Studies on lipoprotein lipase of rat heart and adipose tissue

Lipoprotein lipase, an enzyme which appears to be identical with the clearing factor of post-heparin plasma[1], was previously found in dilute ammonia extracts of rat heart acetone powder[2,3,4]. The present paper demonstrates the presence of lipoprotein lipase in aqueous extracts of adipose tissue, and that the enzyme from both sources requires one of several cations for activity and is inhibited by *p*-chloromercuribenzoate (PCMB).

Experiments with heart enzyme

As shown on Table I, lipoprotein lipase is inactive in the absence of any added cation. It is activated by 0.005 to 0.02 *M* $CaCl_2$ and 0.025 to 0.2 *M* NH_4Cl. $MnCl_2$, $MgCl_2$ and $SrCl_2$ behave identically to $CaCl_2$ while $(NH_4)_2SO_4$ is active at the same concentrations as NH_4Cl. NaCl, KCl, LiCl, RbCl, $(CH_3)_4NCl$ and $BaCl_2$ are completely inactive. NH_4Cl and $(NH_4)_2SO_4$ inhibit lipoprotein lipase at a concentration of 0.3 to 0.5 *M*, the same range that NaCl and KCl inhibit the enzyme[3]. The divalent cations all inhibit completely at a concentration of 0.05 *M*.

TABLE I

CATION REQUIREMENT OF RAT HEART LIPOPROTEIN LIPASE

The incubation mixture contained the following in a total volume of 1 ml: 0.2 ml of lipoprotein lipase*, 0.1 ml of activated coconut oil**, 0.4 ml of 10% albumin (pH 8.5), 10 γ of sodium heparin and other additions as noted. The vessels were incubated at 37° for 1 hour and 0.05 ml aliquots used for the glycerol determinations[3].

Vessel	*Addition*	*Glycerol μMole*
1	None	0
2	$CaCl_2$, 0.001 *M*	0
3	$CaCl_2$, 0.005	0.6
4	$CaCl_2$, 0.01	0.8
5	$CaCl_2$, 0.02	0.68
6	$CaCl_2$, 0.05	0
7	$CaCl_2$, 0.005***	0.25
8	$CaCl_2$, 0.01***	0.44
9	NH_4Cl, 0.01	0
10	NH_4Cl, 0.025	0.73
11	NH_4Cl, 0.05	0.82
12	NH_4Cl, 0.2	0.8
13	NH_4Cl, 0.4	0.4
14	NH_4Cl, 0.05 + NaCl, 0.3 *M*	0.6
15	NH_4Cl, 0.05 + NaCl, 0.5	0
16	NH_4Cl, 0.2 + NaCl, 0.3	0

* Lipoprotein lipase was prepared by extracting 50 mg of rat heart acetone powder with 1 ml of 0.025 *N* NH_3 for 30 minutes at 0°.

** Activated coconut oil was prepared by incubating a 1.5% solution of coconut oil (Abbott Laboratories) in whole, normal human serum for 30 minutes at 37° C.

*** Heparin omitted.

The inhibition by NaCl is not due to competition with the required cation. The concentration of NaCl necessary to inhibit enzymic activity decreases with increasing NH_4Cl concentration. In fact, inhibition is complete when the total salt concentration reaches 0.5 *M*, irrespective of the ions present.

Although the heparin activation cannot be demonstrated in the absence of the required cations, the cation effect is demonstrable in the absence of heparin. The degree of activation by both heparin and the cations is dependent only upon the absolute level of each and not their relative concentrations.

Lipoprotein lipase is inhibited by relatively high concentrations of PCMB (Table II). The inhibition is apparently due to an interaction with the enzyme protein and not the substrate protein. It is not readily reversible with cysteine.

Pancreatic lipase is inhibited by approximately the same concentration of PCMB[5]. It is also activated by calcium ions[6]. This effect of calcium ions has been attributed to their ability to precipitate the fatty acids as they are formed and thus remove an end product which inhibits further hydrolysis. This explanation would not appear valid for the lipoprotein lipase system

since (1) albumin is present in a concentration sufficient to bind the fatty acids and; (2) ammonium ions, which do not form insoluble soaps, are also active.

TABLE II

INHIBITION OF RAT HEART LIPOPROTEIN LIPASE BY PCMB

The incubation medium contained the following in a total volume of 1 ml: 0.2 ml of lipoprotein lipase, 0.1 ml of activated coconut oil, 0.4 ml of 10% albumin, 0.01 ml of 1 *M* $MnCl_2$ and 10 γ of sodium heparin. The lipoprotein lipase was preincubated for 10 minutes at 37° C with or without PCMB as indicated. The vessels were incubated at 37° for 1 hour.

Vessel	*PCMB concentration*		*Glycerol μMole*
	Preincubation	*Incubation*	
1	0	0	0.45
2	0	0.0025 *M*	0.46
3	0	0.005	0.37
4	0	0.01	0.1
5	0	0.015	0
6	0.010 *M*	0.0025	0.2
7	0.017	0.005	0

Experiments with adipose tissue enzyme

Perirenal and testicular fat were extracted for 30 seconds at 2° with an equal volume of distilled water in a Waring Blendor. The mixture was centrifuged at 2° for 30 minutes at 25,000 × *g*

TABLE III

LIPOPROTEIN LIPASE ACTIVITY OF AQUEOUS EXTRACTS OF RAT ADIPOSE TISSUE

The incubation medium contained 0.1 ml of substrate (containing 1.5% triglyceride), 0.4 ml of 10% albumin (except where indicated) and lipoprotein lipase in a total volume of 1 ml. Incubations were carried out at 37° for 1 hour.

