ISOLATION OF C-I AND C-II ACTIVATED LIPOPROTEIN LIPASES AND PROTAMINE INSENSITIVE TRIGLYCERIDE LIPASE BY HEPARIN-SEPHAROSE AFFINITY CHROMATOGRAPHY

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Received 12 March 1975

1. Introduction

Recent evidence from several laboratories has indicated the presence of at least three long chain triglyceride hydrolases in the post-heparin plasma of normolipidemic subjects and laboratory animals [1–7]. These triglyceride hydrolases have the following properties:

1) Lipoprotein lipase C-I (LPL_{C-I}) is present in post-heparin plasma but not in adipose tissue from normal controls or post-heparin plasma from subjects with Type I hyperlipoproteinemia. LPL_{C-I} is activated by serum or C-I (one of the apolipoprotein polypeptides of lipoprotein C) but not C-II and is inhibited by NaCl and protamine sulfate [7].

2) Lipoprotein lipase C-II (LPL_{C-II}) is present in post-heparin plasma and adipose tissue [7–10]. LPL_{C-II} is activated by serum or C-II but not C-I and is inhibited by NaCl and protamine sulfate.

3) A third triglyceride lipase (TGL) differs from the foregoing two lipases in that it is insensitive to inhibition by protamine sulfate and 1 M NaCl [1,2]. TGL is believed to be secreted from liver [5] and is not activated by serum or apolipoprotein cofactors [15];

LPL_{C-II} [6] and TGL [4–6], but not LPL_{C-I} have been isolated by heparin-sepharose chromatography. The present report describes the isolation of all three lipases by heparin-sepharose chromatography of post-heparin plasma from normal controls, and demonstrates that the 1.5 M NaCl eluate which contains LPL_{C-II} [4–6] also exhibits LPL_{C-I} activity following desalting of this fraction.

2. Materials and methods

2.1. Source of post-heparin plasma

Post-heparin plasma was obtained from healthy donors who had fasted overnight (14–16hr). Blood was drawn into iced plastic bags containing 1/50 vol of 4% sodium citrate 30 min after intravenous administration of 10 000 units of hepatin sodium (Upjohn Co., Kalamazoo, Michigan). Plasma was separated by centrifugation (4,500 rev/min) at 0°C and stored at −15°C.

2.2. Isolation of LPL_{C-I} and LPL_{C-II} by Sephadex G-100 chromatography

A preparation containing LPL_{C-I} and LPL_{C-II}, which had been isolated as described previously [11], was applied to a column (1.5 × 100 cm) containing Sephadex G-100 equilibrated with 0.05 M NH_4OH–NH_4Cl buffer, pH 8.5. Additional buffer (4°C) was applied at the rate of 20–25 ml/hr and the eluate was collected in 2.0-ml fractions. Each fraction was analyzed for protein [12] and lipolytic activity using [^{14}C]-triolein activated by serum, C-I or C-II.

2.3. Isolation of LPL_{C-I}, LPL_{C-II} and TGL by heparin-Sepharose chromatography

Heparin-Sepharose column chromatography was performed according to the procedure described by Boberg, et al. [6]. Small columns (0.4 × 10 cm) were filled to a height of 2.5 cm with heparin-Sepharose, prepared according to the method of Egelrud and

Olivecrona [13] and equilibrated with 5 mM Veronal buffer, pH 7.4, containing 0.3 M NaCl. An aliquot (4.0 ml) of post-heparin plasma, diluted with an equal volume of 5.0 mM Veronal buffer containing 0.3 M NaCl, was applied to the column. The column was eluted sequentially with 8.0 ml of Veronal buffer containing 0.3 M NaCl, 8.0 ml of Veronal buffer containing 0.72 M NaCl (elution of TGL) followed by an additional 6.0 ml of this solution, and 8.0 ml of 1.5 M NaCl–Veronal buffer (elution of LPL_{C-I} and LPL_{C-II}). Each fraction was assayed for lipolytic activity using [^{14}C]-triolein activated by serum, C-I or C-II, as well as for response to protamine sulfate (3.0 mg/ml).

Fractions eluted with Veronal buffer containing either 0.72 M NaCl or 1.5 M NaCl were desalted by chromatography on Sephadex G-25 (0.4 × 10 cm columns, filled to a height of 5–6 cm) equilibrated with Veronal buffer.

