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HEPARIN-RELEASED TRIGLYCERIDE LIPASE FROM CHANG LIVER CELLS

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Summary: Heparin-released triglyceride lipase (TGL) from Chang liver cells (ATCC CCL 13) was investigated using very low density lipoproteins (VLDL) as a substrate. The TGL activity was released into the culture medium when the cells were incubated with heparin. The enzyme showed maximum activity at pH 8.5, and 80% inhibition by 0.6 M NaCl. These results indicated that Chang liver cells, a cell line derived from liver, synthesize lipoprotein lipase.

INTRODUCTION

There has been considerable interest in the role of liver as a site of lipoprotein catabolism, as well as its role in synthesis and secretion of lipids and lipoproteins. Especially hepatic-triglyceride lipase (H-TGL) has been thought to play an important role in lipoprotein catabolism, but the physiological function of this enzyme is still far from clear. Cell culture is a useful methodology for biological study. We describe here the nature of triglyceride lipase (TGL) activity released with heparin from a liver cell line: Chang liver cells (ATCC CCL 13).

MATERIALS AND METHODS

Preparation of cells: Chang liver cells (ATCC CCL 13) were maintained in Eagle's minimum essential medium (MEM) containing 10% fetal bovine serum. The cells grown confluently in a glass bottle were harvested by treatment with 0.02% EDTA. Dislodged cells were pelleted by centrifugation (700 x g for 10 min). The cells were resuspended in the medium (Eagle's MEM containing 2% bovine serum albumin, pH 7.2) at the concentration of 5 x 10^6 cells per ml.

Preparation of enzyme: Sodium heparin was added to the cell suspension at the final concentration of 20 U per ml, and the mixture was incubated at room temparature with continuous gentle shaking. The cells were removed by centrifugation after 3 hours incubation, and the supernatants were obtained as the enzyme.

Enzyme assays: Lipolytic activity was assayed, using as substrate very low density lipoproteins (VLDL) prepared from normal human serum. 0.1 ml of VLDL containing 1.5 mg/ml of triglycerides was added to 0.1 ml of the enzyme. When the inhibition test was performed, 0.05 ml of inhibitor was added to this reaction mixture. After incubation at 37°C for one hour, the liberated free

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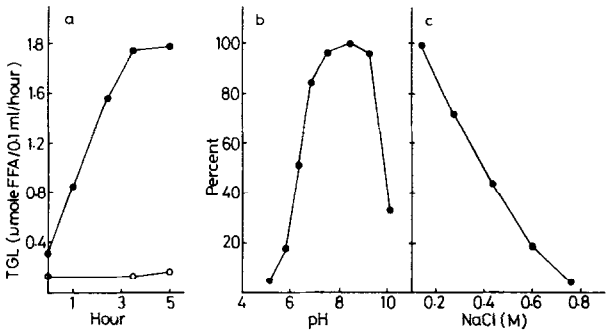

Figure 1

a) Effect of different incubation times on the release of TGL from Chang liver cells. Sodium heparin for a final concentration of 20 U per ml was added to Chang liver cells suspended in Eagle's MEM containing 2% bovine serum albumin (pH 7.2). The released TGL activity was determined after incubation was carried out at room temperature for indicated times. The enzyme activity was assayed as described in the text.

●——●: with heparin o——o: without heparin

b) Hydrolysis of triglycerides with heparin-released TGL at different pH values. Incubation was performed for one hour at 37°C. Each value is expressed in percentage of maximum hydrolytic activity as determined by the release of free fatty acids.

c) Inhibition of heparin-released TGL with sodium chloride. A NaCl solution (0.05 ml) was added to the mixture of the enzyme and the substrate (0.2 ml, pH 8.5) at 0°C for the indicated final concentration of the salt. Then incubation was performed for one hour at 37°C. Each value is expressed in percentage of maximum hydrolytic activity as determined by the release of free fatty acids.

fatty acids were determined by the enzymatic method (1), using NEFA C Test Wako (Wako Pure Chemcal Co., Ltd., Oosaka, Japan). The activity of TGL was expressed as μ mole of liberated free fatty acids per 0.1 ml of the enzyme solution per hour (μ mole FFA/0.1 ml/hour).

RESULTS

The effect of different incubation times on the release of TGL from Chang liver cells is shown in Fig. la. When the cells were incubated in the medium containing heparin, the enzyme activity increased linearly up to 2 hours. The maximum release was obtained at the incubation time of 3.5 hours. This enzyme release was achieved when heparin concentration in the medium was 10 U per ml or more (data not shown). On the other hand, essentially no TGL activity was released without heparin.

This nature of Chang liver cells was confirmed not to be due to transmissible agent by infection test of the culture meium of this cell line to others (FL and Flow 1000, these cell line do not have a higher active TGL than Chang liver cells).

The optimum pH of this enzyme showed a wide range (7.5 - 9.0) with a peak of pH 8.5 (Fig. 1b). The enzyme activity was inhibited by NaCl in the reaction mixture, showing a linear decrease with a salt concentration up to 0.6 M (Fig. 1c). 80% of the control value (0.15 M) was inhibited by 0.6 M.

DISCUSSION

Our data conclusively shows that Chang liver cells synthesize lipoprotein lipase (LPL), releasable with heparin. H-TGL is generally considered to be resistant to NaCl (2). However, the TGL activity from Chang liver cells was inhibited by the salt. Also, some investigators observed that hepatic plasma membrane lipase in rats is inhibited by NaCl (3). The problems of the relationship between TGL from hepatic and extrahepatic tissues remain to be defined. However, we think the evidence shows that this cell line is a useful material for studying LPL.

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REFFERENCES

1. Shimizu, s., Yasui, K., Tani, Y., and Yamada, H. (1979) Biochem. Biophys. Res. Commun. 91, 108-113.
2. LaRosa, J.C., Levy, R.I., Windmueller, H.G., and Fredirickson, D.S. (1972) J. Biol. Chem. 13, 356-363.
3. Assmann, G., Krauss, R.M., Fredirickson, D.S., and Levy, R.I. (1973) J. Biol. Chem. 248, 1992-1999.