# DIFFERENTIAL ACTIVATION OF LIPOPROTEIN LIPASE FROM HUMAN POST-HEPARIN PLASMA, MILK AND ADIPOSE TISSUE BY POLYPEPTIDES OF HUMAN SERUM APOLIPOPROTEIN C

D. GANESAN, R.H. BRADFORD, P. ALAUPOVIC and W.J. McCONATHY

*Cardiovascular Section, Oklahoma Medical Research Foundation and the Departments of Biochemistry and Medicine, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma 73104, USA*

Received 23 April 1971

## 1. Introduction

Specific polypeptides of human serum apolipoproteins have recently been found to activate triglyceride hydrolysis by LPL* from cow's milk [1] and rat adipose tissue [2]. These investigators demonstrated a similar polypeptide specificity for activation of the enzyme from both sources. Polypeptides containing glutamic acid or alanine as carboxyterminal amino acid, but not those containing valine, glutamine and threonine, served as activators.

The present study was undertaken to evaluate the activation of purified LPL from human post-heparin plasma by immunochemically homogeneous polypeptides of human serum Apo-A and Apo-C and phosphatidylcholine. Inasmuch as the polypeptide specificity of LPL from human post-heparin plasma was found to differ from that reported for LPL from cow's milk [1] and rat adipose tissue [2], additional studies were performed using LPL from human milk and adipose tissue as well as purified LPL from dog and rat post-heparin plasma.

Purified lipoprotein lipase from post-heparin plasma of normolipidemic subjects was activated to a greater extent by the R-valine polypeptide of Apo-C than by its R-glutamic acid polypeptide. Neither the R-alanine polypeptide nor the R-threonine and R-glutamine polypeptides of apolipoprotein A served as an activator. This pattern of polypeptide specificity was similar to that observed for purified lipoprotein lipase from rat and dog post-heparin plasma, but differs from that for lipoprotein lipase from adipose tissue (human and rat) and milk (human and cow), suggesting the possibility that two distinct lipoprotein lipolytic activities are present in these tissues.

* *Abbreviations*:
LPL : lipoprotein lipase
Apo-A and Apo-C: apolipoproteins A and C
FFA: free fatty acids
PC : phosphatidylcholine

## 2. Materials and methods

### 2.1. *Preparation of lipoprotein lipase*

Blood was obtained 30 min following the intravenous administration of 10,000 units of heparin to subjects with normal serum lipid concentrations, who had fasted overnight. Plasma lipolytic activity, measured by in vitro assay [3], was normal in each subject. LPL was isolated and purified by a modification [4] of the method of Fielding [5]. The purified enzyme eluted from calcium phosphate gel, designated $LPL_{CP}$, had a specific activity of 2,000–2,500 units (one unit of enzyme releases 1 μmole of free fatty acid per hr) per mg of protein, representing a purification of at least 10,000-fold. Results of immunochemical and electrophoretic analyses of $LPL_{CP}$ have recently been reported [4].

Additional purification of $LPL_{CP}$ using polyacrylamide gel electrophoresis was performed to obtain an apolipoprotein-free lipase, designated $LPL_{PA}$ [4].

Human milk, obtained from a volunteer donor in the second month post-partum, was centrifuged at 3,250 *g* for 20 min at 5° and the cream layer discarded. After centrifugation was repeated once, the skimmed milk was lyophilized and the lyophilized powder was dissolved in 0.05 M ammonium hydroxide–ammonium chloride buffer, pH 8.5, 25 mg per ml [6]. Samples of subcutaneous adipose tissue were obtained from subjects at the time of surgery, and an acetone–ether powder prepared [7].

Studies of polypeptide specificity were also performed using purified LPL from rat and dog post-heparin plasma and LPL from rat epididymal fat. Blood was obtained 10 min (rats) or 20 min (dogs) following the intravenous administration of 100 units of heparin per kg body weight. Procedures similar to those described above were utilized for isolation and purification of LPL from plasma and for preparation of an acetone–ether powder of adipose tissue.

### 2.2. *Assay 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 μmoles of ammonium sulfate and 10 μmoles of $^{14}$C-triolein* (triolein [Analabs, Inc., North Haven, Conn.] containing 5 μCi of glyceryl trioleate-1-$^{14}$C [Amersham-Searle Corp., Arlington Heights, Ill.] per mmole, emulsified in 3 mg of gum arabic per μmole of triglyceride [3]). Purified apolipoprotein polypeptides and/or egg phosphatidylcholine* (Analabs, Inc.) were added to the assay mixture as indicated. Incubations at pH 8.5 and 37° were performed for 60 min and the production of $^{14}$C-oleic acid measured [8].

