Assembly of lipoprotein lipase in perfused guinea-pig hearts

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It has been suggested that lipoprotein lipase (LPL) can be assembled into its catalytically active dimeric form only after its oligosaccharide chains have been processed in the Golgi. To study this in a complete organ, LPL was metabolically labelled with [^{35}S]methionine in perfused guinea-pigs hearts. After 10 min pulse-labelling, LPL protein was eluted as two peaks from heparin–agarose: peak 1 at about 0.65 M NaCl, peak 2 at about 0.95 M NaCl. Catalytic activity was associated only with peak 2. Model studies with bovine LPL showed that active dimeric LPL is eluted in peak 2, but after treatments that dissociate the enzyme into inactive monomers it is eluted in peak 1. Pulse-labelled LPL in both peak 1 and peak 2 was fully sensitive to treatment with endoglycosidase (Endo) H. With chase, peak 1 disappeared and peak 2 acquired resistance to Endo H. These findings suggest that core glycosylated LPL is assembled into dimers already in the endoplasmic reticulum and that processing of the oligosaccharide chains occurs after dimerization.

INTRODUCTION

Lipoprotein lipase (LPL) catalyses one of the main steps in lipoprotein metabolism, i.e. hydrolysis of triacylglycerol in chylomicra and very-low-density lipoprotein (Borensztajn, 1987; Olivecrona and Bengtsson-Olivecrona, 1987, 1989; Bensadoun, 1991). The enzyme has a fairly complex life-cycle. It is synthesized in parenchymal cells in several extrahepatic tissues, notably adipose tissue and red muscles (Cryer, 1987; Camps et al., 1990). The enzyme is released from these cells and moves to binding sites at the vascular endothelium, where it exerts its physiological function (Olivecrona and Bengtsson-Olivecrona, 1989). Endothelial LPL is turned over rapidly, probably through release into blood (Bagby, 1983; Chajek-Shaul et al., 1988) followed by uptake and degradation in the liver (Wallinder et al., 1984).

Active LPL is a dimer of two identical N-glycosylated 55 kDa chains (Olivecrona and Bengtsson-Olivecrona, 1987; Osborne et al., 1985). At what stage of assembly and processing LPL becomes catalytically active is under debate (Bensadoun, 1991; Braun and Severson, 1992). Several groups have suggested that catalytic activity is contingent upon processing of the oligosaccharides in the Golgi. The Steins and their collaborators have proposed that there is a regulated critical step in processing of the oligosaccharide chains which determines the activity of the enzyme (Chajek-Shaul et al., 1985). Vannier and Ailhaud (1989) have concluded from experiments with murine Ob 17 and 3T3-F442A cells that core-glycosylated catalytically inactive LPL monomers are transferred from the endoplasmic reticulum (ER) to Golgi, where their oligosaccharides are processed and the enzyme assembles into catalytically active dimers. Semb and Olivecrona (1989a) studied the relation between glycosylation and activity of LPL in guinea-pig adipocytes. Using lectin affinity chromatography, they isolated LPL containing only high-mannose oligosaccharides and showed that they were catalytically active. In the presence of methyldeoxynojirimycin or deoxymannojirimycin, which inhibit trimming and processing of the oligosaccharides, catalytically active high-mannose-type LPL molecules were released into the medium. These authors concluded that core glycosylated LPL can be catalytically active. According to this view, processing of the oligosaccharide chains indicates that the enzyme has passed through the Golgi, but is not necessary for transport, or for catalytic activity. Similar conclusions were reached by Doolittle et al. (1990) in a study with fragments of rat adipose tissue in culture, and by Masuno et al. (1991), who studied LPL in brown adipocytes.

The complex transport of LPL from synthesis in parenchymal cells to its site of action at the vascular endothelium cannot easily be modelled in cell culture. We have recently developed methods for pulse–chase studies of LPL in perfused hearts (Liu and Olivecrona, 1991, 1992). In the present study we have used this system to clarify whether trimming and processing of the oligosaccharide chains, or dimerization, is the primary event. To develop a method to separate dimeric and monomeric LPL, we exploited the observation that LPL dimers bind to heparin with high affinity because heparin-binding sites on both subunits simultaneously bind to the heparin chain (Clarke et al., 1983). Unfolded LPL (Bengtsson-Olivecrona and Olivecrona, 1985) and even fragments of the molecule (Bengtsson-Olivecrona et al., 1986) retain the ability to bind to heparin, but with lower affinity.