Vessel	*Enzyme* ml.*	*Substrate**	*Other additions***	*Glycerol μMole*
1	0.3	None	None	0
2	0.3	C.O.	None	0.11
3	0.3	Rat chyle	None	0.55
4	0.1	A.C.O.	None	0.11
5	0.2	A.C.O.	None	0.31
6	0.3	A.C.O.	None	0.40
7	0.3	W.A.C.O.	No albumin present	0.07
8	0.3	W.A.C.O.	Albumin, 0.5%	0.20
9	0.3	W.A.C.O.	Albumin, 1.0	0.26
10	0.3	W.A.C.O.	Albumin, 3.0	0.40
11	0.3	A.C.O.	Protamine, $5 \cdot 10^{-5}$ *M*	0.10
12	0.3	A.C.O.	Protamine + heparin, 300 γ	0.30
13	0.3	A.C.O.	NaCl, 0.5 *M*	0
14	0.3	A.C.O.	Pyrophosphate, 0.013 *M*	0
15	0.3	A.C.O.	PCMB, 0.007 *M*	0.11
16	0.3	A.C.O.	PCMB, 0.012	0
17	0.3	A.C.O.	NH_4Cl, 0.2 *M*	0.19
18	0.3	A.C.O.	NH_4Cl, 0.4	0
19	0.3	A.C.O.	$CaCl_2$, 0.01 *M*	0.29
20	0.3	A.C.O.	$CaCl_2$, 0.04	0.05

* The following abbreviations are used: C.O. = coconut oil (Abbott Laboratories), A.C.O. = activated coconut oil (a 1.5% solution of coconut oil in whole, normal human serum incubated for 30 minutes at 37°), W.A.C.O. = washed activated coconut oil (A.C.O. which had been washed repeatedly by centrifugation so that no residual serum remained).

** The protamine, NaCl, pyrophosphate and PCMB were preincubated with the enzyme at the concentration indicated for 30 minutes at 0°. The heparin was added to the mixture of enzyme and protamine and preincubated for another 30 minutes at 0°. The NH_4Cl, $CaCl_2$ and albumin were added directly to the incubation vessel in the concentrations indicated.

and the aqueous layer collected from beneath the solidified fat. The protein concentration of this solution was approximately 4 mg/ml (determined spectrophotometrically[7]). The enzymic activity per mg of protein is 3 to 4 times greater than the rat heart preparations.

The evidence that this aqueous extract contains lipoprotein lipase is summarized in Table III. The lipolytic enzyme from adipose tissue is identical to rat heart lipoprotein lipase in the following respects: (1) it catalyzes the hydrolysis of the triglyceride moiety of chylomicrons and activated coconut oil (coconut oil pre-incubated with serum or α_1-lipoproteins), while coconut oil itself is hydrolyzed only slightly[4]; (2) it requires a fatty acid acceptor (*viz.* albumin)[3]; (3) it is inhibited by $5 \cdot 10^{-5}$ *M* protamine[3], 0.2–0.4 *M* NaCl and NH_4Cl, 0.01 *M* sodium pyrophosphate[3], 0.01 *M* PCMB and 0.02–0.04 *M* $CaCl_2$ ($MnCl_2$, $MgCl_2$ or $BaCl_2$). It appears to differ from rat heart lipoprotein lipase in that no heparin[3] or cation requirements can be shown. Aqueous extracts of an acetone powder of rat adipose tissue also contain lipoprotein lipase activity (Table IV). Activation by heparin and ammonium and calcium ions is demonstrable in these preparations.

TABLE IV

LIPOPROTEIN LIPASE ACTIVITY OF AQUEOUS EXTRACTS OF AN ACETONE POWDER OF RAT ADIPOSE TISSUE

The incubation medium contained 0.1 ml of substrate (containing 1.5% triglyceride), 0.4 ml of 10% albumin, lipoprotein lipase* and other addition as indicated in a total volume of 1 ml. Incubations were carried out at 37° for 1 hour.

Vessel	*Enzyme ml*	*Substrate***	*Heparin 10 γ/ml*	*$(RH_4)_2SO_4$ M*	*$CaCl_2$ M*	*Glycerol μMole*
1	0.1	Rat chyle	+	0.05	—	0.4
2	0.1	A.C.O.	+	0.05	—	0.44
3	0.1	C.O.	+	0.05	—	0.07
4	0.4	A.C.O.	—	—	—	0.56
5	0.4	A.C.O.	+	—	—	0.61
6	0.4	A.C.O.	—	0.05	—	0.49
7	0.4	A.C.O.	+	0.05	—	0.94
8	0.4	A.C.O.	+	—	0.005	1.07

* 25 mg of acetone powder extracted with 1 ml of water for 30 minutes at 0°. The protein concentration of the extract is approximately 6 mg/ml.

** Abbreviations same as in Table III.

Previous investigators have noted only slight lipolytic activity in adipose tissue with simple triglycerides as substrate[8]. Lipoprotein lipase may, therefore, be the major, if not the only, lipase in adipose tissue. This is significant with respect to fat transport. KELLNER[9] has reported that the capillary endothelium is permeable to lipoprotein molecules and that serum lipoproteins are present in extracellular and tissue fluids although in somewhat lower concentration than in blood. Thus, serum lipoproteins, and not simple triglycerides, may be the major substrates for tissue lipases. Lipoprotein lipase, therefore, would appear to be of major importance in fat transport and deposition.

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