EFFECT OF SERUM AND C-APOPROTEINS FROM VERY LOW DENSITY LIPOPROTEINS ON HUMAN POSTHEPARIN PLASMA HEPATIC LIPASE

Paavo K. J. KINNUNEN and Christian EHNHOLM
Department of Serology and Bacteriology, University of Helsinki, Finland

Received 27 February 1976

1. Introduction

The presence of a lipase of hepatic origin in postheparin plasma of experimental animals has been confirmed by several investigators [1–6]. An enzyme with similar characteristics has also been found in humans [7–9]. This enzyme hydrolyzes triglycerides, phospholipids and acylCoA thiolesters [4,9] and has been shown to be a transacylase [10]. Hepatic lipase clearly differs from lipoprotein lipase of extrahepatic origin in its co-factor requirement and immunological reactivity. Also, it is not inhibited by high ionic strength in the assay medium [5,7,11,12].

One of the characteristics of lipoprotein lipase is that it needs for full activity a VLDL-apoprotein, apoC-II. This has been demonstrated for purified lipoprotein lipase from adipose tissue [14], heart [15], postheparin plasma [4] and milk [16]. A recent study with bovine milk lipoprotein lipase indicates, that depending on the substrate concentration and the presence of apoC-II in the assay medium, also apoC-I and apoC-III can stimulate fatty acid liberation [17].

We now report, that low concentrations of serum activate purified human postheparin plasma hepatic lipase. The C-apoproteins from VLDL did not cause this stimulation, but all of them suppressed the enzymatic activity.

Abbreviations: VLDL: very low density lipoproteins; apoC-I, apoC-II and apoC-III: apoproteins from very low density lipoproteins having carboxyl terminal amino acid serine, glutamic acid and alanine, respectively [13].

2. Material and methods

2.1. *Enzyme assay*

Triglyceride hydrolase was assayed using glyceryl-[^{14}C]triolein emulsified in the presence of 5% gum arabic as substrate [15]. Unless otherwise stated, assays were performed in the absence of serum and in a medium containing 0.1 M NaCl.

2.2. *Enzyme purification*

Hepatic lipase and lipoprotein lipase were purified from human postheparin plasma essentially as described [12], using: (a) formation of an enzyme–substrate complex followed by delipidation, (b) affinity chromatography of the solubilized preparation on heparin-Sepharose and (c) chromatography on calcium phosphate gel. The specific activities of the final preparations were approx. 1100 and 1800 μmol fatty acid liberated/mg protein/h, for hepatic lipase and lipoprotein lipase, respectively.

2.3. *Purification of the C-apoproteins from human VLDL*

The C-apoproteins from human VLDL [18] were purified from delipidated preparations [19] as described by Herbert et al. [20]. They were shown to be pure by polyacrylamide gel electrophoresis [20] and by amino acid analysis [21].

3. Results and discussion

The activity of purified human postheparin plasma hepatic lipase was measured as a function of serum

North-Holland Publishing Company – Amsterdam

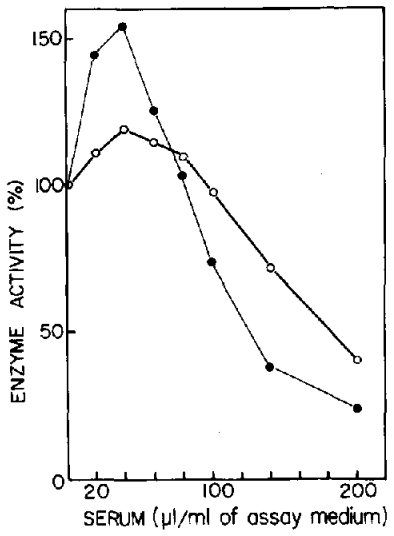

Fig.1. Effect of serum on the activity of human postheparin plasma hepatic lipase. Effect on enzyme obtained from heparin-Sepharose affinity chromatography, before calcium phosphate gel treatment (o—o) and after calcium phosphate gel (•—•). The amount of serum in the assay medium is indicated on the abscissa.

concentration in the assay medium, fig.1. A stimulation at low concentrations of serum is seen, whereas higher concentrations cause inhibition. This stimulation was evident also in lipase preparations before calcium phosphate gel chromatography, fig.1.

To study, if the serum stimulation was due to any of the C-apoproteins from VLDL, the activity of hepatic lipase was recorded in the presence of varying amounts of apoC-I, C-II and C-III in the assay medium (fig.2). They all inhibited the enzymatic activity. The inhibition caused by apoC-II and C-III was linear, while apoC-I was giving a sigmoidal inhibition curve. Thus the stimulation of hepatic lipase does not seem to be due to the VLDL-C-apoproteins. The identity of the stimulating serum factor remains uncertain, but it is of interest, that pre-heparin plasma causes a shift in the pH-optimum of the hepatic lipase [22].