MATERIALS AND METHODS

Materials

Antiserum against guinea-pig LPL was raised in rabbits. Bovine LPL was purified from cow's milk (Bengtsson-Olivecrona et al., 1985) and iodine-labelled as described by Wallinder et al. (1984). Heparin–Sepharose and Protein A–Sepharose 4B beads were from LKB–Pharmacia (Stockholm, Sweden). BSA and Trasylol were from Sigma (St. Louis, MO, U.S.A), heparin was from AB Lövens (Malmö, Sweden), modified Eagle's minimal essential medium (MEM) and methionine-free MEM were from Gibco (Paisley, UK), and leupeptin and pepstatin were from The Peptide Institute, Osaka, Japan. Endoglycosidase (Endo) H was from Genzyme Corp. (Boston, MA, U.S.A.). [^{35}S]Methionine (1080 mCi/mmol) and ^{14}C-labelled molecular mass standard proteins were from Du Pont–New England Nuclear (Boston, MA, U.S.A.).

Animals

Male guinea pigs weighing 400–700 g were used in this study. They were allowed free access to water and standard chow pellets

Abbreviations used: LPL, lipoprotein lipase; MEM, modified Eagle's medium; Endo H, endoglycosidase H; ER, endoplasmic reticulum.
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(for composition see Semb and Olivecrona, 1989b), and were housed with a 12 h daylight cycle. They were anaesthetized with Hypnorm Vet (AB Leo, Helsingborg, Sweden; 15 mg/kg body wt.) and diazepam (3.75 mg/kg body wt.) administered intramuscularly. Hypnorm Vet is a combination of the narcotic analgesic fentanyl citrate (0.315 mg) and the tranquillizer fluanisone (10 mg) in 1 ml of solution. All experimental procedures were approved by the local Animal Ethics Committee.

Pulse–chase experiments on perfused hearts

Hearts were removed under anaesthesia from guinea pigs and perfused as previously described (Liu and Olivecrona, 1991). The blood was washed out with a single pass of 15 ml of perfusate A (methionine-free MEM supplemented with 3% dialysed BSA, 100 mg/dl glucose and 1 mM glutamine). Perfusion (recirculating mode) was then continued with this medium for 30 min. The criterion used was that the heartbeat stabilized during this period and remained constant at around 180 beats/min. Then [^{35}S]-methionine was added and recirculated for 10 min for pulse-labelling. The labelling medium was removed and the heart was washed with a single pass of 40 ml of perfusate B (MEM supplemented with 1% BSA, 100 mg/dl glucose, 1 mM glutamine and 10 mM methionine) and then perfused in recirculating mode with perfusate C (same as perfusate B but with 3% BSA). At the end of the chase, the hearts were flushed (about 1 min) with a single pass of 15 ml of MEM containing 1 mg/ml BSA and 33 μg/ml heparin, and then with 10 ml of the same medium but without heparin. The heparin flush was collected on ice. The hearts were homogenized in 9 vol. of buffer D (0.025 M NH_4Cl, pH 8.2, containing 5 mM EDTA, 8 mg/ml Triton X-100, 0.4 mg/ml SDS, 33 μg/ml heparin, 10 μg/ml leupeptin, 1 μg/ml pepstatin, 25000 i.u./ml Trasylol) with a Polytron PT 3000 homogenizer (Kinematica AG, Lucerne, Switzerland) at 28000 rev./min for 20 s on ice. After centrifugation (10000 *g* for 10 min, 4 °C), the supernatant was used to measure incorporation of [^{35}S]methionine into total proteins by trichloroacetic acid precipitation, for chromatography on heparin–agarose, and for isolation of LPL by immunoprecipitation as described by Liu and Olivecrona (1991).

