ACTIVATION OF LIPOPROTEIN LIPASE IN VITRO BY UNSATURATED PHOSPHOLIPIDS

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1. Introduction

Lipoprotein lipases (LPL) are a group of enzymes which hydrolyze triglycerides in the presence of lipoproteins or certain apolipoproteins [1,2]. The activation of lipoprotein lipases from rat heart [1], adipose tissue [2,3], bovine milk [4] and human plasma [5] by apoldipoproteins has been investigated. Apo–Lp–Glu and apo–Lp–Ala, present as major components in human very low density lipoproteins (VLDL) and as minor components in high density lipoproteins (HDL) have been reported as the co-activators for the LPL [6,7]. Although the plasma co-factor peptides have been intensively studied, little attention has been given to the role of classes of phospholipid on the activation of the lipoprotein lipase.

The effects of phosphatidylcholine [8] and other individual phospholipids [9] on milk lipoprotein lipase and on the enzymes derived from rat epididymal tissue have been studied. The enzymatic activity decreased markedly when the chain length of the saturated acyl chains in the phosphatidylcholine molecule was increased. Furthermore studies on the treatment of the hyperlipoproteinaemia with polyunsaturated phosphatidylcholine (PU-PC) showed a decrease of cholesterol ($p < 0.01$) and phospholipids but not of plasma triglycerides [10].

In view of the uncertainties of the effect of PU-PC on the hypertriglyceridaemia we investigated the effect of PC-species and of the double bonds in the fatty acid molecule on the lipoprotein lipase activity in vitro using a well-defined artificial triglyceride emulsion and cow milk lipase.

2. Materials

2.1. *Lipoprotein lipase preparation*

Skimmed cow's milk was prepared according to Bier and Havel [1] and dialysed overnight against distilled water. After lyophilization, acetone butanol powders were prepared by extraction of 1 g of the crude LPL with 40 ml precooled acetone and repeated twice, respectively with 25 ml butanol and 25 ml acetone. The air-dried powder was stored at −20°C, and was resuspended as a 4% solution in 0.1 MNaCl just prior to assay.

2.2. *Co-factor lipoproteins and LPL-activators*

Lipoproteins were prepared by ultracentrifugation of plasma from fasting normal male donors [11]. Apo-VLDL was prepared according to Koga [12]. Purified soybean phosphatidylcholine and samples of essential phospholipids (EPL-lipostabil) were presented to us by Natterman (Köln). Egg-yolk phosphatidylcholine was purified on Al_2O_3. Saturated PC was prepared by hydrogenation of the unsaturated form derived from egg-yolk and EPL-samples. Bovine serum albumin (Poviet) was used as free fatty acid (FFA) acceptor.

2.3. *Substrates*

Triolein (Appl. Sc.) or corn oil was mixed with PC in chloroform as described in the results. After solvent evaporation, 3.33% corn oil triglycerides were dispersed in 0.15 M NaCl for the pH-stat method and 4.5% in 0.3 M NH_3–NH_4Cl, pH 8.8, for the batch method. Sonication was with a Brandson B12 sonifier for three periods of 30 sec at 50 W.

3. Analytical methods

Protein concentrations were determined by the method of Lowry et al. [13] using bovine serum albumin as standard. Free fatty acids were determined by microtitration [14] and the released FFA were analysed by GLC [15].

Lipoprotein lipase activity was followed by two different methods previously used.

In the pH-stat method [16] a solution of 0.1 ml albumin (20 mg%), 0.1 ml apo-VLDL (20 mg%), 1.2 ml milk LPL (4%) and 0.8 ml NaCl (0.8%) was adjusted to pH 8.8 at 37°C and mixed with 0.6 ml substrate adjusted to pH 8.8 at 37°C. The hydrolysis was followed continuously at pH 8.8 by pH-titration with 0.1 N NaOH during 45 min at 37°C with a Radiometer TTT-2 pH-stat.

In the batch method the reaction mixture contained 1.5 ml LPL (4%), 1 ml substrate, 1 ml 0.3 M NH_3–NH_4 Cl-buffer, pH 8.8, 0.2 ml VLDL (40 mg% protein) and 0.5 ml albumin (4%). After incubation at 37°C during 1 hr, 1 ml of the reaction mixture was extracted with 5 ml Dole-extraction mixture (isopropanol–heptane–1 N H_2SO_4; 40:10:1 by volume). After addition of 6 ml heptane and 2 ml H_2O, 3 ml of the heptane phase is titrated with 0.025 NaOH using Nile Blue as indicator.

