STEREOSPECIFICITY OF LIPOPROTEIN LIPASE IS AN INTRINSIC PROPERTY OF THE ACTIVE SITE OF THE ENZYME PROTEIN

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1. Introduction

The circulating plasma triacylglycerol, which is transported mainly in the chylomicrons and very low density lipoproteins (VLDL), is hydrolyzed prior to the uptake in the extrahepatic tissues by lipoprotein lipase (LPL, EC 3.1.1.3). LPL is present in myocardium, adipose tissue and skeletal muscle [1–3] and has been suggested to be bound to the sulphated glycosaminoglycans on the luminal surface of the vascular endothelium in these tissues [3]. There is direct evidence that LPL is the rate limiting step in the removal of plasma triacylglycerol [4].

The molecular properties of LPL are still poorly understood. This is probably due to the fact that until recently no methods were available for the purification of relative large quantities of homogenous LPL. A rich source of LPL is bovine milk [5]. The enzymatic properties (i.e., inhibition by protamine and by high ionic strength, activation by apolipoprotein C-II) of both bovine milk LPL and human postheparin plasma LPL are identical [5,6], and they show immunological cross-reactivity [7] and have very similar amino acid composition [8–10]. We therefore have used the bovine milk LPL as a model to study the enzymatic properties of LPL in general. For full activity LPL requires a co-factor, apolipoprotein C-II [6], which is a component of both chylomicron and VLDL surface film [11,12]. LPL is stereospecific in the presence of serum activator; it preferentially cleaves the ester bond between the acyl group and the *sn*-1 carbon of the glycerol backbone of triacylglycerol [13,14]. We wanted to study, whether the apoprotein activator affects this stereospecificity, or whether the stereospecificity of LPL is an intrinsic property of the active site of the enzyme molecule.

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2. Materials and methods

2.1. *Lipoprotein lipase*

LPL was purified to homogeneity from bovine milk using chromatography on agarose beads with covalently-linked heparin [15]. The purified enzyme was stored in 20 mM Tris–HCl buffer (pH 7.4) containing 50% (v/v) glycerol and 2.0 M NaCl at 20°C without any loss of activity.

2.2. *Apolipoprotein C-II*

ApoC-II was purified from human plasma VLDL as in [16,17].

2.3. *Lipids*

The labelled enantiomeric triacylglycerol analogs were as in [13,14]. Egg lecithin was purified as in [16].

2.4. *Enzyme incubations*

The substrate lipids were mixed in chloroform and dried under nitrogen. After the addition of 1.25 ml 0.2 M Tris–HCl buffer (pH 8.4), the mixture was mixed on vortex mixer at maximal speed for 5 min. The resulting emulsion gave identical reaction rates

as substrate for LPL with an emulsion prepared by sonication. The final incubation mixture contained in 2.5 ml total vol. 0.1 M Tris–HCl buffer (pH 8.4), the following components: 50 nmol 1-*O*-[9,10-$^3H_2$]-octadecyl-2,3-dioctadecenoyl-*sn*-glycerol, spect. act. 0.11 mCi/mmol, 50 nmol 3-*O*-[1-^{14}C]octadecyl-1,2-dioctadecenoyl-*sn*-glycerol, spect. act. 0.05 mCi/mmol, 0.38 mmol NaCl, 25 mg bovine serum albumin and 10.9 μg (1.24 nmol) apoC-II, when indicated. At the times illustrated in the figures aliquots were taken from the mixture incubated with LPL in a water bath at +35°C and extracted as in [19]. The lipids were separated on silica gel-coated plates (Merck) developed with hexan/diethylether/acetic acid (70/20/2). The separated lipids were measured for ^{3}H- and ^{14}C-radioactivity as in [13].

3. Results and discussion

3.1. *Stereospecificity in the presence of apoC-II*

The stereospecificity reported [13,14] for lipoprotein lipase was confirmed in our experiments using purified LPL and purified activator, apoC-II, fig.1. The substrate containing the alkyl group in the *sn*-1 position was hydrolyzed much slower than the substrate with the alkyl group in the *sn*-3 position. As the 1(3)-alkyl-3(1)-acylglycerol did not exceed >10% of the total alkylacylglycerol formed, the alkylacylglycerol is expressed as total. Thus equal product formation was achieved both by purified components and crude preparations (fig.1 and [13]).