Preparation of heart microsomal vesicles

A heart was perfused with perfusate A containing [^{35}S]methionine for 30 min and then flushed through the aorta with 10 ml of ice-cold 0.25 M sucrose in 5 mM imidazole buffer, pH 7.4. All subsequent operations were at 4 °C. The heart was then minced and homogenized in 0.25 M sucrose (1:5, w/v) with the Polytron homogenizer at 15000 rev./min for 30 s. The homogenate was filtered through a 200 μm-mesh nylon cloth, and the filtrate was centrifuged at 1500 *g* for 10 min. The supernatant was then centrifuged at 10000 *g* for 15 min in a Sorvall centrifuge. The 10000 *g* supernatant was further subjected to centrifugation at 100000 *g* for 60 min in a 70.1 rotor in a Beckman L5-65B preparative centrifuge. The pellet was collected, washed once with the buffer and then resuspended in 50 mM Tris/HCl buffer, pH 7.4. NADPH reductase activity was measured (Williams and Kamin, 1962) and found to be enriched 15-fold in this fraction. Protein was determined by the BCA (bicinchoninic acid) method according to the manufacturer's instructions (Pierce, Rockford, IL, U.S.A.).

Heparin–agarose chromatography

Supernatant of heart homogenate, prepared in buffer D as described under 'Pulse–chase experiments on perfused hearts', was applied on a 4 ml heparin–agarose column equilibrated with buffer E (20 mM Tris/HCl, 0.1% Triton X-100, 1 mg/ml BSA, pH 7.4). The column was washed with the same buffer at 0.5 ml/min for 40 min, and was then eluted by a 50 ml + 50 ml gradient of 0–2 M NaCl in buffer E. Fractions (2 ml) were subjected to immunoprecipitation and/or measurement of LPL catalytic activity as described below. The conductivity of the fractions were determined and converted into NaCl molarity by comparison with standards made up in buffer E.

Immunoprecipitation and digestion with Endo H

Immunoprecipitation was performed with a rabbit antiserum to guinea-pig LPL as described by Liu and Olivecrona (1991), but Protein A–Sepharose beads were used instead of *Staphylococcus aureus* cells to harvest the immunoprecipitates. For treatment with Endo H, the washed immunoprecipitates were dissolved in 50 mM Tris/HCl/0.5% SDS/0.1 M mercaptoethanol, pH 6.8, and heated at 95 °C for 5 min. The Protein A–Sepharose beads were removed by brief centrifugation. Then Endo H (50 m-units/ml) in 0.15 M sodium acetate, pH 6.0, was added and the samples were incubated at 37 °C for 16–20 h. Controls were incubated in the same way without Endo H. An equal volume of sample buffer (130 mM Tris/HCl, pH 6.8, containing 4 g of SDS, 20 ml of glycerol, 10 ml of mercaptoethanol and 0.2 ml of 1% Bromophenol Blue per dl) was added. The samples were heated for 5 min at 95 °C, and were then resolved by SDS/PAGE (10% gels). The gels were dried and fluorograms were obtained as described by Liu and Olivecrona (1991).

Sucrose-density-gradient centrifugations

Sedimentation experiments with bovine LPL were performed with a Beckman ultracentrifuge (L5-65B) and a Ti 50.1 rotor. Linear density gradients of 10–5% (w/v) sucrose in 10 mM Bistris, pH 6.5, with 1.5 M NaCl and 1 mg of BSA/ml were made in polypropylene tubes. The total volume was 4.8 ml. Before centrifugation, some samples (^{125}I-LPL and unlabelled LPL in 10 mM Bistris/1 M NaCl, pH 6.5) were treated with 1 M guanidinium chloride for 1 h at 20 °C by addition of guanidinium chloride from a 6 M stock solution. This led to complete loss of catalytic activity. The treated samples were separated from guanidinium chloride on a Sephadex G-25 column (50 mm × 14 mm) in 10 mM Bistris/1 M NaCl, at pH 6.5 and 10 °C. After the runs, the tubes were emptied through puncture with a syringe needle in the bottom; 0.25 ml fractions were collected for measurement of lipase activity and/or of radioactivity.

