Role of Specific Glycopeptides of Human Serum Lipoproteins in the Activation of Lipoprotein Lipase

By Richard J. Havel, M.D., Virgie G. Shore, Ph.D., Bernard Shore, Ph.D., and Dennis M. Bier, M.D.

ABSTRACT

Lipoprotein lipase forms an enzyme-substrate complex with fat emulsions in the presence of serum lipoproteins. Lipoproteins of very low density and high density have this property, but the former are much more active per unit weight of protein. In this investigation, the activity, expressed as quantity giving half-maximal rate of production of free fatty acids, of specific glycopeptides isolated from very low density and high density lipoproteins was tested in an incubation mixture containing lipoprotein lipase from cows' milk and 1.8 mg triglyceride per ml. The two major polypeptides of high density lipoproteins were virtually inactive in amounts up to 100 &mu;g per ml. Activity of the unfractionated apoproteins of very low density lipoprotein was similar to that of the native lipoprotein (about 4 &mu;g/ml). Two of its polypeptides were active: one with carboxyl-terminal glutamic acid at 0.45 to 0.60 &mu;g/ml and one with carboxyl-terminal alanine at 1.8-2.8 &mu;g/ml. Some preparations of the latter peptide were less active and inhibited at high levels. Three other glycopeptides from very low density lipoprotein were inactive. Low density lipoprotein from subjects with primary biliary cirrhosis and a lipoprotein of density 1.04 to 1.06 from a subject with specific elevation of this fraction, both containing the active glycopeptides, had considerable activity (5 to 11 &mu;g/ml). These studies indicate that specific glycopeptides are required for the action of lipoprotein lipase on emulsified triglycerides and suggest that they are important components of the mechanism for extra-hepatic utilization of plasma triglycerides.

ADDITIONAL KEY WORDS triglycerides phospholipids polypeptides primary biliary cirrhosis fat emulsions

Lipoprotein lipase is necessary for the utilization of triglycerides in chylomicrons and very low density lipoproteins (1). It will also catalyze hydrolysis of triglycerides in artificial fat emulsion when serum lipoproteins are added. The lipoproteins associate with the emulsion particles to produce an active substrate for the enzyme.

Recent studies on the lipoprotein lipase of cows’ milk have shown that lipoproteins of very low density (VLDL) and high density (HDL), but not of low density (LDL), in human serum activate the hydrolysis of a soybean oil emulsion of triglycerides by the lipase (2). Per unit weight of protein, the activity of VLDL from normolipidemic subjects was approximately 13 times that of HDL. Since these two lipoprotein fractions share several common glycopeptides in varying proportions, it seemed possible that the activator property might reside in one or a limited number of these polypeptides. We now report that the two major glycopeptides of HDL are inactive, while two glycopeptides which are minor constituents of HDL and major components of VLDL are active at very
low concentrations. The activator property of LDL and HDL fractions from certain hyperlipoproteinemic individuals was related to content of the active glycopeptides.

Methods

Materials.—Serum was obtained from fasting individuals, both normolipidemic and with defined hyperlipoproteinemic states. Lipoprotein fractions were separated in a preparative ultracentrifuge by standard techniques (3) and dialyzed against 0.15M sodium chloride containing 0.04% disodium ethylenediamine tetraacetate, pH 7.4. Phospholipid-protein complexes were prepared by extraction of VLDL with diethyl ether at 0 to 2°C. Apolipoproteins and specific polypeptides were isolated in water-soluble form from VLDL or HDL proteins of normolipoproteinemic and hyperlipoproteinemic subjects by column chromatography on DEAE-cel lulose and dialyzed against water at pH 8 (4, 5). Intralipid (10% emulsion of fractionated soybean oil triglycerides with 0.6% egg lecithin) was supplied by Vitrum, A.B., Stockholm, Sweden. Each polypeptide fraction tested appeared to be pure by amino acid analysis and polyacrylamide gel electrophoresis.

