ISOLATION OF APOLIPOPROTEIN-FREE LIPOPROTEIN LIPASE FROM HUMAN POST-HEPARIN PLASMA

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Summary: Lipoprotein lipase from human post-heparin plasma was purified at least 10,000-fold using the Fielding procedure. When the purified lipoprotein lipase was subjected to polyacrylamide electrophoresis, a single band with lipolytic activity and four additional bands were observed. These four bands are identical in their electrophoretic and immunochemical properties to the polypeptides of apolipoprotein C. Evidence is presented which suggests that one or more of these polypeptides may serve as a partial activator of this enzyme.

High density lipoproteins (1,2) and, very recently, very low density lipoproteins (3) have been found to activate triglyceride hydrolysis by lipoprotein lipase (LPL). Several reports have appeared recently which indicate that specific apolipoprotein polypeptides may be responsible for this activation (4-6). We have employed the procedure described by Fielding (7) for the purification of LPL in our continuing studies to characterize this enzyme in human post-heparin plasma. The present report describes results which indicate that the LPL isolated from human plasma by this procedure contains components which are identical in their immunochemical and polyacrylamide gel electrophoretic behavior to apolipoprotein C polypeptides.

MATERIALS AND METHODS

Preparation of Lipoprotein Lipase: Blood was obtained 30 min following the intravenous administration of 10,000 units of heparin to normolipidemic subjects who had fasted overnight. The plasma lipolytic activity, measured by in vitro assay (8), was normal in each subject. LPL was isolated and purified

following the Fielding procedure (7), with the exceptions that triolein (Analabs, Inc.) emulsified in gum arabic (8) was substituted in several instances for Intralipid (Vitrum, Stockholm, Sweden), the enzyme-triglyceride complex was isolated by repeated ultracentrifugation at 59,000 x g (force calculated at top of tube) for 1 hr in a Type SW 27.2 rotor, and a mixture of ethanol and diethyl ether (3:1, v/v), followed by diethyl ether was used for extracting lipid and deoxycholate from the detergent-treated, lyophilized enzyme. The purified enzyme, designated LPL_{CP}, was eluted from the calcium phosphate gel (Calbiochem, Los Angeles, Calif.) and samples obtained for protein and immunochemical analyses, polyacrylamide gel electrophoresis and assay of lipolytic activity.

Immunochemical Analyses: Double diffusion according to the Ouchterlony method (9) and immunoelectrophoresis according to the method of Grabar and Williams as modified for microscopic slides by Scheidegger (10) were performed in agar gel, using purified antisera to human serum lipoprotein A (anti-LPA, prepared in goats), lipoprotein B (anti-LPB, from rabbits), lipoprotein C (anti-LPC, from sheep), very low density lipoproteins ($d < 1.006$ g/ml, anti-VLDL, from rabbits) and albumin (from goats) (11,12)*.

Polyacrylamide Gel Electrophoresis: Disc gel electrophoresis was performed using 7% polyacrylamide, pH 4.3 or 8.9. In some instances the gel contained 8 M urea. The bands were stained for protein with Amido Black B or Coumassie Blue and for lipase by the Gomori procedure (13).

Protein Analyses: Protein was determined by the Lowry method (14), using bovine serum albumin (Mann Research Laboratories, New York, N.Y.) as standard.

Assays of Lipolytic Activity: Lipolytic activity of enzyme preparations was measured using an assay mixture containing the following (per ml): 60 mg of bovine serum albumin (Armour, Fraction V), 25 µmole of ammonium sulfate, 7.82 µmole of glyceride emulsified in 30 mg of gum arabic (8) and,

* These antisera were kindly provided by Dr. P. Alaupovic and his associates.

unless noted otherwise, 0.05 ml of human serum. In some instances, egg phosphatidylcholine* (Analabs, Inc., North Haven, Conn.) replaced serum in the assay mixture. Assays other than those utilizing ^{14}C-triolein were performed following the procedure described previously (8), with the exceptions that the substrate was not activated prior to addition of the enzyme and incubations were for 30 min. Triolein*, diolein*, monoolein*, tributyrin* (Analabs, Inc.) and Lipostrate-CB (Ediol; Calbiochem) served as glyceride substrates. Assays of the purified enzyme were also performed using ^{14}C-triolein* (glyceryl trioleate-1-^{14}C; Amersham-Searle Corp., Arlington Heights, Ill.). In these instances, the assay procedure described above was utilized, except that incubations were extended to 60 min and analyses were performed following the procedure described by Schotz (15).