Assay of LPL activity

LPL activity in the perfusate and in supernatants of the heart homogenate was measured as described in detail elsewhere (Bengtsson-Olivecrona and Olivecrona, 1992). The substrate emulsion was prepared by sonication of [^{3}H]triolein into Intralipid. This emulsion was then mixed with a medium containing BSA, heat-inactivated rat serum, Tris/HCl buffer and 0.1 M NaCl, pH 8.5. Assay incubation was at 25 °C for times chosen from the expected activity, but always less than 1 h. The reaction was stopped, and the fatty acids were extracted and the radioactivity was counted. One m-unit of LPL activity is 1 nmol of fatty acids released/min.

RESULTS AND DISCUSSION

Intracellular localization of newly synthesized LPL

There is much evidence that LPL follows the general pathway of

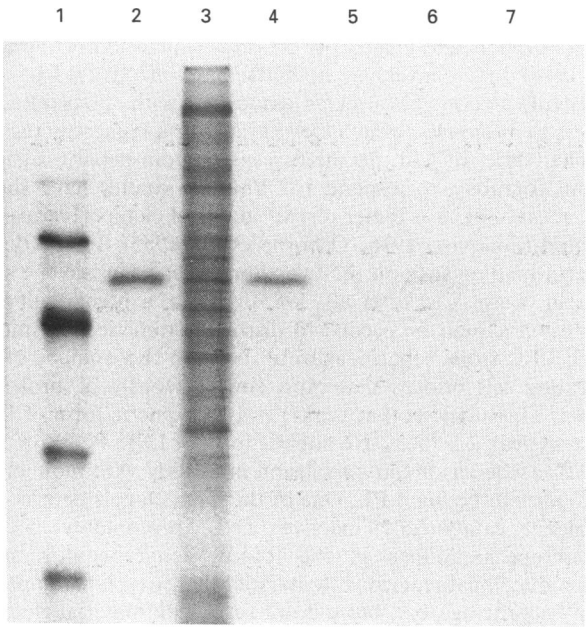

Figure 1 Demonstration of LPL in microsomal vesicles

Microsomal vesicles were isolated from a metabolically labelled guinea-pig heart as described under 'Methods'. Lanes: 1, molecular-mass standards (phosphorylase *b*, 92.5 kDa; BSA, 67 kDa; ovalbumin, 46 kDa; trypsin inhibitor, 35 kDa; lactoglobulin A, 18.4 kDa); 2, ^{125}I-labelled bovine LPL; 3, total microsome fraction; 4, immunoprecipitate from microsomes not treated with Na_2CO_3; 5, same as lane 4, but non-immune serum instead of antiserum; 6, immunoprecipitate from microsomes treated with Na_2CO_3; 7, same as lane 6, but non-immune serum instead of antiserum.

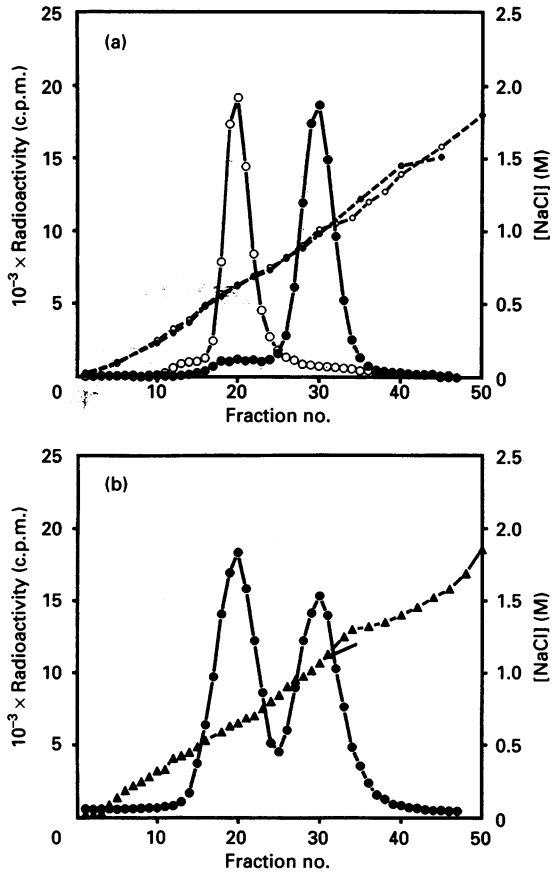

Figure 2 Separation of ^{125}I-labelled bovine LPL by chromatography on heparin–agarose