2.4. Assays of lipolytic activity

Preparation of the substrate containing [^{14}C]-triolein has been described previously [7], except that Triton X-100 was substituted for gum arabic as an emulsifier. Fatty acid was extracted by the method of Schotz et al. [14]. Lipolytic activity was also measured in the presence of protamine sulfate (3.0 mg/ml), according to the procedure described by Krauss et al. [1].

2.5. Isolation and characterization of C-I and C-II

C-I and C-II polypeptides were isolated and characterized by the methods described previously [16].

3. Results and discussion

Our earlier studies have demonstrated the presence of two lipoprotein lipases, LPL_{C-I} and LPL_{C-II}, in post-heparin plasma normal controls [15]. Fig.1 shows the elution pattern of these two LPLs on Sephadex G-100 chromatography, and their polypeptide activation characteristics. LPL_{C-I} and LPL_{C-II} are inactivated by 1 M NaCl and protamine sulfate (3 mg/ml) either when preincubated with the inhibitor or when the inhibitor is added during incubation of the assay mixture. LPL_{C-I} exhibited greater sensitivity than LPL_{C-II} to NaCl inhibition.

In the present study, TGL was eluted from heparin-sepharose columns prior to the two LPLs, required no serum cofactor, and was insensitive to

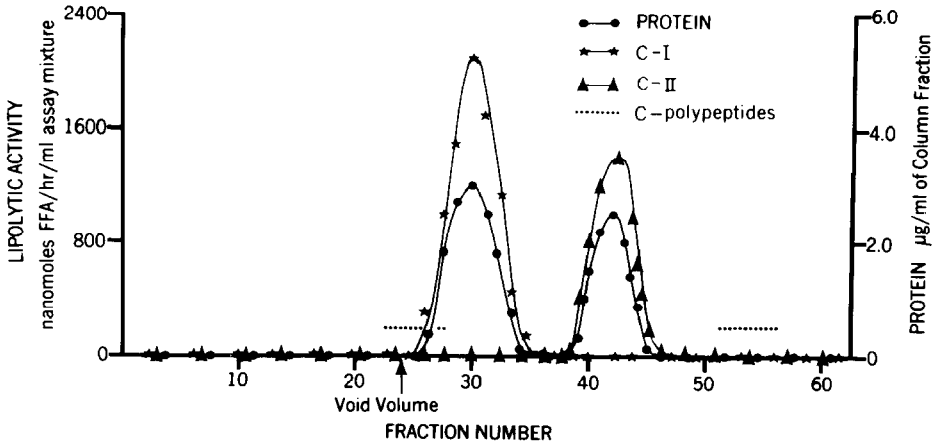

Fig.1. Separation of LPL_{C-I} (first fraction eluted), LPL_{C-II} and lipoprotein C polypeptides by Sephadex G-100 chromatography. The sodium citrate eluate from calcium phosphate gel chromatography of post-heparin plasma from normal controls was applied to the column, and eluted as described under Methods. Each eluate fraction (2.0 ml) was analyzed for protein and lipolytic activity. Assays for lipolytic activity were performed using [^{14}C]-triolein (22 000 dpm) emulsified in Triton X-100, 5 µmol of C-I or C-II, 10 µg of phosphatidylcholine, 60 mg of albumin and 25 µmole of ammonium sulfate at pH 8.5. Incubations at 37°C were for 1 hr.

Table 1
Characteristics of lipolytic activities present in human post-heparin plasma

Enzyme preparation	Lipolytic activity μmol/ml enzyme/hr				Protamine sulfate***
	Activator added				
	None	Serum	C-I	C-II	Percent of control
LPL_{C-I}*	0.02	0.80	1.20	0.04	6
LPL_{C-II}*	0.01	1.02	0.03	0.81	14
0.72 M** NaCl-Veronal buffer fraction	4.97	5.42	4.03	5.26	123
0.72 M** NaCl-Veronal buffer fraction after Sephadex G-25 chromatography	5.10	4.98	4.60	5.12	102
1.5 M** NaCl-Veronal buffer fraction	0.65	6.09	0.80	10.80	16
1.5 M** NaCl-Veronal buffer fraction after Sephadex G-25 chromatography	0.67	3.43	7.85	1.01	11

Assay mixtures (1.0 ml total vol) contained 0.1 ml of enzyme solution, either 0.05 ml of serum or 10 μg C-I or C-II polypeptide and 0.5 ml of substrate containing 10 μmole of [^{14}C]-triolein emulsified with Triton X-100.