Analytical.—The activator property of lipoprotein fractions or specific apoproteins was assayed in a system containing artificial fat emulsion (Intralipid), 1.8 mg triglyceride/ml, in the presence of 0.1M ammonium buffer at pH 8.6, 5.3% human serum albumin and cows’ milk proteins (2.5 mg/ml) (2). Incubations were carried out for 45 minutes at 37°C and samples removed at intervals of 15 minutes were assayed for free fatty acids (FFA) (2). Control samples containing no activator or 0.06 ml normal human serum/ml incubation medium were included in each assay run. Enzyme activity was calculated as jumoles FFA produced per minute with 40 mg cows’ milk proteins. As described elsewhere (2), hydrolysis of triglyceride with addition of increasing quantities of activator lipoproteins shows saturation kinetics in this system. Therefore, for each assay, enzyme activity was determined at several concentrations of lipoprotein or apoprotein and the quantity of protein present (µg per ml incubation medium) at the apparent K_m was used to assay its potency. Protein content was estimated by the method of Lowry (6) for intact lipoprotein and from quantitative amino acid analysis for delipidated preparations (5). Lipid content of lipoproteins was estimated by standard methods (7-9). Electrophoresis of serum and lipoprotein fractions was carried out on agarose gel (10) and of delipidated fractions on 12.5% polyacrylamide gel containing 8% urea (5).

Results

Activation of Lipolysis by Apolipoproteins and Isolated Glycopeptides.—Three different preparations of each of the two polypeptides with C-terminal threonine (R-Thr) and C-terminal glutamine (R-Gln), which account for approximately 90% of protein in HDL (5), were tested for the activator property. Neither one, nor a mixture of the two, had detectable activity at concentrations up to 100 µg/ml medium. Two other minor peptides of HDL, which have slower mobilities in polyacrylamide gels than R-Thr and R-Gln, were also inactive at concentrations up to 30 µg/ml. Unfractionated VLDL apolipoprotein or apolipoprotein-phospholipid complexes had activities similar to those observed with native VLDL (4 to 8 µg/ml medium). Two peptides which were isolated from VLDL were consistently active. One of these, with C-terminal glutamic acid, was most active with an apparent K_m of 0.45 and 0.60 µg per ml in two preparations (Fig. 1). High concentrations
ACTIVATION OF LIPOPROTEIN LIPASE

(ca. 15 μg/ml) of R-Glu sometimes inhibited slightly, a phenomenon observed frequently when intact lipoproteins or serum are used as activator. A second peptide of VLDL, with C-terminal alanine, was also active (Fig. 1). Several distinct R-Ala fractions are generally obtained on DEAE-cellulose. The most active of these had a Km of 1.8 and 2.8 μg/ml in two preparations. Less active fractions had unique behavior: they usually showed increasing activity with increasing peptide concentrations up to about 10 μg/ml, but at higher concentrations lipolysis was markedly decreased so that rates equivalent to those observed with serum or other active preparations were not obtained. Three other polypeptides isolated from VLDL were inactive at concentrations up to 10 μg/ml. One of these is characterized by C-terminal valine, another by its unusually high content of arginine, and the third by a high percent of serine, glutamic acid, and glycine and a low percent of hydrophobic residues (5). The activity of mixtures of an active preparation of R-Ala and R-Glu was approximately additive and the activity of these peptides was not inhibited by a mixture of R-Thr and R-Gln or by R-Val (Table 1). However, mixtures of highly active R-Glu or R-Ala, with less active R-Ala2 and R-Ala3 gave substantially lower activity than R-Glu or R-Ala alone.

Lipoproteins from Hyperlipoproteinemic Subjects (Table 2).—Lipoprotein fractions from two subjects (I.W. [11] and H.B.) with increased concentration of HDL1 (density

![FIGURE 2](patterns from disc electrophoresis at pH 8.8 of apo-lipoproteins in 12.5% polyacrylamide gels containing 8M urea. 1, apo HDL; 2, apo LDL; 3, apo VLDL; 4, apo HDL1 (subject M.S.); 5, apo HDL1 (subject H.B.); 6, apo HDL1 (subject I.W.). The arrow indicates the top of the separating gel.)