In (**a**) two separate runs are superimposed. In run 1 a freshly made preparation of labelled LPL was used (●). For run 2 (○) the enzyme preparation was first treated by heating at 95 °C for 5 min in the presence of 1% SDS. This treatment resulted in loss of catalytic activity. The preparations were mixed with 5 ml of homogenate of guinea-pig heart in buffer D and applied to a 4 ml column of heparin–agarose. After washing with buffer E, the column was eluted by a 50 ml + 50 ml gradient of 0–2 M NaCl in buffer E at a flow rate of 0.5 ml/min. About 98% of the applied radioactive LPL was recovered in the gradient fractions for both runs. The conductivity in the fractions was measured and converted into NaCl concentrations by use of calibrated standards (▲, △, salt gradient for run 1 and 2 respectively). In (**b**) the peak fractions from the two runs in (**a**) were combined, mixed with heart homogenate and chromatographed as in (**a**) (●, LPL radioactivity; ▲, NaCl gradient).

vesicular transport (Ailhaud, 1990; Bensadoun, 1991; Braun and Severson, 1992). The cDNA sequences show that the protein has a signal peptide (Wion et al., 1987; Enerbäck et al., 1987). Vannier et al. (1986) have provided biochemical evidence that in mouse adipocytes LPL is contained within vesicles, and the enzyme has been directly observed inside vesicles by electron-microscopic immunohistochemistry (Blanchette-Mackie et al., 1989). It has, however, been suggested that in heart LPL is engaged in hydrolysis of intracellular triacylglycerols (Palmer et al., 1981). Chohan and Cryer (1979) have demonstrated LPL activity in heart microsomes. To study this further, we prepared a microsomal fraction by differential centrifugation from a heart which had been perfused with [^{35}S]methionine. One sample of the washed microsomes was treated with Na_2CO_3, to open the vesicles (Fujiki et al., 1982). Treated and untreated samples of the microsomes were then pelleted again, dissolved in detergent-containing buffer and subjected to immunoprecipitation. The results (Figure 1) show that the intact microsomal vesicles contained LPL, whereas the enzyme had been lost from the opened vesicles. Taken together, available evidence indicates that in heart, as in adipocytes, LPL is a secretory protein. Hydrolysis of intracellular triacylglycerol droplets is more likely carried out by a lipase similar to the hormone-sensitive lipase in adipose tissue. There is mRNA for this enzyme in rat hearts (Holm et al., 1988) and biochemical evidence that it is active there (Goldberg and Khoo, 1985). An implication of the vesicular location for LPL is that for efficient assay or imunoprecipitation of LPL from heart homogenates, the vesicles must be disrupted. In the present study this was accomplished by inclusion of detergents in all homogenization buffers.

Separation of active and inactive forms of LPL by chromatography on heparin–agarose

Previous studies from several laboratories had consistently shown that, on elution with a salt gradient from heparin–agarose, catalytically active LPL emerges at about 1 M NaCl (reviewed by Olivecrona and Bengtsson-Olivecrona, 1989). Treatment with dilute guanidinium hydrochloride dissociates the dimeric enzyme into monomers, with a change in conformation and loss of activity (Osborne et al., 1985). Model experiments now showed that bovine LPL that had been treated in this manner was eluted from heparin–agarose at about 0.6 M NaCl, well separated from active LPL (results not shown). When the fractions were analysed by sucrose-density-gradient centrifugation, material from peak 1 banded in the position expected for LPL monomers, whereas peak 2 banded as expected for dimers.