RESULTS AND DISCUSSION

LPL_{CP} had a specific activity of 2,000-2,500 units per mg of protein, representing a purification of at least 10,000-fold relative to the initial specific activity in post-heparin plasma. Greater purification resulted when triolein was substituted for Intralipid in the isolation procedure. Triolein was not hydrolyzed by LPL_{CP} in the absence of serum, although some activity for tributyrin was observed in serum-free assay mixtures. Diolein and monoolein were not hydrolyzed by LPL_{CP} in the presence or absence of serum.

Immunochemical analyses demonstrated that LPL_{CP} reacted with anti-LPC (Fig. 1) and anti-VLDL, but not with anti-LPA, anti-LPB and anti-albumin. Additional evidence that apolipoprotein polypeptides were present was obtained when LPL_{CP} was subjected to polyacrylamide gel electrophoresis. Five bands, designated 1-5, were observed (Fig. 2). Lipolytic activity, as demonstrated by direct staining as well as *in vitro* assay after elution from polyacrylamide gel, was found only in Band 1 (designated LPL_{PA}). Triolein was not hydrolyzed

* Thin-layer chromatographic analysis of each of these compounds demonstrated a single area with appropriate R_f value.

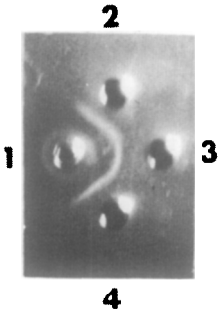

Figure 1. Immunodiffusion analysis demonstrating a reaction of identity between LPL_{CP} and apolipoprotein C (Apo-C) from human serum. 1 = Anti-LPC; 2 and 4 = LPL_{CP}; 3 = Apo-C.

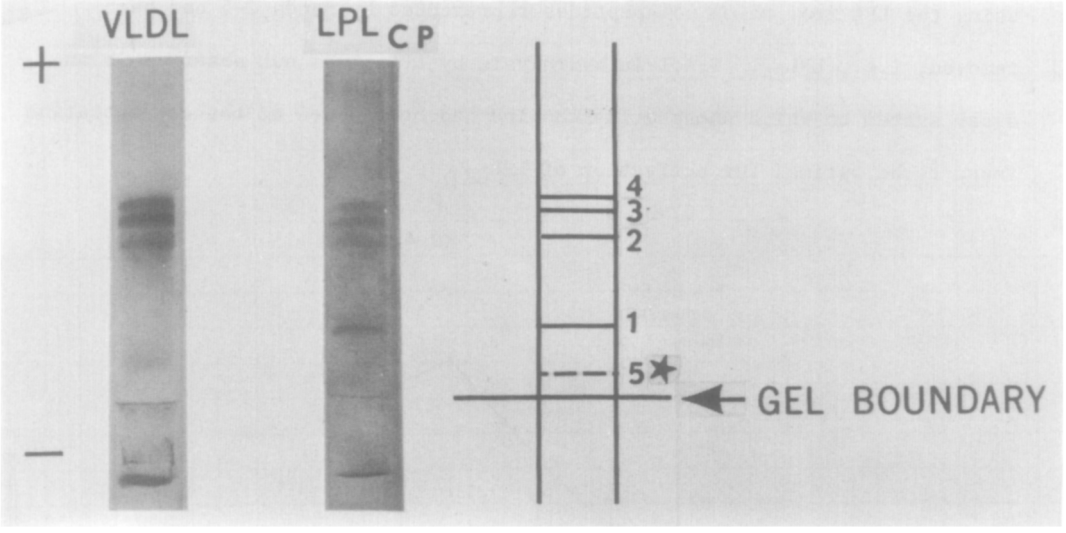

Figure 2. Separation of LPL_{CP} by polyacrylamide gel electrophoresis at pH 8.9. Lipolytic activity was found only in Band 1, designated LPL_{PA}. Bands 2 through 5 from LPL_{CP} are similar in electrophoretic mobility to the apolipoprotein polypeptides in which the carboxy-terminal amino acid is glutamic acid (Band 2), alanine (Bands 3 and 4) and valine (Band 5). *Band 5 was observed at pH 8.9 only when larger amounts of LPL_{CP} were applied. This component of LPL_{CP} was demonstrated consistently, however, by polyacrylamide electrophoresis at pH 4.3.

