Reconstituted high density lipoprotein behaviour is modified by the nature of the lipid vesicle

Sheelagh M.A. Jones and M. Perenna Rogers*

Department of Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, England

Received 14 November 1984

Apo HDL is a more potent inhibitor of lipoprotein lipase than native HDL HDL reconstituted from apo HDL and vesicles made from either HDL total lipids or HDL phospholipids inhibits lipoprotein lipase in the same manner as native HDL HDL reconstituted from apo HDL and vesicles made from phosphatidylcholine and sphingomyelin still behaves like apo HDL

High density lipoprotein *Lipid modification* *Lipid–protein interaction* *Lipoprotein lipase* *Phospholipid vesicle* *Reconstitution*

1. INTRODUCTION

Native high density lipoprotein inhibits the hydrolysis of Intralipid by lipoprotein lipase from rat [1] and human [2]. During studies to investigate which apoprotein(s) are responsible for the inhibition we found that delipidated apo HDL is a more potent inhibitor than native HDL. The present work was undertaken to determine whether, if the apoprotein was reconstituted with lipid vesicles, the native HDL behaviour would be restored.

2. MATERIALS AND METHODS

Rat HDL (d 1.063–1.21) was isolated from rat serum as previously described [3] and was delipidated by a modified method of Windmueller using 3:2 (v/v) chloroform:methanol [4]. The apoprotein was soluble in 6 M urea, 0.2 M Tris (pH 8) and recoveries after the removal of lipid were above 60% with the protein containing less than 2% residual phospholipid as measured by the method of Chen et al. [5].

Aqueous dispersions of HDL lipids were prepared by drying down under N_2 the organic extracts collected during delipidation under N_2. Phospholipids were separated from the neutral lipids at this stage by thin layer chromatography using 80:20:1, hexane:ether:acetic acid (v/v) as the solvent system [6]. The whole lipid extract or separated HDL-phospholipids were resuspended in 25 mM EDTA, pH 8.6, by sonication and the resulting lipid vesicles reconstituted with the apoprotein in a 1:1 (w/w) ratio by sonication also [7]. The lipid-protein vesicles were isolated by centrifugation at d 1.063 (24 h, 100000 × g) to remove free lipid and at d 1.21 (24 h, 100000 × g) to float the particles and sediment-free apoprotein.

Aqueous dispersions of phosphatidylcholine and sphingomyelin (80:20, w/w) were prepared according to [8] and reconstituted with the apoprotein, in a 1:2 (w/w) ratio, by incubation at 27°C for 20 min. Isolation of the particles was by centrifugation at d 1.063 and d 1.24 under the same conditions as above. In one experiment the phosphatidylcholine/sphingomyelin vesicles and the vesicles from HDL phospholipid were prepared by the same method [7] and reconstituted with apo

* To whom correspondence should be addressed

Abbreviations HDL, high density lipoprotein, apo HDL, protein moiety of HDL obtained after delipidation

Published by Elsevier Science Publishers B V (Biomedical Division)
00145793/85/$3 30

HDL in a ratio of 1:2, w/w, by the same procedure [7].

The reconstituted lipid-protein vesicles were desalted before use by gel filtration on Sephadex G-25 columns (4 × 1 cm) with 10 mM Tris, 1 mM EDTA, pH 8, buffer.

The measurement of triacylglycerol hydrolysis, preparation of rat serum activated Intralipid and acetone-ether dried powders of rat epididymal adipose tissue were carried out as reported previously [1].

3. RESULTS

The protein to phospholipid ratio was 1.06:1 ± 0.09 (5 determinations) for the phosphatidylcholine/sphingomyelin vesicles and 1.08:1 ± 0.16 (4 determinations) for the vesicles made from HDL lipids or phospholipids.

Fig.1 shows that apo HDL is a more potent inhibitor of lipoprotein lipase activity than native HDL at a given protein concentration. Moreover, apo HDL produces a greater maximum inhibition. At a final protein concentration of 0.1 mg/ml the % inhibition was 37 ± 8 and 66 ± 8 (6 observations) for apo HDL and HDL, respectively.

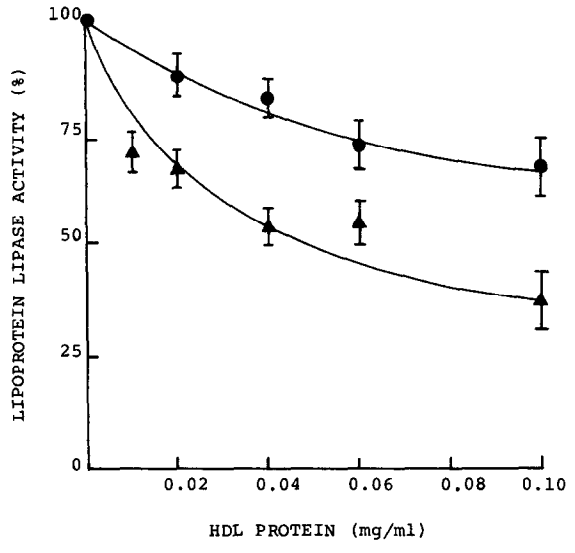

Fig.1. Inhibition of lipoprotein lipase activity by native HDL and apo HDL. The substrate was activated Intralipid, 3 mg/ml ●, Native HDL; ▲, apo HDL. The results are the means ± SD of 5 different experiments.

