Biochem. J. (1990) 271, 11–15 (Printed in Great Britain)

Synthesis and secretion of active lipoprotein lipase in Chinese-hamster ovary (CHO) cells

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Cultured Chinese-hamster ovary cells (CHO cells) were found to produce and secrete a lipase, which was identified as a lipoprotein lipase by the following criteria. Its activity was stimulated by serum and apolipoprotein CII, and was inhibited by high salt concentration. The lipase bound to heparin–agarose and co-eluted with ^{125}I-labelled bovine lipoprotein lipase in a salt gradient. A chicken antiserum to bovine lipoprotein lipase inhibited the activity and precipitated a labelled protein of the same apparent size as bovine lipoprotein lipase from media of CHO cells labelled with [^{35}S]methionine. The lipase activity and secretion were similar in growing cells and in cells that had reached confluency. Hence, lipoprotein lipase appears to be expressed constitutively in CHO cells and is not linked to certain growth conditions, as in pre-adipocyte and macrophage cell lines. At 37 °C, but not at 4 °C, heparin increased the release of lipase to the medium 2–4-fold. This increased release occurred without depletion of cell-associated lipase activity, suggesting that heparin enhanced release of newly synthesized lipase.

INTRODUCTION

Lipoprotein lipase is an enzyme localized at the vascular surface of endothelial cells in some tissues (reviewed in [1–5]). It hydrolyses triacylglycerols in chylomicrons and very-low-density lipoproteins, and thereby makes fatty acids available for use within the tissue. Its activity is under hormonal control, and differs much between tissues. This is thought to reflect differences in the rates at which active enzyme is delivered from parenchymal cells in the tissue to binding sites in adjacent capillaries. This rate, in turn, appears to be regulated both by changes in the abundance of lipoprotein lipase mRNA and by post-transcriptional mechanisms [5].

Lipoprotein lipase is produced in several cell types [2]. In general, these are cells engaged in rapid metabolism of fatty acids such as adipocytes and muscle cells. Another cell type which makes lipoprotein lipase is macrophages [6,7], but the function of this enzyme is less clear. Many studies on lipoprotein lipase have been carried out with cell lines [8–13] and with primary cultures of cells [14–19]. In the cell lines the expression of lipoprotein lipase is usually dependent on the growth conditions [6–8,12]. In the present paper we report that the enzyme is also produced in Chinese-hamster ovary (CHO) cells, where it is expressed both in growing and in confluent cells.

MATERIALS AND METHODS

Cell culture conditions

Chinese-hamster ovary cells (CHO-D) were obtained from Dr. Jan Sandström, Unit of Cell and Molecular Biology, University of Umeå. Line CHO-K1 was obtained from Dr. Karin Hjalmarsson, SYMBICOM, Umeå. Cells were maintained in MEM Alpha Medium with nucleosides (GIBCO) supplemented with 2 mM-glutamine, 10% (v/v) heat-inactivated fetal-bovine serum (GIBCO), 100 μg of streptomycin/ml and 100 units of penicillin/ml (GIBCO). Cells were grown at 37±0.3 °C in an atmosphere of 5% CO_2 in air and 100% relative humidity. The cells were usually subcultured every 4–5 days with trypsin/EDTA (GIBCO). HeLa cells were obtained from Professor Lars Thelander, Department of Medical Biochemistry and Biophysics, University of Umeå. In most experiments cells were grown in 25 cm^2 flasks with 5 ml of medium.

Harvesting of cells

For lipase activity measurements medium was decanted and stored at 4 °C where lipase activity was stable for 24 h. The cell layer was washed once with phosphate-buffered saline (Dulbecco's; Gibco) and then dissolved in 2 ml of cold 2.5 mM-ammonia, adjusted with HCl to pH 8.2, containing 5 mM-EDTA, and, per ml: 8 mg of Triton X-100, 0.4 mg of SDS, 33.3 μg of heparin, 10 μg of leupeptin, 1 μg of pepstatin and 25 kallikrein-inhibitor units of Trasylol. This buffer (A) was previously used for similar purposes [9,20]. Cells became detached after a few minutes in the buffer, and the extract was briefly sonicated to disrupt the cells fully. Both media and cell extracts were centrifuged (Beckman Microfuge) before assay of lipase activity. The activity was stable also in the cell extract at least for 24 h at 4 °C.

