. Samples were incubated for 8 min at 37°. The incubation was stopped by 2 ml of 96% ethanol and after centrifugation, the absorbance of liberated fluorescein was measured at 490 nm. (1 Absorbance unit = 1.3×10^{-5} M fluorescein solution.)

2. The preparation of inhibitor factor from rat liver

Liver tissue was homogenized in twenty volumes of acetone (−18°) and acetone dry powder was prepared. The acetone powder was suspended at 0° in 5 mM tris-HCl, pH 7.5, containing 1 mM $CaCl_2$ (50 mg per ml) and the undissolved material was removed by centrifugation. The supernatant was brought to pH 13 by the addition of 1 N NaOH and after standing at 0° for 30 min, the pH was restored to pH 7.0, with 1 N HCl. The precipitate formed was removed by centrifugation and the supernatant fractionated with ammonium sulfate. The ammonium sulfate fraction precipitating between 0.30 and 0.60 saturation was dialyzed against 5 mM tris-HCl, pH 7.5, containing 1 mM $CaCl_2$.

This inhibitor factor appears to be associated with a protein. Its molecular weight was estimated as about 100,000 by Sephadex gel filtration. The inhibitory effect was abolished by trypsin treatment but not by ribonuclease (table 1). Heat treatment at 50° for 5 min destroyed 50% of its activity, and at 60° for 5 min destroyed 100% of its activity. The inhibitor factor was not contaminated by trypsin or other proteolytic activities in such quantities as to depress the activity of lipase.

It seems that the inhibitor factor is specific for lipase (table 2). Amylase and trypsin are not inhibited by this factor.

As the lipase inhibitor is also able to relieve the haemolytic effect of lipase, it is supposed that it might play an important role in the protection of the organism, and its function might become more significant in the case of some pathological processes.

Table 1
Effect of trypsin treatment on the activity of lipase inhibitor.

Fraction digested for 60 min		Trypsin	Trypsin + lipase inhibitor	Lipase inhibitor	Soybean inhibitor only
Lipase acivity*	0.45	0.49	0.52	0.32	0.52

600 μg of lipase inhibitor protein were incubated with 500 μg of trypsin in a 0.65 ml of 5 mM M tris-HCl, pH 7.5, containing 1 mM $CaCl_2$ at 30° for 60 min. Subsequent to incubation, 2.0 mg of soybean trypsin inhibitor were added to each reaction mixture. The lipase activities were measured in the presence of 0.2 ml of these digested fractions.

* The activity of lipase was expressed as the absorbance of fluorescin liberated by 100 μg of pancreas protein. See fig. 1.

Table 2
Action of lipase inhibitor on pancreatic enzymes.

Enzymes	Activity of enzyme	Activity of enzyme in the presence of 50 μg of inhibitor protein
Amylase* (5 μg per reaction mixture)	27.9	28.4
Trypsin** (25 μg per reaction mixture)	0.56	0.55
Lipase*** (50 μg protein per reaction mixture)	0.43	0.22

* Amylase activity (units per reaction mixture) was assayed according to the method of Smith and Roe [2].

** Trypsin activity was assayed according to the method of Erlanger et al. [3]. The activity of trypsin is expressed by increase in absorbance at 410 nm of *p*-nitroaniline liberated from *N*-benzoyl-DL-arginine-*p*-nitroanilide in 7 min at 37°.

*** Lipase activity is expressed as in fig. 1.

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