STEREOSPECIFICITY OF HEPATIC LIPASES

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Received 14 October 1976

1. Introduction

Most studies on the positional specificity of triacylglycerol lipases have dealt with the susceptibility of hydrolysis of the ester bonds on the primary versus the secondary carbon atom in the glycerol moiety. More recently, differences between the primary positions have been observed. Lipoprotein lipase from milk, adipose tissue and post-heparin plasma preferentially hydrolyses the ester bond in position 1, whereas lingual lipase cleaves the bond at position 3 faster [1–3]. Assman et al. [4] reported no stereospecificity for another lipase in post-heparin plasma, the hepatic lipase. In principle it should be possible to distinguish between the two lipases in post-heparin plasma by comparing the stereospecific and non-stereospecific hydrolytic activities. Since the interest for such measurements is increasing [5] we decided to reinvestigate the stereospecificity of different hepatic lipases. Contrary to expectations the heparin-releasable liver lipase has the same specificity as lipoprotein lipase, whereas the lysosomal liver lipase was non-selective between positions 1 and 3.

2. Materials and methods

3-*O*-Tetradecyl-*sn*-glycerol was synthesized according to the method of Baer and Fischer [6] from 1,2-isopropyliden-*sn*-glycerol and 1-bromotetradecane. M.p. 56.5–57.5°C; $[\alpha]_D^{25}$: −2.07°C (2.66 benzene); calc.: C 70.78; H 12.58; O 16.64; found: C 70.70; H 12.50; O 16.70. From this compound 1-*O*-tetradecyl-*sn*-glycerol was prepared according to the method of Lands and Zschocke [7]. M.p. 57.0–57.5°: $[\alpha]_D^{25}$: +2.07°C (2.66 benzene); found: C 70.80; H 12.56; C 16.70. 1,2-[^{3}H]dioleoyl-3-*O*-tetradecyl-*sn*-glycerol and 1-*O*-tetradecyl-2,3-[^{14}C]dioleoyl-*sn*-glycerol were prepared by acylation of the glyceryl ethers with oleoyl chloride [8] prepared according to Borgström and Krabisch [9]. Labeled oleic acid was obtained from Radiochemical Centre, Amersham, UK. The diacyl- and also monoacyl-derivatives were purified by thin-layer chromatography on silica gel.

Egg lecithin (BDH) was purified by alumina chromatography and gave one spot on silica gel thin-layer chromatography. Substrate emulsions were prepared by a modification of Lundberg's procedure [10]. Equal amounts of the two alkyldiacylglycerol enantiomers (^{3}H- and ^{14}C-labeled) were mixed and the solvent was evaporated. After sonication in 2 ml water or buffer in a Branson sonifier S125 for 5 min the mixture was transferred to another tube containing 100 μg egg lecithin. The sonication was repeated and then 0.8 ml of 10% defatted crystalline bovine serum albumin was added followed by a sonication for 30 s. Alternatively, 50 μl or 100 μl of 0.5% Triton X-100 was added to the glyceride emulsion instead of egg lecithin.

Livers from male Sprague-Dawley rats were homogenized in 4 vol. of cold 0.25 M sucrose. After centrifugation 500 g for 5 min it was used directly or stored at −20°C. Perfusion of livers with heparin and preparations of post-heparin plasma was performed as described by Krauss et al. [11]. Incubations usually contained 0.08 M buffer, 0.49 mM alkylglyceride and 0.26 mM egg lecithin in 0.25 ml. Lipase activity was measured either as fatty acid liberation [12] or by thin-layer chromatography of the products on silica gel (solvent: petroleum ether/ether/acetic

acid 70:30:1) after extraction with chloroform/methanol (1:1). From model experiments the fraction of free fatty acids partitioning into the upper phase was calculated and the data given in this paper represent the total conversion to fatty acids. The $^{14}C/^{3}H$ ratio in the upper phase was always the same as in fatty acids isolated by thin-layer chromatography. Protein was determined according to Lowry et al. [13].