### TABLE 1

<table>
<thead>
<tr>
<th>Polypeptides (μg/ml medium)</th>
<th>Lipase activity (μmoles FFA/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-Glu (0.2)</td>
<td>0.11</td>
</tr>
<tr>
<td>R-Glu (0.9)</td>
<td>0.40</td>
</tr>
<tr>
<td>R-Glu (3.1)</td>
<td>0.51</td>
</tr>
<tr>
<td>R-Ala1 (0.6)</td>
<td>0.10</td>
</tr>
<tr>
<td>R-Ala1 (2.3)</td>
<td>0.34</td>
</tr>
<tr>
<td>R-Ala2 (6.0)</td>
<td>0.09</td>
</tr>
<tr>
<td>R-Ala2 (31)</td>
<td>0.02</td>
</tr>
<tr>
<td>R-Ala3 (6.3)</td>
<td>0.18</td>
</tr>
<tr>
<td>R-Ala4 (15)</td>
<td>0.09</td>
</tr>
<tr>
<td>R-Glu (0.2) + R-Ala1 (0.6)</td>
<td>0.32</td>
</tr>
<tr>
<td>R-Glu (0.9) + R-Ala1 (2.3)</td>
<td>0.50</td>
</tr>
<tr>
<td>R-Glu (3.1) + R-Ala3 (31)</td>
<td>0.17</td>
</tr>
<tr>
<td>R-Ala1 (2.3) + R-Ala3 (6.3)</td>
<td>0.20</td>
</tr>
<tr>
<td>R-Ala1 (2.3) + R-Ala3 (15)</td>
<td>0.27</td>
</tr>
<tr>
<td>R-Ala1 (2.3) + R-Thr (13) + R-Cln (3.5)</td>
<td>0.56</td>
</tr>
<tr>
<td>Serum control (0.08 ml/ml)</td>
<td>0.42</td>
</tr>
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</table>

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1.04 to 1.07 g/ml) and from two subjects (D.E. and M.S.) with primary biliary cirrhosis were assayed for lipoprotein lipase-activator properties. A lipoprotein fraction of density 1.006 to 1.040 (S, 3-20) from H.B. had low levels of activity, possibly related to content of VLDL-peptides which are usually found in small amounts in this fraction (5). The HDL-rich fraction of H.B. had very low activity. It contained only lipoproteins of β-mobility and had virtually identical amino acid composition to normal LDL; it gave none of the polypeptide bands of VLDL or HDL in polyacrylamide gel electrophoresis (Fig. 2). The HDLβ of I.W., mainly lipoproteins of α mobility with small amounts of β-lipoprotein, had activity in the range of normal VLDL (Table 2). Fractionation of its delipidated protein on DEAE-cellulose gave R-Thr identical with that isolated from normal HDL (but no R-Gln), and a number of VLDL polypeptides including R-Val, R-Glu and several R-Ala fractions; electrophoresis of the protein in polyacrylamide gel showed bands corresponding to these fractions and to β-apo-protein, which does not enter the separating gel (Fig. 2). Other HDL-fractions from I.W. were also unusually active and contained large amounts of αβ-lipoprotein. Her serum (apparent Kₘ at 0.005 ml/ml medium) was approximately four times as active as normal serum. The LDL from a woman (D.E.) with primary biliary cirrhosis was moderately active (Table 2). This fraction contained only lipoprotein of β-mobility. However, these LDL are largely the "obstructive" lipoprotein (LP-X), which contains the polypeptide R-Ala (12). Serum from another subject (M.S.) with primary biliary cirrhosis contained predominantly αβ-lipoprotein and had approximately ten times the normal quantity of activator (apparent Kₘ 0.0019 ml serum/ml medium). Lipoproteins with this mobility were present in all density fractions from 1.006 to 1.21 and all were potent activators. Analytical ultracentrifugation showed approximately 10 mg of lipoprotein/ml plasma between densities 1.040 and 1.090. The polyacrylamide gel pattern of

### Table 2

<table>
<thead>
<tr>
<th>Subject</th>
<th>HDLβ-rich lipoproteins</th>
<th>HDLβ-rich lipoproteins</th>
<th>HDLβ-rich lipoproteins</th>
<th>HDLβ-rich lipoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.B.</td>
<td>1.006-1.040</td>
<td>1.006-1.040</td>
<td>1.006-1.040</td>
<td>1.006-1.040</td>
</tr>
<tr>
<td>D.E.</td>
<td>1.006-1.040</td>
<td>1.006-1.040</td>
<td>1.006-1.040</td>
<td>1.006-1.040</td>
</tr>
<tr>
<td>M.S.</td>
<td>1.006-1.040</td>
<td>1.006-1.040</td>
<td>1.006-1.040</td>
<td>1.006-1.040</td>
</tr>
</tbody>
</table>

*Predominant component listed first: CE = cholesteryl ester, PC = phosphatidylcholine, TC = triglycerides.
M.S.'s HDLX (density 1.050 to 1.075) resembled that of VLDL (Fig. 2).