### 2.3. *Preparation and characterization of apolipoprotein polypeptides*

Apo-A and Apo-C and their polypeptides were isolated and purified from human serum high density lipoproteins (d 1.063–1.210 g/ml) and very low density lipoproteins (d < 1.006 g/ml), using the procedure described previously [9]. Studies were performed using the following purified polypeptides, which are designated by their carboxy-terminal amino acid: R-glutamine, R-threonine, R-valine, R-alanine and R-glutamic acid. Criteria of homogeneity for individual purified polypeptides included the demonstraton of a single precipitin line when each polypeptide preparation was subjected to immunoelectrophoretic analysis, using specific antisera to Apo-A, Apo-C, R-Gln, R-Thr, R-Val or R-Ala. The amino acid composition of Apo-A [10] and Apo-C [11] polypeptides agreed with published results.

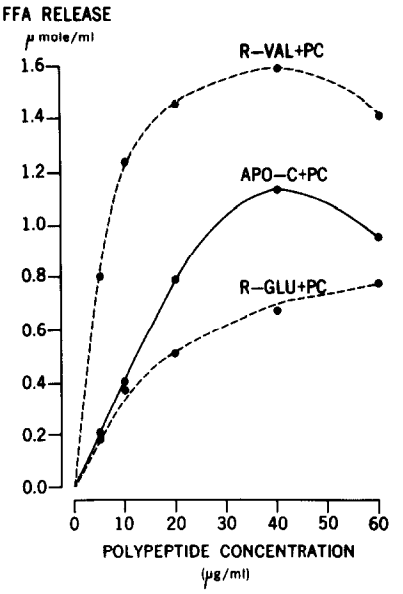


Fig. 1. Activation of purified $LPL_{CP}$ from human post-heparin plasma by Apo-C and its R-Val and R-Glu polypeptides isolated from human serum. Values for FFA release and polypeptide concentration are presented per ml of assay mixture. Each ml of assay mixture contained 4 units of $LPL_{CP}$ and 20 μg of PC. Apparent $K_m$ values (presented as nmole of protein per ml of assay mixture): R-Val = 0.8, R-Glu = 2.8.

## 3. Results and discussion

$LPL_{CP}$ from human post-heparin plasma hydro-

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

Table 1
Activation of LPL from post-heparin plasma, adipose tissue and milk by Apo-C and its R-Val, R-Glu and R-Ala polypeptides.

| | FFA Release (μmole/ml) | | | |
|---|---|---|---|---|
| | Apo-C | R-Val | R-Glu | R-Ala |
| $LPL_{CP}$* | | | | |
| Human | 1.14 | 1.68 | 1.08 | 0 |
| Rat | 0.22 | 0.32 | 0.24 | 0 |
| Dog | 0.42 | 0.62 | 0.33 | 0 |
| Adipose LPL** | | | | |
| Human | 0.08 | 0 | 0.15 | 0.05 |
| Rat | 0.16 | 0 | 0.25 | 0.18 |
| Milk LPL** | | | | |
| Human | 0.20 | 0 | 0.42 | 0.20 |
| Cow | 0.18 | 0 | 0.40 | 0.18 |

Values for FFA release are presented per ml of assay mixture. Each ml of assay mixture contained 20 μg of the polypeptide indicated and 40 μg of PC. The R-Glu and R-Thre polypeptides were also evaluated and found to be inactive.
* Each ml of assay mixture contained 4 units of enzyme.
** Each ml of assay mixture contained 2.5 mg of protein from the acetone–ether extract of adipose tissue or 5 mg of skimmed milk protein.

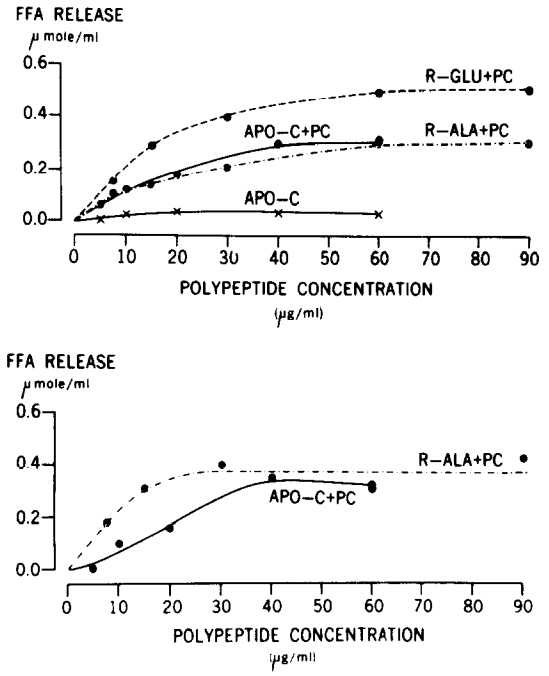


Fig. 2. Activation of lipoprotein lipase from cow's milk (upper panel) and rat adipose tissue (lower panel) by Apo-C and its R-Glu and R-Ala polypeptides isolated from human serum. Values for FFA release and polypeptide concentration are presented per ml of assay mixture. Each ml of assay mixture contained either 5 mg of skimmed milk protein or 2.5 mg of protein from the acetone–ether extract of adipose tissue, and, where indicated, 40 μg of PC. Apparent $K_m$ values (presented as nmole of protein per ml of assay mixture): for milk lipase, R-Ala = 2.0, R-Glu = 3.1; for adipose tissue lipase, R-Ala = 1.1.