3. Results and discussion

The hydrolysis of alkyldiacylglycerol isomers was studied at different pH in rat liver homogenates (fig.1). The shape of the pH curve is very similar to that obtained by Assman et al. [14] using a triacylglycerol substrate. At pH 5.0 both isomers were attacked at a similar rate (fig.2) indicating that the lysosomal lipase activity exhibits no stereospecificity. In the alkaline range 1,2-[^{3}H]dioleoyl-3-alkyl-*sn*-glycerol was more rapidly hydrolyzed than the 2,3-dioleoyl compound which shows that the ester bond at position 1 is attacked preferentially to that at position 3. Addition of albumin stimulated lipolysis at 9.0 with remaining stereospecificity. At pH 5.0 Triton X-100 and albumin stimulated the hydrolysis

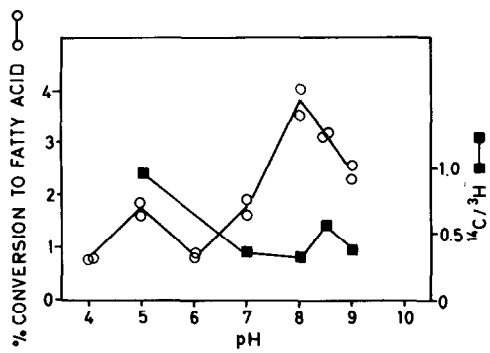

Fig.1. Hydrolysis of alkyldiacylglycerol at different pH. Liver homogenate (1.9 mg protein) was incubated for 15 min with 0.25 mM of each of 1,2-[^{3}H]dioleoyl-3-*O*-tetradecyl-*sn*-glycerol and 1-*O*-tetradecyl-2,3-[^{14}C]dioleoyl-*sn*-glycerol solubilized in lecithin and albumin as described under Materials and methods. Open symbols show the conversion of the ^{3}H-substrate to fatty acid and the black symbols show the $^{14}C/^{3}H$ ratio in fatty acid (ratio in substrate = 1.00).

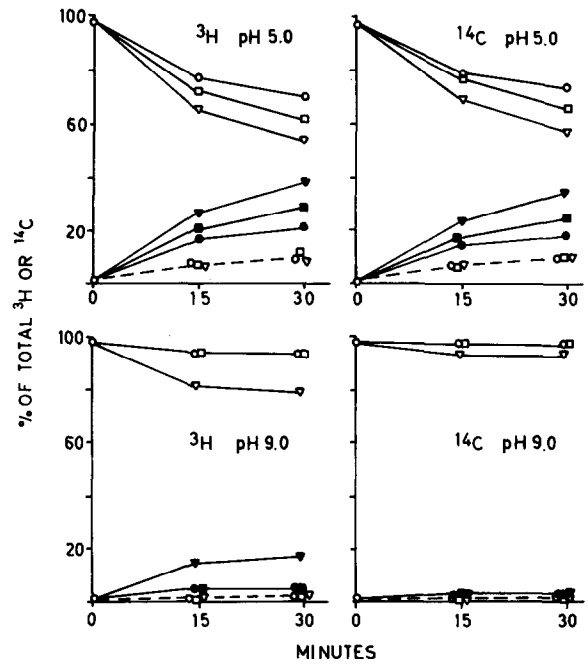

Fig.2. Effect of albumin and Triton X-100 on alkyldiacylglycerol hydrolysis at pH 5.0 and pH 9.0. Liver homogenate was incubated with the racemic substrate described in fig.1, although the basal incubation (○ ●) did not contain albumin. 1.6% Albumin (▽ ▼) or 0.08% Triton X-100 (□ ■) were added where indicated and the final incubation mixture was sonicated for 10 s. The amount of radioactivity in alkyldiacylglycerol (◎—○, ▽—▽, □—□) alkylmonoacylglycerol (○- - -○, ▽- - -▽, □- - -□) and fatty acid (●—●, ▼—▼, ■—■) is shown.

of both isomers to the same extent. Free fatty acid was the main product indicating that some alkylmonoacylglycerol was further hydrolyzed (see below). The lipase(s) with alkaline pH optimum occurs in the microsomal fraction, plasma membranes and cytosol [14]. The plasma membrane enzyme can be released to plasma or to a perfusate by heparin [11]. A stereospecificity was earlier not observed for this hepatic lipase of post-heparin plasma [4]. Therefore we prepared this lipase by heparin perfusion of the liver and its stereospecificity was studied (table 1). The ^{3}H isomer was hydrolyzed approximately four times faster than the ^{14}C isomer. The $^{14}C/^{3}H$ ratio in the released fatty acid was not significantly affected if the substrate amount or the emulsifying agent was changed (table 1). The emulsions prepared with Triton X-100, which were

Table 1
Hydrolysis of different preparations of alkyldiacylglycerol substrates by heparin-liver-perfusate

Substrate	% in fatty acid		
	^{3}H	^{14}C	$^{14}C/^{3}H$
0.49 mM ADG; 0.01% Triton X-100	11.7	3.02	0.25
0.49 mM ADG; 0.02% Triton X-100	13.4	3.09	0.23
0.24 mM ADG; 0.26 mM PC	6.92	1.69	0.25
0.49 mM ADG; 0.26 mM PC	4.09	1.10	0.26
1.47 mM ADG; 0.26 mM PC	2.39	0.63	0.26