When reconstituted HDL was made from apo HDL and phosphatidylcholine/sphingomyelin vesicles, the pattern of lipoprotein lipase inhibition was similar to that observed with the original apo HDL (fig.2). Four experiments produced equivalent data. The same results were found whether reconstitution was carried out by incubation at 27°C in 6 M urea [8] or by sonication in the absence of urea [7]. However, when HDL was reconstituted from apo HDL and vesicles made from the total lipid obtained during the delipidation of HDL, then the pattern of inhibition seen with native HDL was regained (fig.2). Two other experiments produced similar results.

The difference between the behaviour of the phosphatidylcholine/sphingomyelin vesicles and the total HDL lipid vesicles was not due to the presence of cholesterol in the latter. The results in fig.2 show that reconstituted HDL formed from apo HDL and vesicles made from only the phospholipids of HDL behaved in a similar way to native HDL.

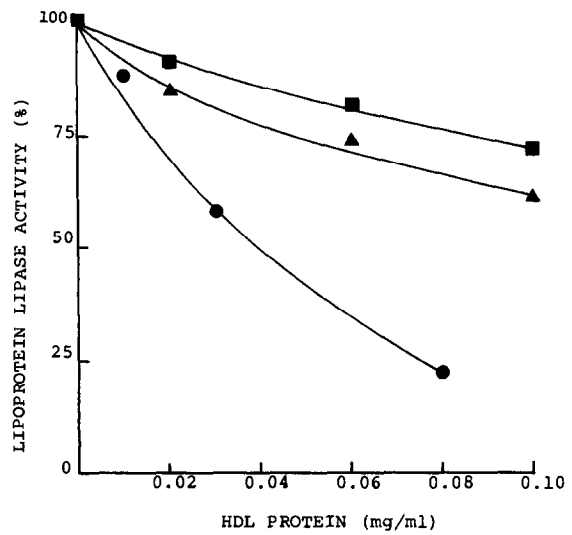

Fig.2. Effects of vesicle composition on the inhibition of lipoprotein lipase by reconstituted HDL The vesicles and reconstituted HDL were prepared as described in section 2. HDL was reconstituted from ●, apo HDL and phosphatidylcholine/sphingomyelin vesicles; ▲, apo HDL and vesicles made from the phospholipid fraction obtained from HDL; and ■, apo HDL and vesicles made from the total lipids obtained from HDL.

4. DISCUSSION

Hirz and Scanu [7] found that HDL reconstituted from HDL total lipids or HDL phospholipids had similar physical characteristics, including circular dichroic spectra, to native HDL. Assmann and Brewer [9] reported similar physical characteristics for native HDL and apo HDL recombined with sphingomyelin or phosphatidylcholine.

Most reconstitution studies involving apoproteins are carried out with vesicles containing phosphatidylcholine, sphingomyelin [10] or the synthetic phospholipid dimyristoyl phosphatidylcholine [11]. This is because such vesicles have an identical lipid composition from experiment to experiment and behave in a reproducible manner. Although the gross shape and structure of the various protein vesicles may be similar, their biochemical properties may be different. The results reported here show that the nature of the vesicle affects the behaviour of the reconstituted HDL in a biological system. Thus, lipoprotein reconstitution studies should take account of the nature of the lipid vesicle and include work with vesicles made from HDL lipids.

ACKNOWLEDGEMENT

This work was supported by a Medical Research Council Grant.

REFERENCES

[1] Rogers, M.P and Hutchinson, I (1981) Biochem J. 200, 453–456
[2] Robinson, S M.A , Younan, I and Rogers, M P (1984) Biochem Soc Trans., in press
[3] Havel, R J , Eder, H.A. and Bragdon, J H. (1955) J. Clin Invest. 34, 1345–1353
[4] Herbert, P N , Windmueller, H.G., Bersot, T.P and Shulman, R S (1974) J Biol Chem 249, 5718–5724
[5] Chen, P.S., Toribara, T.Y. and Warner, H (1956) Anal. Chem 28, 1756–1758
[6] Christie, W.W (1982) in: Lipid Analysis, 2nd ed , pp 34–38, Pergamon, Oxford.
[7] Hirz, R and Scanu, A M (1970) Biochim. Biophys Acta 207, 364–367
[8] Scanu, A. (1967) J. Biol. Chem 242, 711–719.
[9] Assmann, G. and Brewer, H.B (1974) Proc Natl. Acad Sci. USA 71, 989–993
[10] Shirai, K., Matsuoka, N. and Jackson, R L. (1981) Biochim. Biophys. Acta 665, 504–510.
[11] Innerarity, T.L , Pitas, R E. and Mahley, R W (1979) J. Biol Chem 254, 4186–4190