lyzed triolein in the presence but not in the absence of serum or phosphatidylcholine [4]. Activation of this purified enzyme was then evaluated using Apo-A, Apo-C and their polypeptides. Apo-A and its R-Gln and R-Thr polypeptides, in concentrations up to 80 μg per ml, failed to activate $LPL_{CP}$. R-Val produced the greatest activation of $LPL_{CP}$ (fig. 1). Apo-C and another of its polypeptides, R-Glu, but not R-Ala activated $LPL_{CP}$ in the presence of phosphatidylcholine. In the absence of phosphatidylcholine, only R-Val served as an activator (less than 10% of the value observed with phosphatidylcholine). The polypeptide specificity of apolipoprotein-free lipase, i.e. $LPL_{PA}$, was similar to that observed for $LPL_{CP}$. Similar results were obtained using fifteen different preparations of $LPL_{CP}$ and five different preparations of each polypeptide.

The polypeptide specificity of LPL from human milk and adipose tissue differed from human plasma LPL, inasmuch as the most potent activator (R-Val) of plasma LPL had no effect on milk and adipose tissue LPL (table 1). R-Glu activated LPL from human milk and adipose tissue to a relatively greater extent than human plasma LPL. R-Ala activated human milk LPL, but had little effect on human plasma LPL.

Purified LPL from dog and rat post-heparin plasma had a similar polypeptide specificity to that observed with human plasma LPL (table 1). A similar pattern of polypeptide specificity was also observed for LPL from human and rat adipose tissue (fig. 2) and for LPL from human and cow's milk (fig. 2). The results for cow's milk and rat adipose tissue obtained in the present study confirm those reported previously [1, 2] using LPL from these sources.

The present study demonstrates two patterns of polypeptide specificity for LPL activation. One pattern

was observed in purified LPL from post-heparin plasma of human subjects, dogs and rats, and a second pattern was exhibited by LPL in adipose tissue from human subjects and rats, and in milk from human subjects and cows. These observations suggest the possibility that at least two LPLs exist, one in post-heparin plasma and another in the other tissues studied.

Preliminary results from studies undertaken recently in our laboratory indicate that $LPL_{CP}$ in post-heparin plasma from two subjects with type IV hyperlipoproteinemia exhibits a polypeptide specificity similar to that observed in controls. Nevertheless, the plasma $LPL_{CP}$ from two siblings with type I hyperlipoproteinemia was partially activated by R-Glu and Apo-C, but not by R-Val. This atypical pattern of polypeptide specificity provides additional evidence favoring the conclusion that the LPL present in type I hyperlipoproteinemia is abnormal, as has been suggested previously on the basis of results obtained from inhibitor and enzyme kinetics studies [3]. Alternatively, this observation might indicate that the LPL present in post-heparin plasma of other subjects is absent in type I hyperlipoproteinemia.

## Acknowledgements

The authors are grateful to Helen Bass, Radha Narayanan, Marvin McDearmon and Gerald Hillerman for technical assistance and to Lynn Greene for assistance in preparing the manuscript. These studies were supported in part by grants from the National Heart and Lung Institute, U.S. Public Health Service (HE-7005) and the Oklahoma Heart Association.

## References

[1] R.J. Havel, V.G. Shore, B. Shore and D.M. Bier, Circulation Res. 27 (1970) 595.
[2] J.C. LaRosa, R.I. Levy, P. Herbert, S.E. Lux and D.S. Fredrickson, Biochem. Biophys. Res. Commun. 41 (1970) 57.
[3] R.H. Bradford and R.H. Furman, Biochim. Biophys. Acta 164 (1968) 172.
[4] D. Ganesan, R.H. Bradford and H.B. Bass, Biochem. Biophys. Res. Commun., in press.
[5] C.J. Fielding, Biochim. Biophys. Acta 206 (1970) 109.
[6] D.M. Bier and R.J. Havel, J. Lipid Res. 11 (1970) 565.
[7] E.D. Korn and T.W. Quigley, J. Biol. Chem. 226 (1958) 833.
[8] M.C. Schotz, A.S. Garfinkel, R.J. Huebotter and J.E. Stewart, J. Lipid Res. 11 (1970) 68.
[9] P. Alaupovic, G. Kostner, D. Lee, W. McConathy and H. Magnani, Exposes Ann. Biochem. Med., in press.
[10] V. Shore and B. Shore, Biochemistry 7 (1968) 3396.
[11] W.V. Brown, R.I. Levy and D.S. Fredrickson, J. Biol. Chem. 245 (1970) 6588.