*These enzyme fractions were obtained by Sephadex G-100 chromatography.

**These fractions were obtained by heparin-sepharose chromatography.

***Protamine sulfate was added to the enzyme solution 10 min prior to beginning the 1-hr incubation (at 27°C) with the [^{14}C]-triolein substrate. Values are presented as percent of control lipolytic activity: lipolytic activity in assay containing protamine sulfate/control lipolytic activity.

protamine sulfate (table 1). These data are in agreement with those reported by Boberg et al. [6]. Lipolytic activity in the 1.5 M NaCl–Veronal buffer fraction (containing LPL_{C-I} and LPL_{C-II}) was activated by the addition of serum or C-II and was inhibited by protamine sulfate (table 1). Although disc gel electrophoresis on 7% polyacrylamide containing 8 M urea demonstrated protein bands characteristic of LPL_{C-I} as well as LPL_{C-II}, C-I activation was not detected prior to desalting this fraction by chromatography on Sephadex G-25. Furthermore, activation by C-I was somewhat less (50–70%) than that observed in a LPL_{C-I} preparation isolated by Sephadex G-100 chromatography, suggesting that the salt inactivation of LPL_{C-I} was only partially reversible under these conditions. In most but not all instances, the desalting process resulted in loss of activity in LPL_{C-II}.

These data provide additional evidence indicating that post-heparin plasma contains at least three triglyceride hydrolases. Heparin-sepharose column chromatography provides a simple one-step procedure for separating TGL from LPL_{C-I} and LPL_{C-II}.

Acknowledgements

The authors express their gratitude to Joyce Gibson for technical assistance, to Dr R. H. Bradford for his critiscism and suggestions in the preparation of this report, and to Drs W. J. McConathy and P. Alaupovic for providing C polypeptides and for their continued interest in this research. The assistance of Mrs Lynn Green and Mrs Marty Moon in preparing the manuscript is also appreciated. This research was supported in part by NHLI Grant No. 1R01 HL 15336–01A1.

References

[1] Krauss, R. M., Windmueller, H. C., Levy, R. I. and Frederickson, D. S. (1973) J. Lipid Res. 14, 286–295.
[2] Ehnholm, C., Greten, H. and Brown, W. V. (1974) Biochim. Biophys. Acta 360, 68–77.
[3] Greten, H., Walter, B. and Brown, W. V. (1972) FEBS Lett. 27, 306–310.
[4] Fielding, C. J. (1972) Biochim. Biophys. Acta 280, 569–575.
[5] Greten, H., Sneiderman, A. D., Chandler, J. G., Steinberg, D. and Brown, W. V. (1974) FEBS Lett. 49, 157–160.
[6] Boberg, J., Augustine, J., Baginsky, M., Tejeda, P. and Brown, W. V. (1974) Circulation 21-III.
[7] Ganesan, D., Bradford, R. H., Alaupovic, P. and McConathy, W. J. (1971) FEBS Lett. 15, 205–209.
[8] Havel, R. J., Shore, V. G., Shore, B. and Bier, D. M. (1970) Circulation Res. 27, 595–600.
[9] LaRosa, J. C., Levy, R. I., Herbert, P., Lux, S. E. and Fredrickson, D. S. (1970) Biochem. Biophys. Res. Comm. 41, 57–62.
[10] Havel, R. J., Fielding, C. J., Olivecrona, T., Shore, V. G., Fielding, P. E. and Egelrud, T. (1973) Biochemistry 12, 1828–1833.
[11] Ganesan, D., Bradford, R. H. and Bass, H. B. (1970) Biochem. Biophys. Res. Comm. 43, 544–549.
[12] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[13] Egelrud, T. and Olivecrona, T. (1972) J. Biol. Chem. 247, 6212–6217.
[14] Schotz, M. C., Garfinkel, A. S., Huebotter, R. J. and Stewart, J. E. (1970) J. Lipid Res. 11, 68–69.
[15] Ganesan, D., Bradford, R. H. and Bass, H. B. (1974) Clin. Res. 22, 649A.
[16] Alaupovic, P., Lee, D. M. and McConathy, W. J. (1972) Biochem. Biophys. Acta 260, 68–69.