Discussion

These studies indicate that specific peptides are required for the action of lipoprotein lipase on triglyceride-rich substrates. These peptides presumably permit binding of the enzyme at the surface of phospholipid-stabilized particles. Scanu found that HDL-apoprotein would not form a complex with Ediol, an emulsion of coconut oil containing polyoxyethylene sorbitan monostearate, and glycerol monostearate as amphiphiles, unless phospholipid micelles were added to the protein solution (13). We have found such addition to have no effect in our system, presumably because the soybean oil triglycerides in Intralipid are stabilized with lecithin. Very recently, Fielding et al. have reported the effect of fractions of HDL-apoprotein obtained by gel filtration on the hydrolysis of triolein emulsion by purified lipoprotein lipase from human postheparin plasma (14). Their observation that only the minor fraction containing the smallest polypeptides is active in their system suggests that polypeptides R-Glu and R-Ala promote the lipolysis of emulsified triglycerides by human lipoprotein lipase.

A physiological role for one or both of the active glycopeptides can be postulated from our data. It is unlikely that their activity is related to the procedure of delipidation, since the activator property of unfractionated VLDL-protein was similar to that of native VLDL per unit weight of protein. Further, the observed activity of the most active preparations of R-Ala and R-Glu exceeded that of unfractionated VLDL-apoprotein, which also contains virtually inactive polypeptides and LDL-protein (5). It is not possible to conclude that the observed activities of the isolated peptides R-Glu or R-Ala, or both are identical to those in native VLDL. In mixing studies, the activities of R-Glu and potent fractions of R-Ala were additive, and their activity was not inhibited by R-Val or a mixture of R-Thr and R-Glu. However, some preparations of R-Ala were inhibitory at high concentrations, and these preparations also inhibited the activity of R-Glu and the more potent fractions of R-Ala. Fielding, using purified lipoprotein lipase from human postheparin plasma, has found similar inhibitory activity with native VLDL and apo-HDL but not HDL (15). In our system, inhibition of VLDL is slight and does not exceed that observed with whole serum. The reasons for the varying behavior of the different fractions of R-Ala are not clear. Differences in sialic acid content occur in fractions apparently corresponding to R-Ala2 and R-Ala3 (16), which are identical in amino acid content. Alterations in the conformation of the polypeptides during isolation could also affect their activator property.

The studies of hyperlipoproteinemic subjects, together with previously reported data on the activity of VLDL and HDL from normolipidemic individuals, seem to indicate that R-Glu and R-Ala are active when present in a variety of lipoproteins with different densities. In the present work, these include a preparation of HDLα, an α2-lipoprotein of widely variable density and the LP-X of primary biliary cirrhosis. The high activity of HDLα with α2-mobility from hyperlipoproteinemic subjects is of interest because this lipoprotein fraction increases after fat-rich meals in cetaceans (17), and in hypertriglyceridemic states (18). A close relation of this lipoprotein fraction to utilization of plasma triglycerides is thereby suggested. However, HDLβ (density 1.075 to 1.125) and, to a lesser extent, HDLγ (density 1.125 to 1.21) also contain the VLDL polypeptides (4, 5, 19). It remains for further studies to show whether the activation of triglyceride emulsions by lipoproteins is related to transfer of the active peptides to the surface of the emulsion, or whether part or all of the active lipoprotein species is transferred.

The properties of R-Ala and R-Glu responsible for their activity are also unknown. The varying activities of subfractions of R-Ala having different behavior on DEAE-cellulose and polyacrylamide gel suggest that small...
differences in composition of the glycopeptide can affect the activator property. From circular dichroic spectra, R-Glu and R-Ala have mainly random coil structure, while the inactive peptides, including R-Val, R-Thr, R-Gln, and particularly the high-arginine VLDL-peptide, contain a large fraction of alpha helix (B. Shore and V. Shore, unpublished data). It is also possible that the specific carbohydrate moieties of the glycopeptides may be related to the activator property.

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