Lipase activity measurement

Lipase activity was measured in an incubation mixture containing ^{3}H-labelled triolein sonicated into Intralipid as described by Peterson *et al.* [21]. The mixture contained 5% (v/v) heat-inactivated rat serum as source of apolipoprotein CII. One unit of lipase activity corresponds to 1 μmol of fatty acid released/min at 25 °C. The incubations were usually carried out for 2 h, and the sample volumes amounted to 5% (v/v) or less of the incubation mixture. Under these conditions, there was a linear relation between lipase activity and time and amount of sample. The intra-assay variation was less than 5%.

Immunoinhibition

For this, media or cell extracts were mixed with an equal volume of antiserum, of corresponding preimmune serum or dilutions of the sera in 20 mM-sodium phosphate buffer/0.15 M-

Abbreviation used: CHO cells, Chinese-hamster ovary cells.
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NaCl, pH 7.4. After 2 h on ice the remaining lipase activity was determined. The antisera used were raised in chickens against bovine lipoprotein lipase isolated from milk or in rabbits against purified milk lipoprotein lipase from humans, cows and guinea pigs. We also tried antisera raised in rabbits against human and rat hepatic lipase.

[^{35}S]Methionine incorporation

Medium was aspirated, and the cell layer was washed once with phosphate-buffered saline and was then incubated for 10 min with Eagle's MEM with Earle's salts and 2 g of $NaHCO_3$/l without glutamine and methionine (Flow Laboratories), supplemented with 5% (w/v) BSA, 5 mM-glucose, 1 mM-glutamine, 0.1 μM-insulin (bovine, Sigma) and 1.25 mM-Hepes, pH 7.4. This medium was replaced by 4 ml of the same composition but also containing [^{35}S]methionine (1134 Ci/mmol, 90 μCi/ml; New England Nuclear) and the cells were then incubated for 2 h. Medium was recovered and the cells were washed and extracted as described above. Total incorporation of radioactivity into proteins was determined by precipitation with trichloroacetic acid on filters as described previously [15].

Immunoprecipitation

Media and cell extracts were denatured by addition of 1% SDS and heated for 5 min at 95 °C. They were then diluted 10-fold with cold buffer (pH 7.4) containing, per ml: 3.564 mg of $Na_2HPO_4,2H_2O$, 55 μg of NaN_3, 8.766 mg of NaCl, 10 mg of Triton X-100, 1 mg of SDS, 100 μg of heparin, 745 μg of EDTA and 17.4 μg of phenylmethanesulphonyl fluoride. For immunoprecipitation, samples containing 60000–100000 d.p.m. and $(10–20) \times 10^6$ d.p.m. in trichloroacetic acid-precipitable proteins were taken from media and from cell extracts respectively. To this 5 μl of a chicken antiserum or a corresponding preimmune serum was added, and the samples were incubated for at least 18 h at 4 °C. To isolate the immune complexes, 2.5 ml of Pharmacia Decanting Suspension 5 (Pharmacia Diagnostics AB, Uppsala, Sweden), containing insolubilized sheep antibodies to chicken IgG, was added. After 2 h at room temperature the samples were centrifuged and the gel washed as previously described [15]. The immune complexes were dissolved in SDS and separated by SDS/polyacrylamide-gel electrophoresis in a Laemmli-type system with ^{14}C-labelled standard proteins (New England Nuclear). The dried gels were first used for fluorography, and bands corresponding to the position of lipoprotein lipase were then cut out, eluted and counted for radioactivity [15].