In all of many sucrose-density-gradient separations of partially active bovine LPL, catalytic activity was associated with the

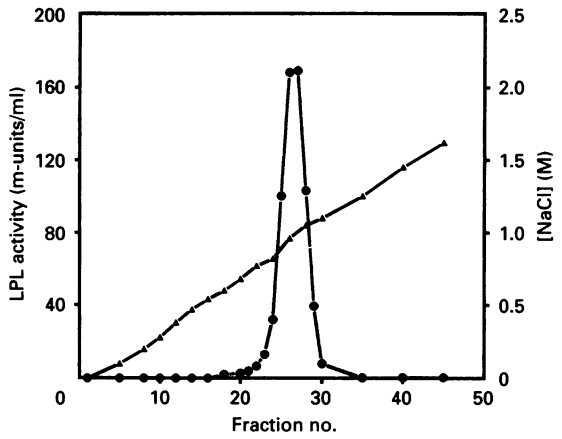

Figure 3 Representative elution pattern for LPL catalytic activity in homogenates of guinea-pig heart on chromatography on heparin–agarose

A guinea-pig heart was homogenized in 10 vol. of buffer D; 5 ml of the homogenate was applied to the heparin–agarose column and chromatographed as in Figure 2. ●, LPL activity; ▲, NaCl gradient.

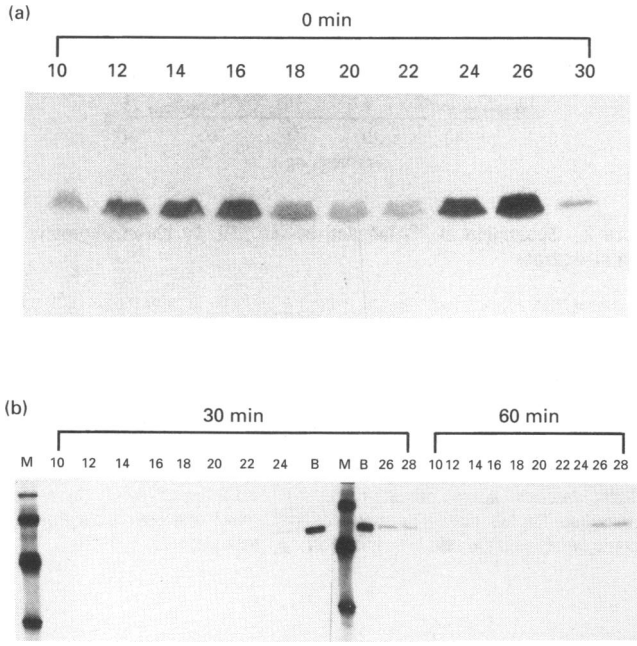

Figure 4 Separation of heart LPL by heparin–agarose chromatography after pulse and chase

Hearts were pulse-labelled for 10 min and then chased for 0 min (**a**), or 30 or 60 min (**b**; the chase times are indicated). Homogenates were chromatographed as in Figures 2 and 3, and fractions were subjected to immunoprecipitation. The immunoprecipitates were separated by SDS/PAGE, and fluorograms are shown. The numbers above the lanes denote fractions from chromatograms comparable with those in Figure 2 and 3. Lanes labelled M are ^{14}C-labelled molecular-mass standard proteins (same as in Figure 1). Lanes labelled B are ^{125}I-labelled bovine LPL.

dimer, but never with the monomer. Manipulations of the enzyme such as storage, addition of various salts to the buffer, or slight elevation of temperature, often led to decrease in or complete loss of catalytic activity. In such experiments active dimeric LPL formed either monomers (as evaluated by using sucrose gradients or heparin–agarose) or higher aggregates. These results support and extend the previous conclusion (Osborne et al., 1985) that conversion of active into inactive LPL is an essentially irreversible process associated with dissociation of dimers to monomers, which may in turn form higher aggregates.

What states of LPL do the two peaks from heparin–agarose chromatography correspond to? Physical studies have shown that active LPL is a dimer of two identical chains (Iverius and Östlund-Lindqvist, 1976; Osborne et al., 1985). In the dimer, heparin-binding sites on the two subunits can bind to the same heparin chain (Clarke et al., 1983). Hence, a higher salt concentration should be needed to dissociate dimeric than monomeric LPL from heparin–agarose, because the number of co-operating salt bridges that must simultaneously be broken is higher. This suggests that peak 1 is a monomeric form of LPL, whereas peak 2 is the active dimeric form of LPL. Peterson et al. (1992) reached a similar conclusion in a study with monoclonal antibodies to bovine LPL. One of the monoclonals detects only unfolded, catalytically inactive, LPL, presumably because its epitope is hidden in the folded, active, conformation. This monoclonal reacted with material that was eluted in peak 1 from heparin–agarose, but did not react with material eluted in peak 2.