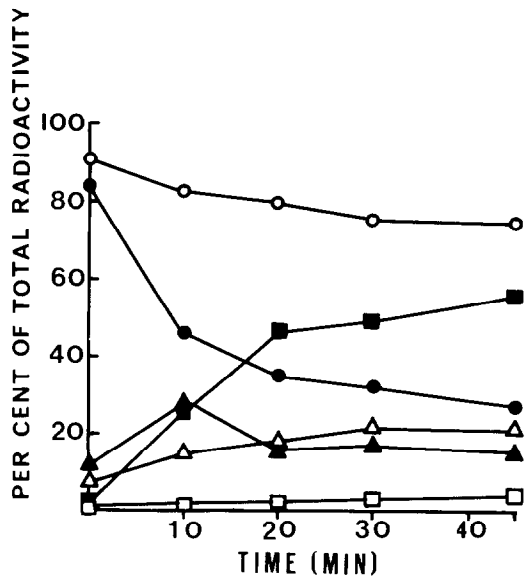

Fig.1. Hydrolysis of egg-lecithin stabilized enantiomeric 1(2)-alkyl-2,3(1,2)-diacylglycerols by lipoprotein lipase in the presence of apoC-II. Detailed description of the assay mixture is in section 2. The reaction was started by the addition of 1.1 μg (0.02 nmol) purified LPL. At the indicated times 450 μl aliquots were withdrawn and analyzed for lipid radioactivity. (○,●) Alkyldiacylglycerol; (△,▲) alkylacylglycerol; (□,■) alkylglycerol. Open symbols represent lipids with the alkyl group in the *sn*-1 position and closed symbols those with the alkyl group in the *sn*-3 position.

3.2. *Stereospecificity in the absence of apoC-II*

The above experiments were performed in the presence of apoC-II, a small molecular weight peptide required for full activity of the enzyme. It has been shown that there is a protein–protein interaction between apoC-II and LPL, reversible at high ionic strength [20]. There is evidence for the formation of 1:1 stoichiometric complex [21,22]. Thus apoC-II could change the conformation of LPL so as to favor the hydrolysis of the ester bond between the acyl group and the *sn*-1 carbon of the glycerol backbone. ApoC-II is known to bind lipids [24]. One possible mechanism for the activation of LPL by apoC-II could be the binding of the substrate triacylglycerol by apoC-II, which then guides the triacylglycerol properly into the active site of the enzyme. As this could have an effect on the stereospecificity of the enzyme, it was of interest to study the stereospecificity of LPL also in the absence of apoC-II. As shown in fig.2, approximately identical hydrolytic pattern was obtained by LPL in the absence of apoC-II as in its presence. Thus the stereospecificity of LPL seems to be an intrinsic property of the enzyme molecule per se, which is not affected by apoC-II activator. Accordingly, apoC-II may be needed only for the enhancement of the catalytic rate constant of LPL. This is further supported by the observation that apoC-II does not affect the surface pressure optimum of LPL, when monolayers of trioctanoylglycerol at an air/water interface were used as a substrate [23].

It can be concluded that purified LPL exhibits

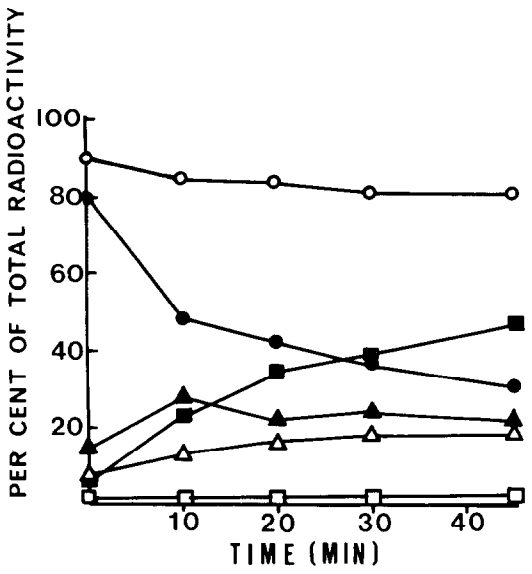

Fig.2. Hydrolysis of egg-lecithin stabilized enantiomeric 1(3)-alkyl-2,3(1,2)-diacylglycerols by lipoprotein lipase in the absence of apoC-II. The assay conditions were essentially identical to those in fig.1, but the reaction mixture did not contain apolipoprotein C-II and the amount of LPL used was 5.5 μg (0.1 nmol). The symbols used are as in fig.1.

stereospecificity unaffected by the apoprotein activator. As the enzyme also hydrolyzes 1(3)-alkyl-3(1)-acylglycerols with equal rates [13], it is probable that the enzyme molecule specifically interacts with the carbonyl group in the *sn*-2 position of the glycerol backbone, which then causes the *sn*-1 acyl group to be cleaved preferentially. Studies to confirm this binding site in LPL are at present in progress.

Acknowledgements

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