Heparin–agarose chromatography

Heparin was covalently coupled to Sepharose-4B (Pharmacia) by the CNBr method. Two columns each containing 3.5 ml of gel were equilibrated in buffer A but without heparin. Cells were harvested in this buffer. Both the cell extract (12 ml) and the medium were briefly centrifuged (10000 *g* for 15 min). To 12 ml of cell extract and to 27.5 ml of medium was added about 0.5×10^6 c.p.m. of ^{125}I-labelled bovine lipoprotein lipase [21] as a reference, and the solutions were applied to the columns at a rate of 1 ml/min. Then the columns were washed with 30 ml of 20 mM-Tris/HCl, pH 7.4, containing 20% (v/v) glycerol and 0.1% (w/v) Triton X-100. The columns were then eluted by a linear gradient formed from 100 ml of this buffer and 100 ml of buffer of the same composition but also containing 2 M-NaCl; 5 ml fractions were collected. From each fraction 2 ml was taken for determination of ^{125}I radioactivity, and 10 μl samples were used for measurement of lipase activity. Initial binding was almost complete, and the total recoveries of lipase activity over the columns were close to 100%. The salt gradient was measured by conductometry of each fraction and compared with standard samples of NaCl in the same buffer. The enzyme activity in the ^{125}I-labelled bovine lipase was less than 10% of the total activities in the samples.

RESULTS

Lipoprotein lipase-like activity

Lipoprotein lipase-like activity was detected both in culture media and in cell extracts from CHO-D cells (Table 1). Lipase activity was related to the cell number and was present both in growing cells and in cells that had reached confluency. When new medium was added to the cells, 30–50% of the total activity in the system was in the medium after 2 h. The steady-state activity in media that had been on the cells for 24 or 48 h was usually 60–70% of the total activity in the system. Similar lipase activities were present also in a different strain of CHO cells, CHO-K1, but were absent from media and extracts from HeLa cells (results not shown).

Effects of serum and salt

The lipolytic activity had two of the characteristic properties of a lipoprotein lipase; it was inhibited by salt and was stimulated by serum or apolipoprotein CII. NaCl (0.85 M in the assay) inhibited the lipase activity of both media and cell extracts by more than 90%. When serum was omitted from the normal assay system, the lipase activity in a cell extract was 11% and in a corresponding medium it was 27% of the lipase activities measured in the complete assay system. When serum was replaced with purified human apolipoprotein CII, similar levels of activity were obtained to those with the normal assay system.

Immunoinhibition

The lipolytic activity both in cells and in media was inhibited by a chicken antiserum against bovine lipoprotein lipase (Fig. 1). It was previously known that this antiserum cross-reacts with lipoprotein lipases from both rats and mice [22]. In contrast, little or no specific inhibition was seen with rabbit antisera against bovine, human and guinea-pig lipoprotein lipases or against human and rat hepatic lipases.

Immunoprecipitation

The chicken antiserum was used for immunoprecipitation of [^{35}S]methionine-labelled proteins from cells and media. In the medium a protein of the same apparent molecular mass as bovine lipoprotein lipase was found (Fig. 2). Estimated from the immunoprecipitates, lipoprotein lipase amounted to 3.3–4%

Table 1. Lipase activity in CHO cells at different stages of growth

Cells were grown on Petri dishes (diam. 30 mm) with 1.5 ml of medium. Four dishes were harvested on 3 consecutive days. To obtain comparable data, medium was changed 2 h before the cells were harvested. The 2 h media were then collected from three plates. The cells were solubilized in 1.5 ml of the detergent-containing buffer. Each sample was assayed in duplicate. Data are means ± S.D. for the three plates. The fourth plate was used to count the cell number.

Day	$10^{-5}\times$ Cell number/dish	Lipase activity (m-units)		
		Cell	Medium	Total
1	3.2	2.10±0.09	1.59±0.11	3.69
2	7.5	5.78±0.27	2.61±0.14	8.39
3	17	8.22±0.38	5.10±0.17	13.32