We then added varying amounts of human VLDL to the standard assay medium and determined the activity of purified hepatic lipase and lipoprotein lipase (fig.3). Addition of VLDL suppressed the hydrolysis of [^{14}C]triolein by both enzymes. The two enzymes differ in that hepatic lipase is inhibited by 55%, whereas lipoprotein lipase is inhibited by 84%. Whether this inhibition is due to suppression by VLDL of enzymatic activities or whether this lipoprotein is a preferred substrate, needs further study.

A growing interest has been focused on the role of lipases in lipoprotein metabolism. Although results obtained from in vitro studies have shown that apoprotein C-II plays a major role in the activation of lipoprotein lipase, far less is known about the role of other C-apoproteins. The function of the hepatic lipase and its relationship to apoproteins is still an open question. We now report that the hepatic lipase

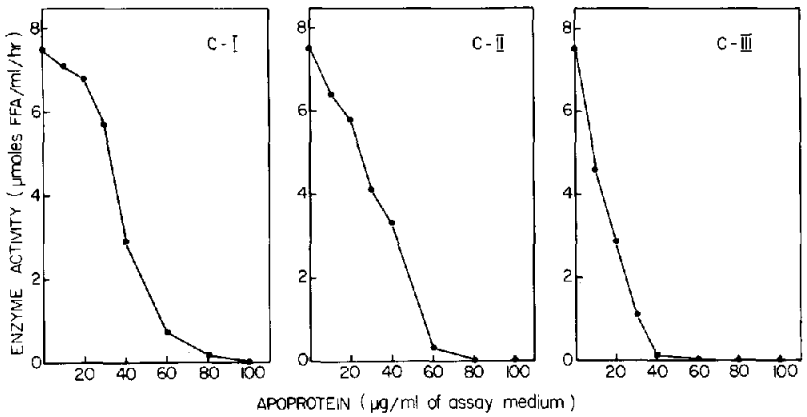

Fig.2. Effect of C-apoproteins from human VLDL on the activity of human postheparin plasma hepatic lipase. (Left) Effect of apoC-I. (Middle) Effect of apoC-II. (Right) Effect of apoC-III. The amount of apoprotein added to the assay medium is indicated on the abscissa.

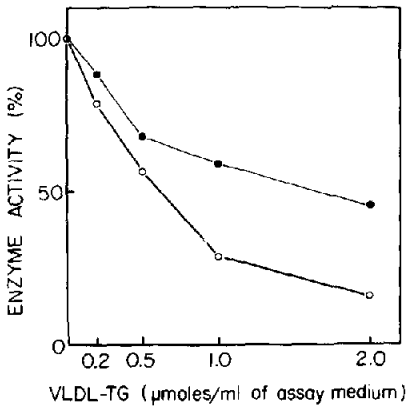

Fig.3. Effect of addition of human VLDL to the assay medium. Enzyme activity was determined from the amount of ^{14}C-fatty acid liberated. The values are corrected for the isotope dilution due to the addition of VLDL-triglyceride, amounts indicated on the abscissa. Hepatic lipase (•—•) and lipoprotein lipase (○—○).

in vitro is strongly inhibited by apoC-I, C-II and C-III. If intact chylomicron and VLDL triglycerides are preferentially hydrolyzed by lipoprotein lipase, as has been suggested [23,24] and not by hepatic lipase, the effects of the C-apoproteins on the activities of the two triglyceride lipases could be meaningful in vivo.

The C-apoproteins of chylomicrons and VLDL would inhibit the hepatic lipase and thus protect their triglyceride from hydrolysis by this enzyme. However, these lipoproteins rich in the lipoprotein lipase activator apoC-II, would be a good substrate for lipoprotein lipase in extrahepatic tissues. Following hydrolysis of the triglycerides of these lipoproteins, remnant particles are formed [25–27]. These remnants contain less C-apoproteins than the intact lipoproteins [25], at least part of them being transferred to the high density lipoproteins [28]. If hepatic lipase is involved in the removal of remnants from plasma, as has been suggested [29], the low concentration of C-apoprotein in remnants might direct the activity of hepatic lipase towards remnant rather than chylomicron and VLDL triglyceride. The findings by Redgrave and by Noel et al. further strenghten this concept, they observed a rapid removal of remnant lipid by liver, whereas intact chylomicrons were taken up to a limited extent [25,30].