4. Results

4.1. *Effect of the substrate, pH, FFA-acceptor and of co-factor lipoprotein on the LPL-activity*

As there is no specific difference between the LPL-activity on a pure triolein and on a corn oil substrate, the latter was chosen for further studies (fig. 1). From our earlier results and from the literature [6, 7] it is known that the rate of hydrolysis is increased more by apo-VLDL than by apo-HDL and activation is further increased by the presence of lecithin (PC) in the reaction mixture. From the activation curves presented in fig. 2 we extrapolate to apo-VLDL concentration (10–20 μg/ml), pH 8.8 and 0.1 ionic strength as the optimal conditions for LPL-activity. Heparin has no influence on the enzyme system.

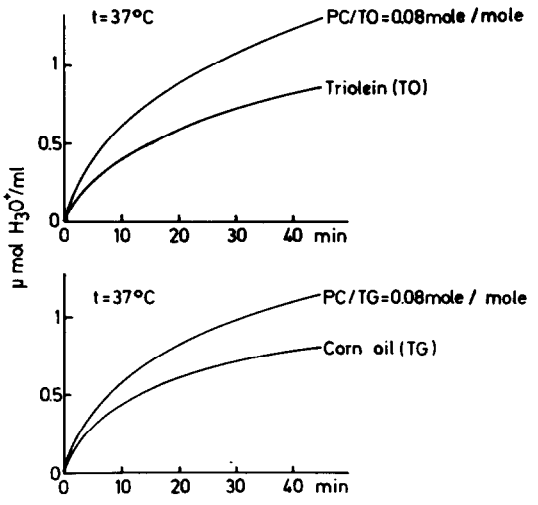

Fig. 1. Activation of LPL by egg-yolk PC on a triolein and on a corn oil substrate.

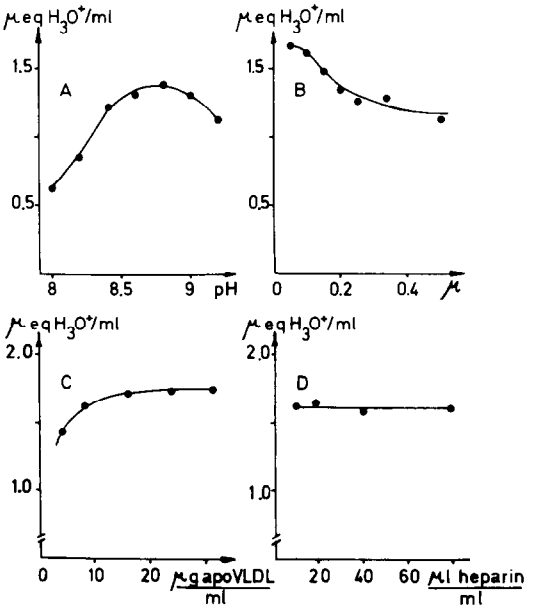

Fig. 2. Optimal conditions for the LPL activity on PC/TG substrates. A. Soybean PC/corn oil TG 0.067 mol/mol. B–D. Soybean PC/corn oil TG 0.1 mol/mol.

4.2. *Selectivity of the enzyme*

The contribution of phospholipase to the LPL-preparation is low. The enzyme shows a small activity on a pure PC substrate and heat denaturation of the enzyme results in a decrease of the activity, although phospholipase is thermally stable (fig. 3). Furthermore, analysis of the released FFA from the substrate with and without PC shows an identical FFA-profile

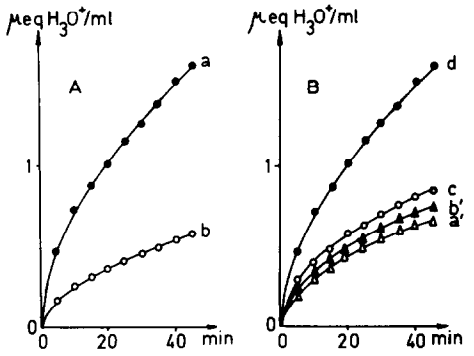

Fig. 3. Influence of heat denaturation of LPL and reactions on specific substrates as a control for the phospholipase activity in LPL. A. LPL activity on a soybean PC/corn oil TG 0.1 mol/mol substrate before (a) and after (b) heat denaturation of the enzyme. B. LPL activity on specific substrates: a. blank, b. 20 mg corn oil, c. 1.8 mg soybean PC, d. 1.8 mg soybean PC + 20 mg corn oil TG or 0.1 mol/mol.

Table 1
Percentage composition of the liberated FFA by a LPL-hydrolysis of a corn oil substrate with and without PC-species

% FFA	Substrate		
	Corn oil	Corn oil + soybean PC 0.1 M PC/M TG	Corn oil + egg-yolk PC 0.135 N PC/M TG
16:0	16	15	16
16:1	1	1	1
18:0	6	6	6
18:1	21	21	22
18:2	47	48	46
18:3	9	9	9

despite the great differences in fatty acid composition of the triglycerides and the phospholipids in the substrate (table 1). From these results it can be conluded that the increase in activity by the addition of PC to the TG-substrate must be considered as real activation of LPL-activity. The high blank value can be explained as a liberation of protons by other enzymes present in the crude milk LPL preparation.