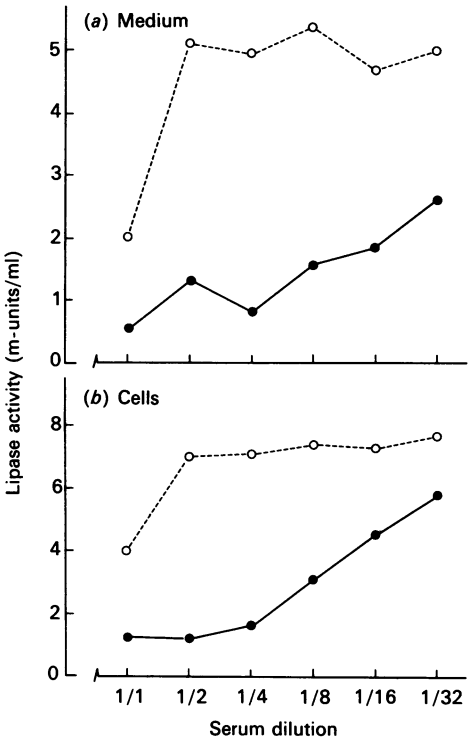

Fig. 1. Immunoinhibition of lipase activity in CHO cells by a chicken antiserum to bovine lipoprotein lipase

Conditions are detailed in the Materials and methods section. Incubation with pre-immune serum (○) and with antiserum (●). All data points are means of duplicate measurements of lipase activity and are expressed per ml undiluted medium or cell extract respectively. With the highest amounts of serum, inhibition was seen also with pre-immune serum. Therefore the serum dilutions were made in 20 mM-sodium phosphate (pH 7.4)/0.15 M-NaCl. Original activities in the medium and cell extract were 4.8 and 9.5 m-units/ml respectively.

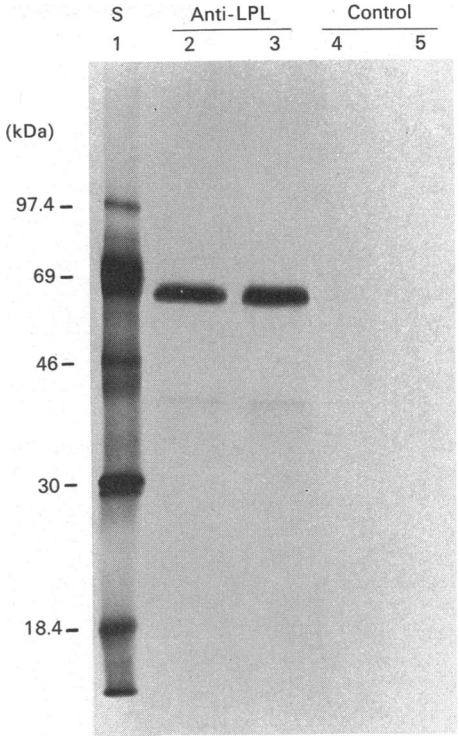

Fig. 2. SDS/polyacrylamide-gel electrophoresis and fluorography of [^{35}S]methionine-labelled immunoprecipitated proteins in culture medium

Conditions are described in the Materials and methods section. Lane 1: standard proteins (S: phosphorylase *b*, albumin, ovalbumin, carbonic anhydrase and lactoglobulin A); molecular masses given in kDa. Lanes 2 and 3: duplicate precipitations with chicken anti-[bovine lipoprotein lipase (LPL)] serum. Lanes 4 and 5: duplicate precipitations with pre-immune chicken serum. The film was exposed for 4 weeks. In other experiments ^{125}I-labelled bovine lipoprotein lipase was included as a standard. Its migration was indistinguishable from that of the immunoprecipitable material from CHO cells.

(two different experiments) of total labelled proteins secreted into the medium in 2 h. For cell homogenates the fraction of protein synthesis devoted to lipoprotein lipase was very low (less than 0.003%), and could not be determined exactly.

Effects of heparin

Another characteristic of lipoprotein lipase is its affinity for heparin [4]. *In vivo*, heparin releases the enzyme from tissue sites by forming soluble lipase-heparin complexes [4]. With both strains of CHO cells heparin caused a 2–4-fold increase in the release of lipase activity to the media during the first 2 h of incubation at 37 °C (Fig. 3). In similar experiments at 4 °C heparin had little or no effect on the activity recovered in the medium after 10 min incubation. The lipase activity in media was found to be rather stable during incubation under cell-culture conditions (37 °C) in the absence of cells. The half-life was around 3 h. Addition of heparin did not increase the stability (results not shown).

Heparin–agarose chromatography

On heparin–agarose chromatography, lipase activity both from cells and from media bound almost completely to the columns and was eluted by a salt gradient in the same position as active bovine lipoprotein lipase (Fig. 4). In these experiments ^{125}I-labelled bovine lipoprotein lipase was added as a marker for the positions of inactive lipase monomers and active lipase dimers respectively.