Alkyldiacylglycerol isomers (ADG) were sonicated either in Triton X-100 or egg phosphatidylcholine (PC) as described under Materials and methods. $^{14}C/^{3}H$ in the substrates was set at 1.00. Enzyme source: 100 μl heparin perfusate. Incubation time 60 min.

much less opalescent than those with egg lecithin, were hydrolyzed faster. The addition of different amounts of lecithin or sonication for different lengths of time changed the opalescence but not the lipolytic rate (table 2). Since the stereospecificity was not changed by these manipulations it can be concluded that this specificity is not very sensitive to the physical form of the substrate. Instead the chemical structure attached to the secondary glycerol carbon is of importance [15].

As pointed out by Morley et al. [16] observed stereospecificities may be exerted at the diacylglycerol instead of the triacylglycerol level. The lipolytic enzyme in heparin perfusate did not discriminate between different enantiomers of 1,3-diacylglycerol analogs (fig.3) but hydrolyzed the 2,3-analog faster than the 1,2-analog. This stereo-specificity was less pronounced than that for alkyldiacylglycerol hydrolysis, substantiating the conclusion [16] that the latter specificity is not solely due to the stereospecificity operating at the level of diacylglycerols or their analogs.

The stereospecificity for the heparin-releasable hepatic lipase therefore is the same as that previously reported for lipoprotein lipase [1–3], which was confirmed from studies with the latter enzyme (unpublished data). These two enzymes in post-heparin plasma can therefore not be measured separately by the use of stereospecific substrates. Unphysiological substrates, such as palmitoyl-CoA, which is attacked only by hepatic lipase [17] or other methods [5] may be useful. Instead the present results support the recent proposal [18] that lipoprotein lipase and hepatic lipase have similar structure

Table 2
Influence of phosphatidylcholine concentration and sonication time on lipolysis of alkyldiacylglycerol isomers

Substrate	% Conversion to [^{3}H]fatty acid	A_{450} nm
0.49 mM ADG; 0.26 mM PC	2.65; 2.66	1.29
0.49 mM ADG; 0.52 mM PC	2.75; 2.78	1.85
0.49 mM ADG; 1.04 mM PC	2.59; 2.59	2.09
0.49 mM ADG; 0.26 mM PC[a]	2.60; 2.92	0.50

[a]Sonicated for 5 + 30 min instead of 5 + 5 min

Different substrates of alkyldiacylglycerol (ADG) isomers (^{3}H- and ^{14}C-labeled, respectively) and egg phosphatidylcholine (PC) were incubated with 50 μl heparin perfusate for 30 min.

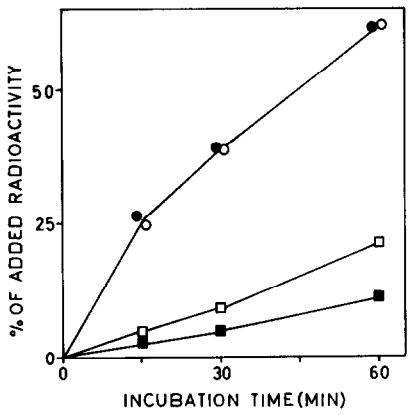

Fig.3. Hydrolysis of different alkylacylglycerols by heparin perfusate. Substrate mixtures (0.49 mM) of 1-[^{3}H]oleoyl-3-*O*-tetradecyl-*sn*-glycerol and 1-*O*-tetradecyl-3-[^{14}C]oleoyl-*sn*-glycerol or 2-[^{3}H]oleoyl-3-*O*-tetradecyl-*sn*-glycerol and 1-*O*-tetradecyl-2-[^{14}C]oleoyl-*sn*-glycerol made with 0.26 mM egg lecithin were incubated with 20 μl heparin perfusate. (●, ■)^{14}C-fatty acid, (○, □)^{3}H-fatty acid, (□, ■)2-acyl substrate, (○, ●)1(3)-acyl substrate.

although they are immunologically different. The acid lysosomal lipase shows no stereospecificity and resembles pancreatic lipase in this respect [1–3]. This further stresses the use of substrate stereospecificity to classify different lipases, also within the same tissue.

Acknowledgements

Excellent technical assistance was given by Miss Elisabeth Nilsson. This study was supported by grants from The Swedish National Board for Technical Development, The Swedish Medical Research Council (projects 3968 and 4931), A. Påhlsson's Foundation and J. Andersson's Foundation.

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