4.3. *Effect of phosphatidylcholine species on the lipoprotein lipase activity*

The effect of phosphatidylcholine species on the LPL activity was studied as a function of the phospholipid concentration (fig. 4). Different molar ratios of PC to TG were investigated and a difference in the activity of the LPL was observed. As previously described by Chung [9] the activation curves show a specific optimal molar PC/TG ratio. The activation properties of phosphatidylcholine on LPL are dependent not only on the chain lengths and hydrophobic qualities of the constituent acids but also on their double bonds.

The effects of egg-phosphatidylcholine, purified soybean phosphatidylcholine and a complex phosphatidylcholine desoxycholate (1/1, EPL) are shown in fig. 4. Differences in activation between the three species must be explained by differences in the ratio of saturated and unsaturated fatty acids and by the presence of desoxycholate which is a surface active compound. The influence of the double bonds was proved by hydrogenation of the egg-yolk- and the EPL-PC species. After saturation to 16:0 and 18:0 as predominant fatty acids these lethicins show only a small LPL activation similar to the results of Chung et al. [9] in his study with pure dipamitoyl PC. When the activation is followed by the batch procedure an optimal activation is obtained at a PC/TG molar ratio of 0:20, which can be ascribed to differences in experimental conditions such as ionic strength, specific ions (NH_4^+), pH-change during the experiment, lipoprotein co-factor VLDL instead of apo-VLDL and the high albumin concentration. The lower degree of the activation must be ascribed to the high albumin and VLDL

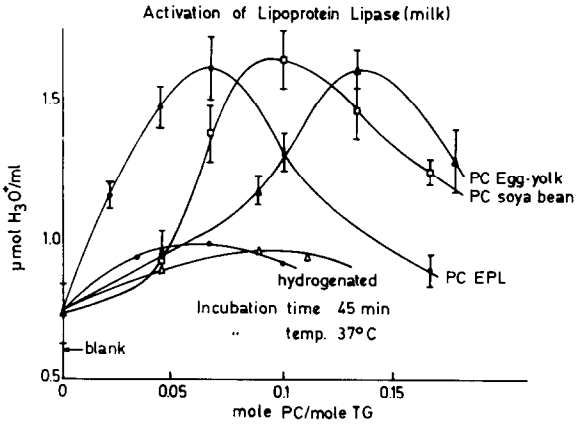

Fig. 4. The influence of saturated and unsaturated phosphatidylcholine species and of desoxycholate on the LPL activity (EPL = soybean PC + desoxycholate).

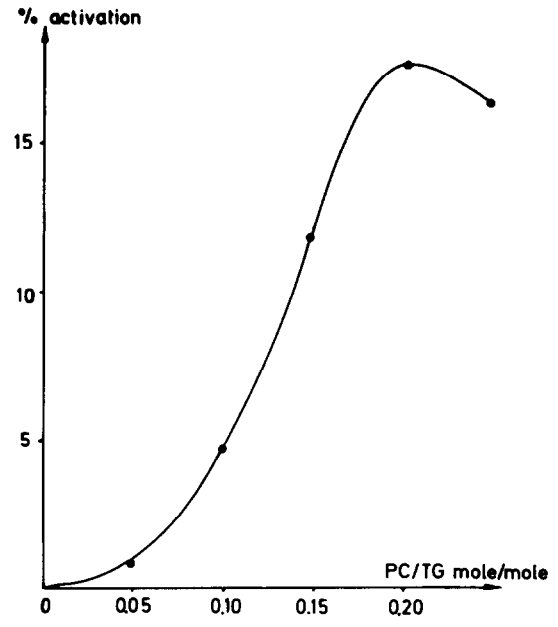

Fig. 5. Percentage increase of the released FFA by the LPL activity on a corn oil substrate in the presence of soybean PC, followed by the batch procedure.

content in the reaction mixture and to the procedure for the determination of liberated FFA.

5. Conclusions

It is demonstrated that the crude milk lipoprotein lipase contains some other enzymes which liberate H_3O^+ ions. The crude enzyme preparation has little or no phospholipase activity.

Optimal chemical conditions for enzyme activity were developed and the results indicate that phosphatidylcholine activates in vitro. The degree of activation is strongly dependent on the fatty acid composition of the molecule. Unsaturated PC activates much more than does saturated PC, so that essential phospholipids play an important role in triglcyeride metabolism.

Furthermore it is also clear that surface active substances such as desoxycholate influence the activation curves of PC on LPL.

We believe that the phospholipids in comparison to the apo-Lp activators interact with the substrate rather than with the enzyme.

Acknowledgements

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