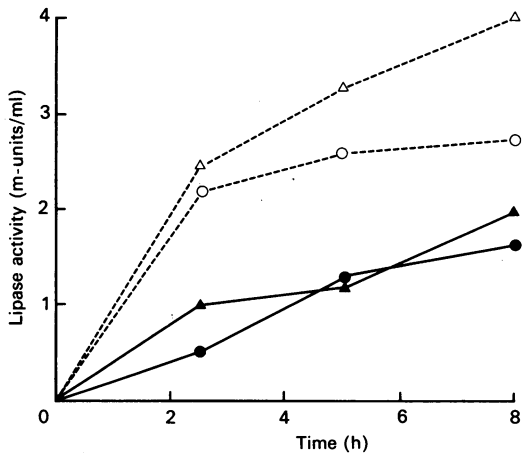

Fig. 3. Effects of heparin on release of lipase activity to the culture medium

CHO-D (●, ○) and CHO-K1 (▲, △) cells were grown in 25 cm^2 flasks with 4 ml of medium. At zero time the medium was changed to new medium with (○, △) or without (●, ▲) heparin (10 i.u./ml). At the indicated times 100 μl samples were taken and stored at 4 °C until assayed for lipase activity. Data points are means of duplicate measurements. The total cell activity at 18 h was 6.6 (●), 5.0 (○), 3.4 (▲) and 2.9 (△) m-units respectively.

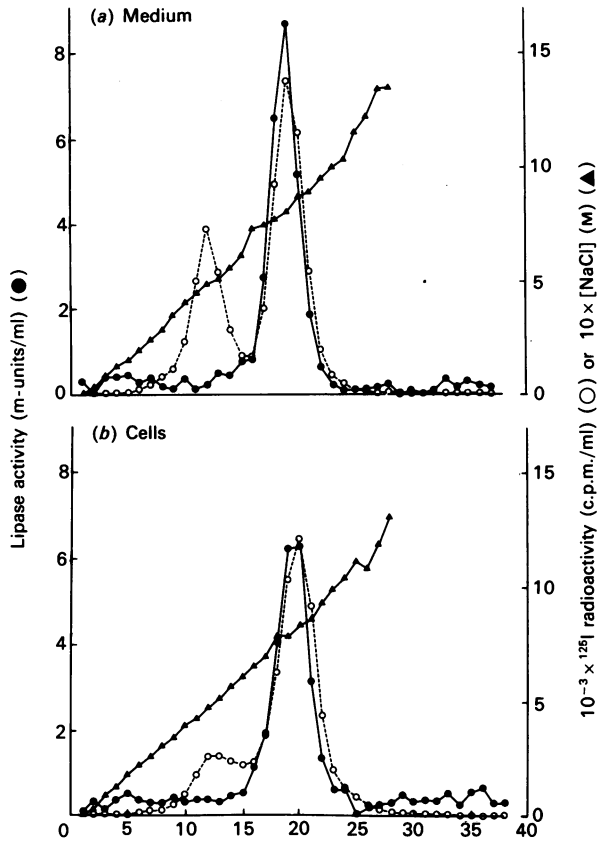

Fig. 4. Heparin–agarose chromatography of lipase activity in media and cell extracts from CHO-D cells

Conditions are described in the Materials and methods section. Iodinated bovine lipoprotein lipase was added for comparison of elution profiles. It was previously known that the main peak of ^{125}I radioactivity is co-eluted with the enzyme of bovine lipoprotein lipase dimers. The smaller peak earlier in the gradient corresponds to inactive lipase monomers [30].

DISCUSSION

In this paper we demonstrate that CHO cells synthesize and secrete active lipoprotein lipase. The enzyme exhibited the properties characteristic for lipoprotein lipases [1]. It required apolipoprotein C-II (or serum as a source of the apolipoprotein) for optimal activity, it was inhibited by high salt concentration, it bound to heparin–Sepharose, and when a salt gradient was applied the enzyme activity was eluted together with ^{125}I-labelled bovine lipoprotein lipase (added as internal standard). The strongest piece of evidence was that the CHO-derived lipase reacted with antisera raised against bovine lipoprotein lipase.