by LPL_{PA} in the absence of serum unless a specific activator, i.e. the apolipoprotein polypeptides containing glutamic acid or valine as the carboxy-terminal amino acid, was added. Bands 2-5, but not Band 1 reacted with anti-LPC. Similar results have been obtained in studies of LPL_{CP} isolated from 12 subjects.

Results presented in Figure 3 indicate that the apolipoprotein polypeptides present in LPL_{CP} may serve as a partial activator of this enzyme. Although inactive when assayed in the absence of serum, LPL_{CP} exhibited a significant lipolytic activity following the addition of phosphatidylcholine to the serum-free assay mixture (Fig. 3). Other investigators (6) have reported recently that phospholipids stimulate lipolytic activity in LPL systems activated by specific apolipoprotein polypeptides. The possibility that one or more of the polypeptides contained in LPL_{CP} were responsible for the activation observed when phosphatidylcholine was added was evaluated using the LPL from which polypeptides represented by Bands 2-5 had been removed, i.e., LPL_{PA}. Triolein hydrolysis by LPL_{PA} was not observed in an assay system to which phosphatidylcholine had been added at the concentration found to be optimal for activation of LPL_{CP}.

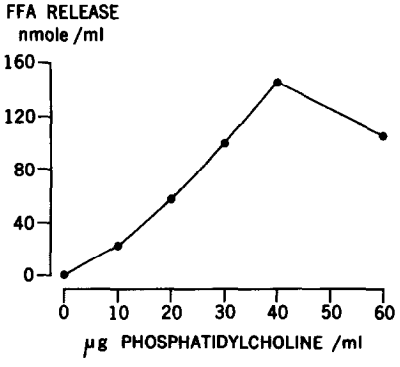

Figure 3. Activation of LPL_{CP} by phosphatidylcholine. Values for free fatty acid (FFA) release and phosphatidylcholine are presented per ml of assay mixture. The assay mixture contained 4 units (1 unit of enzyme releases 1 µmole of FFA per hr) of LPL_{CP} per ml, ^{14}C-triolein (5 µCi per mmole) and additional components described in the Materials and Methods section.

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REFERENCES

1. Korn, E. D., J. Biol. Chem., 215, 15 (1955).
2. Scanu, A. and I. H. Page, J. Exptl. Med., 109, 239 (1959).
3. Bier, D. M. and R. J. Havel, J. Lipid Res., 11, 565 (1970).
4. Havel, R. J., V. G. Shore, B. Shore and D. M. Bier, Circulation Res., 27, 595 (1970).
5. Fielding, C. J., C. T. Lim and A. M. Scanu, Biochem. Biophys. Res. Commun., 39, 889 (1970).
6. La Rosa, J. C., R. I. Levy, P. Herbert, S. E. Lux and D. S. Fredrickson, Biochem. Biophys. Res. Commun., 41, 57 (1970).
7. Fielding, C. J., Biochim. Biophys. Acta, 206, 109 (1970).
8. Bradford, R. H. and R. H. Furman, Biochim. Biophys. Acta, 164, 172 (1968).
9. Ouchterlony, O., Progr. Allergy, 5, 1 (1958).
10. Scheidegger, J. J., Intern. Arch. Allergy Appl. Immunol., 7, 103 (1955).
11. Seidel, D., P. Alaupovic and R. H. Furman, J. Clin. Invest., 68, 1211 (1969).
12. Lee, D. M. and P. Alaupovic, Biochemistry, 9, 2244 (1970).
13. Gomori, G., in Microscopic Histochemistry-Principles and Practice. Univ. of Chicago Press, Chicago, Ill., 1952, p. 204-205.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr and P. J. Randall, J. Biol. Chem., 193, 265 (1951).
15. Schotz, M. C., A. S. Garfinkel, R. J. Huebotter and J. E. Stewart, J. Lipid Res., 11, 68 (1970).