To test if separation of LPL monomers and dimers on heparin–agarose could be applied to the enzyme in guinea-pig hearts, ^{125}I-labelled bovine LPL was mixed with a homogenate of guinea-pig heart and separated on heparin–agarose. Virtually all the labelled enzyme bound to the column and was eluted in fractions 26–34 of the salt gradient. The peak, fraction 30, corresponded to 1.0 M NaCl (Figure 2a). When the mixture of bovine LPL and homogenate was heated at 90 °C for 5 min in the presence of 1 % SDS, all LPL activity was lost, indicating a change of structure. The labelled LPL still adsorbed almost completely to the column, but was now eluted earlier in the gradient, in fractions 17–24. The peak, fraction 20, corresponded to 0.65 M NaCl (Figure 2a). When the peak fractions from these two chromatographies were pooled and re-run on heparin–agarose they could be well separated, as depicted in Figure 2(*b*). One peak appeared in fractions 15–24; the centre of this peak corresponded to about 0.65 M NaCl. The other peak appeared in fractions 26–34; its centre corresponded to around 1.0 M NaCl. Catalytic activity due to endogenous guinea-pig LPL in heart homogenates also bound almost quantitatively to heparin–agarose; less than 5% appeared in the pass-through and subsequent wash fractions. On gradient elution the activity appeared as a single peak (Figure 3), close to the position for peak 2 in Figure 2, i.e. where active bovine LPL was eluted. In no situation have we seen LPL activity to be eluted in the position of peak 1 in Figure 2, i.e. where unfolded LPL was eluted. From this we decided to use heparin–agarose chromatography to separate metabolically labelled LPL from guinea-pig hearts.

Pulse–chase experiments

When hearts were pulse-labelled for 10 min, heparin–agarose separated two types of LPL (Figure 4). The first peak was eluted at about 0.65 M, similar to the position for inactive LPL in Figure 2. There was no LPL catalytic activity in these fractions. The second peak was eluted in the same position as LPL catalytic activity in Figure 3. After 30 or 60 min of chase, peak 1 had disappeared and all immunoprecipitable labelled LPL was eluted in peak 2 (Figure 4). This suggests that peak 1 represents an early event in the biosynthesis and maturation of LPL and is a precursor for the catalytically active peak-2 form.

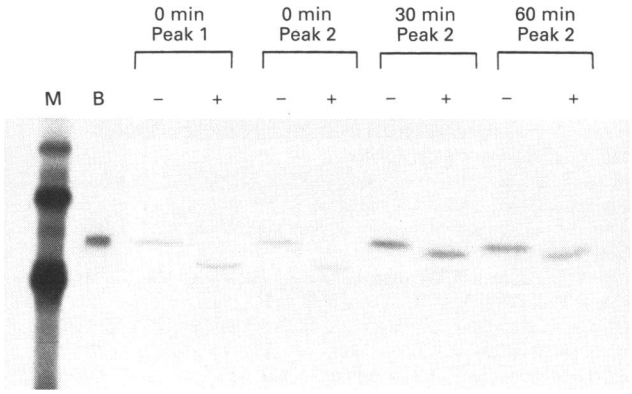

Figure 5 Endo H digestion of LPL from peaks 1 and 2 as separated by heparin–agarose chromatography

Hearts were pulse-labelled for 10 min, and then chased for 0, 30 or 60 min. Homogenates were separated by heparin–agarose chromatography. Fractions corresponding to peaks 1 and 2 in Figure 4 were immunoprecipitated, redissolved, and incubated with (+) or without (−) Endo H as described in the Materials and Methods section. The samples were then separated by SDS/PAGE (10% gels). The Figure shows a fluorogram. Lanes labelled M and B are as in Figure 4.