The lipase activity was similar in growing and in confluent cells, indicating that its expression was not dependent on the growth conditions, as is the case in several other cell lines. In 3T3-L1 and in *ob/ob* cells lipoprotein lipase synthesis is turned on after the cells become confluent and differentiate into adipocyte-like cells [8,12]. In macrophages, on the other hand, lipoprotein lipase production is high in activated [7] and proliferating [6] cells; when the cells become growth-arrested, lipoprotein lipase production is remarkably suppressed. The constitutive expression of lipoprotein lipase in CHO cells may reflect a property in the ancestral ovarian cells. It has recently been demonstrated that there is high lipoprotein lipase activity in guinea-pig ovaries [23]. The levels of activity in the CHO cells seem to be similar to those reported in stimulated macrophages [7], but at least 10-fold lower than in differentiated 3T3-L1 cells [9].

Lipoprotein lipases appear to be highly conserved among species [24]. cDNAs for the human [25], mouse [26], bovine [27] and guinea-pig [28] enzymes predict that the mature proteins all have 448–450 amino acids. Residue identities are more than 85% between any pair [24]. Chicken lipoprotein lipase contains 15–17 additional *C*-terminal amino acids, and shows 73–77% similarity to the four mammalian lipoprotein lipases [29]. The hamster enzyme had the same size on SDS/polyacrylamide gels as the other mammalian enzymes. In a panel of antisera raised against bovine lipoprotein lipase, the hamster enzyme was inhibited only by the sera which inhibited mouse and rat lipoprotein lipase. Several of the antisera inhibited human, but not rat or mouse, lipoprotein lipase. These antisera did not inhibit the hamster enzyme. This suggests that, as expected, the hamster enzyme is more closely related to the mouse and rat enzymes than to human or guinea-pig lipoprotein lipase.

In several experiments we monitored the release of lipase from the cells. There was an initial rapid increase of activity in the medium, but after some hours the activity approached an apparent steady-state level. The cell-associated activity remained constant. This is qualitatively similar to observations in other cell systems [9,10]. The suggested interpretation is that the cells constantly produce lipase, which is either transferred to the medium or degraded [9,11,15,16]. When protein synthesis is blocked by cycloheximide, cellular lipoprotein lipase activity decays with a half-life of less than 1 h in most previously studied cell systems [8–10,13,15]. During the first 1 h an activity corresponding to 10–20% of total cell-associated activity appeared in the medium of the CHO cells. Hence, a substantial basal release of the enzyme occurred, but this is probably not the major pathway for turnover of cellular lipoprotein lipase. In the medium the lipase slowly lost its catalytic activity (half-life about 3 h). Previous studies have shown that the enzyme protein is retained [9,11], so that the loss of activity must reflect a conformational change, probably dissociation of active dimeric enzyme into inactive monomers [30].

In most previously studied cell systems heparin greatly increases release of lipoprotein lipase to the medium [8–10,13–19]. The response to heparin generally shows two phases. There is an initial rapid release which is independent of protein synthesis. This probably represents release of preformed lipase at or close to the cell surface. Then follows a slower continued release which is dependent on synthesis of new enzyme molecules. Heparin augmented lipase release also in the present system, 2–4-fold during the first hours. Lipase from CHO cells and from media bound almost quantitatively to heparin-Sepharose, and when a salt gradient was applied the enzyme was eluted in the same position as bovine lipoprotein lipase. Thus the hamster enzyme must have a similar interaction with heparin to that of other mammalian lipoprotein lipases [4].

CHO cells should provide a useful model to study intracellular processing and transport of lipoprotein lipase. A major advantage is that production of lipoprotein lipase appears to be constitutive in these cells and is not linked to differentiation processes. Another advantage compared with adipocyte cell lines which have been used in previous studies is that the CHO cells do not contain large fat-droplets, which complicate cell-fractionation studies.

This work was supported by grants from the Swedish Medical Research Council (B13X-727).

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Received 28 December 1989/18 May 1990; accepted 31 May 1990