Acknowledgements

The skillful technical assistance of Ms Seija Puomilahti and Mrs Barbro Bång is gratefully acknowledged. Also our thanks are due to Mrs Pirkko Kahrama for typing the manuscript and for Mrs Satu Cankar for drawing the figures. This study was granted from The Finnish Heart Association and from The National Council for Medical Sciences, Finland.

References

[1] Fielding, C. J. (1972) Biochim. Biophys. Acta 280, 569.
[2] LaRosa, J., Levy, R. I., Windmueller, H. G. and Robinson, D. S. (1972) J. Lipid Res. 13, 356.
[3] Assman, G., Krauss, R. M., Fredrickson, D. S. and Levy, R. I. (1973) J. Biol. Chem. 248, 1992.
[4] Ehnholm, C., Bensadoun, A. and Brown, W. V. (1973) J. Clin. Invest. 52, 26a.
[5] Greten, H., Sniderman, A. D., Chandler, J. G., Steinberg, D. and Brown, W. V. (1974) FEBS Lett. 42, 157.
[6] Jansen, H. and Hülsmann, W. C. (1974) Biochim. Biophys. Acta 369, 387.
[7] Greten, H., Walter, B. and Brown, W. V. (1972) FEBS Lett. 27, 306.
[8] Ehnholm, C., Shaw, W., Greten, H., Lengfelder, W. and Brown, W. V. (1974) in: Atherosclerosis (Schettler, G. and Weizel, A., eds.) p. 557, Springer-Verlag, Berlin.
[9] Ehnholm, C., Shaw, W., Greten, H. and Brown, W. V. (1975) J. Biol. Chem. 250, 6756.
[10] Waite, M., Sisson, P., Freude, K. A. and Zieve, F. J. (1975) Biochem. Biophys. Res. Comm. 67, 471.
[11] Jansen, H., van Zuylen-van Wiggen, A. and Hülsmann, W. C. (1973) Biochem. Biophys. Res. Comm. 55, 30.
[12] Ehnholm, C., Kinnunen, P. K. J. and Huttunen, J. K. (1975) FEBS Lett. 52, 191.
[13] Alaupovic, P. (1971) Atherosclerosis 13, 141.
[14] Bensadoun, A., Ehnholm, C., Steinberg, D. and Brown, W. V. (1974) J. Biol. Chem. 249, 2220.
[15] Ehnholm, C., Kinnunen, P. K. J., Huttunen, J. K., Nikkilä, E. A. and Ohta, M. (1975) Biochem. J. 149, 649.
[16] Havel, R. J., Fielding, C. J., Olivecrona, T., Shore, V. G., Fielding, P. E. and Egelrud, T. (1973) Biochemistry 12, 1828.
[17] Östlund-Lindqvist, A.-M. and Iverius, P.-H. (1975) Biochem. Biophys. Res. Comm. 65, 1447.
[18] Havel, R. J., Eder, H. A. and Bragdon, J. H. (1955) J. Clin. Invest. 34, 1345.
[19] Brown, W. V., Levy, R. I. and Fredrickson, D. S. (1969) J. Biol. Chem. 244, 5687.

[20] Herbert, P., Levy, R. I. and Fredrickson, D. S. (1971) J. Biol. Chem. 246, 7068.
[21] Shore, V. G. and Shore, B. (1951) in: Blood Lipids and Lipoproteins: Quantification, Composition and Metabolism (Nelson, G. S., ed.) Ch. 15, Wiley-Interscience, New York.
[22] Ehnholm, C., Greten, H. and Brown, W. V. (1974) Biochim. Biophys. Acta 360, 68.
[23] Robinson, D. S. (1970) in: Comprehensive Biochemistry (Florkin, M. and Stotz, E. M., eds.) Vol. 18, p. 51, Elsevier, Holland.
[24] Fielding, C. J. and Higgins, J. M. (1974) Federation Proc. 33, 351.
[25] Noel, S.-P., Dolphin, P. J. and Rubinstein, D. (1975) Biochem. Biophys. Res. Comm. 63, 764.
[26] Eisenberg, S., Bilheimer, D. W., Levy, R. I. and Lindgren, F. T. (1973) Biochim. Biophys. Acta 326, 361.
[27] Bierman, E. L., Eisenberg, S., Stein, O. and Stein, Y. (1973) Biochim. Biophys. Acta 329, 163.
[28] Havel, R. J., Kane, J. P. and Kashyap, M. L. (1973) J. Clin. Invest. 52, 32.
[29] Bergman, E. N., Havel, R. J., Wolfe, B. M. and Bøhmer, T. (1971) J. Clin. Invest. 50, 1831.
[30] Redgrave, T. G. (1970) J. Clin. Invest. 49, 465.