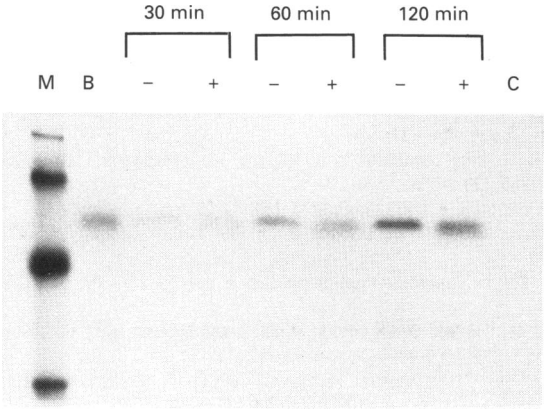

Figure 6 Endo H digestion of LPL released to the medium

Hearts were pulse-labelled for 10 min, chased for 30, 60 or 120 min, and then flushed for 1 min with medium containing 33 μg/ml heparin. LPL was immunoprecipitated from the medium, redissolved and incubated with (+) or without (−) Endo H as described in the Materials and Methods section. Lanes labelled M and B are as in Figure 4. The lane labelled C is a control with non-immune serum instead of antiserum.

Figure 5 demonstrates that both the peak-1 and peak-2 forms of LPL from pulse-labelled hearts were fully sensitive to Endo H digestion, which resulted in complete conversion of the original LPL band, about 55 kDa, into a faster moving form with apparent molecular mass around 51 kDa. Hence, both forms had only high-mannose-type chains at the end of the 10 min labelling period. Endo H digestion of LPL from hearts chased for 30 min gave rise to a form of LPL, which moved slightly faster than the original LPL, but slower than the fully Endo-H-sensitive form (Figure 5). This form of LPL has previously been observed in guinea-pig adipocytes, and was designated as partly Endo-H-resistant (Semb and Olivecrona, 1989a). In addition, some of the fully Endo-H-sensitive form was left after a 30 min chase, implying that not all the LPL molecules had reached the *trans*-Golgi. After 60 min chase all of the LPL was partly Endo-H-resistant.

LPL was released into the medium by a 1 min heparin wash-out, immunoprecipitated and digested with Endo H (Figure 6). No fully Endo-H-sensitive LPL was detected in the medium at any time. There was a partly resistant form, similar to that seen in hearts chased for 60 min (Figure 5). During a longer chase, more labelled LPL was released into the medium by heparin, but the sensitivity to Endo H did not change.

Conclusions

After pulse-labelling, LPL protein was present in both peak-1 and peak-2 forms. At this time, 10 min or less, the enzyme was presumably still contained in the ER (Hurtley and Helenius, 1989). In accord with this, the oligosaccharide chains were all sensitive to Endo H. These data strongly suggest that already in the ER the enzyme assembles into core-glycosylated dimers. This is in accord with the demonstration by Semb and Olivecrona (1989a) that high-mannose LPL isolated from guinea-pig adipocytes by lectin affinity chromatography was catalytically active. Recently Ben-Zeev et al. (1992) have shown that in COS cells incubated at 16 °C to inhibit vesicular transport, LPL was retained in the ER in a high-mannose catalytically active form; furthermore, active LPL accumulated in the ER of COS cells transfected with an LPL cDNA construct that contained a tetrapeptide (KDEL) ER retention signal at its C-terminal end. All of these studies shown that LPL can attain its catalytically active form already in the ER, while in a high-mannose form. Some recent studies indicate that, at least in rats and mice, removal of glucose residues from the core oligosaccharide(s) may be necessary before the enzyme can fold correctly (Ben-Zeev et al., 1992; Carroll et al., 1992). With chase in our experiments, the peak-1 material disappeared and the peak-2 material acquired resistance to Endo H. Taken together, these findings suggest that the order of intracellular processing and transport of LPL in the heart is as follows: LPL monomers are synthesized, core-glycosylated and assembled into dimers in the ER. Conversion of oligosaccharide chains from the high-mannose into the complex type occurs after dimerization. The processed dimeric LPL is secreted from the cell and transported to the endothelium without further change in structure. This interpretation is in accord with the present view that most oligomeric proteins are assembled in the ER (Hurtley and Helenius, 1989). In fact, it appears that correct assembly is often a prerequisite for transfer to the Golgi (Pelham, 1989).

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