Hydrolysis of Chylomicron Triacylglycerol by Endothelium-Bound Lipoprotein Lipase

EFFECT OF DECREASED APOPROTEIN C-II/C-III RATIO

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Chylomicrons with a decreased ratio of C-II/C-III apoproteins on their surface were produced by the addition of apoproteins C-III-0 or C-III-3 to intact rat lymph chylomicrons. These chylomicrons inhibited the activity of soluble lipoprotein lipase *in vitro*, but had no effect on the activity of the endothelium-bound enzyme in the perfused heart.

The removal of chylomicrons and VLD lipoproteins from circulation requires the action of lipoprotein lipase. This enzyme hydrolyses the triacylglycerol moiety of the lipoproteins on the luminal surface of capillary endothelial cells of heart, adipose tissue and skeletal muscle (Borensztajn, 1979). It has been demonstrated that for lipoprotein lipase to exert its full catalytic action, it requires as cofactor apoprotein C-II, a normal constituent on the surface of triacylglycerol-rich lipoproteins (Krauss *et al.*, 1973; Breckenridge *et al.*, 1978; Lukens & Borensztajn, 1978b). Other surface apoproteins, specifically apoproteins C-I and C-III, inhibit the activation of solubilized lipoprotein lipase *in vitro* by apoprotein C-II (Brown & Baginsky, 1972; Havel *et al.*, 1973a; Bensadoun *et al.*, 1974). On the basis of these observations *in vitro*, it was suggested that the removal of triacylglycerol-rich lipoproteins from the circulation might be modulated by the relative concentrations of apoprotein C-I, C-II and C-III on the surface of the lipoprotein (Schonfeld *et al.*, 1976; Rogers *et al.*, 1976; Schaefer *et al.*, 1978). The observation that in certain individuals with increased concentrations of plasma triacylglycerol, the apoprotein C-II/C-III ratio on VLD lipoproteins is decreased compared with normal individuals seemed to support this hypothesis (Carlson & Ballantyne, 1976; Montes & Knopp, 1977).

We recently examined the effects of rat apoproteins C-I and C-III-3 on the hydrolysis of intact and apoprotein C-II-activated rat lymph chylomicron triacylglycerol by solubilized and endothelium-bound lipoprotein lipase (Lukens & Borensztajn, 1978b). In agreement with previously published studies (Brown & Baginsky, 1972; Havel *et al.*, 1973a; Bensadoun *et al.*, 1974) we showed that the activity of solubilized enzyme was markedly inhibited

by apoproteins C-I and C-III. However, no inhibition of the endothelium-bound enzyme was detected. We did not ascertain, however, whether the apoproteins added to the chylomicrons became bound to the lipoprotein surface. Consequently, it could not be concluded that normal enzyme activity persisted in the presence of altered C-apoprotein ratios on the chylomicron surface. In the present study, we demonstrate that the relative concentrations of C-apoproteins on the surface of chylomicrons can be altered by the addition of apoproteins C-III-0 and C-III-3. These chylomicrons with decreased apoprotein C-II/C-III ratios inhibited the activity of solubilized lipoprotein lipase, but had no effect on the endothelium-bound enzyme.

Methods

Animals

Male Sprague-Dawley rats (180-240 g) were maintained on laboratory chow and kept in alternating 12h periods of light and darkness. All animals were starved for 18-24h before the heart-perfusion experiment.

Apoprotein fractionation

Apoproteins C-III-0 and C-III-3 were purified from unlabelled rat lymph chylomicrons. Chylomicrons were washed and delipidated as described by Lukens & Borensztajn (1978a). Apoprotein fractionation was by the method of Herbert *et al.* (1974) for rat HD lipoproteins, except that Sephacryl S-200 and DEAE-Sephacel (Pharmacia Fine Chemicals, Uppsala, Sweden) were substituted for Sephadex G-200 and DEAE-cellulose respectively. The isolated apoproteins were judged to be pure by electrophoresis on polyacrylamide gels containing 8M-urea (Herbert *et al.*, 1974).

Abbreviations used: VLD lipoprotein, very-low-density lipoprotein; HD lipoprotein, high-density lipoprotein.

Chylomicron preparation

Rat ^{14}C -labelled lymph chylomicrons were obtained as previously described (Lukens & Borensztajn, 1978a). The chylomicrons were incubated at room temperature with rat serum (9:1, v/v) for 30 min to furnish the lipoproteins with a full apoprotein complement (Havel *et al.*, 1973b) and then mixed (1:3, v/v) with 20% (w/v) bovine serum albumin (Sigma Chemical Co., St. Louis, MO, U.S.A.) adjusted to pH 5.0 with 1 M-NaOH, to decrease their non-esterified fatty acid content (Borensztajn & Robinson, 1970). The chylomicrons were washed by flotation in Krebs-Ringer bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932) as described by Lukens & Borensztajn (1978a). Apoproteins were added to the washed chylomicrons in different amounts. The triacylglycerol concentration of all mixtures (50 mg/ml) was kept constant by the addition of Krebs-Ringer bicarbonate buffer, pH 7.4. The apoprotein/chylomicron mixtures were left for 20 min at room temperature and then subjected to gel chromatography to remove proteins not bound to the chylomicrons. Chromatography was carried out in columns (1.4 cm $\times$ 40 cm) of 2% agarose (Bio-Gel A-50m, 50–100 mesh; Bio-Rad Laboratories, Richmond, VA, U.S.A.). About 100 mg of chylomicron triacylglycerol in 2.0 ml was applied to the column. The chromatography conditions used were essentially as described by Imaizumi *et al.* (1978), except that in the elution solvent 0.02 M-NaCl was substituted for 0.2 M-NaCl. The elution of the chylomicrons with the void volume was monitored visually.

Other procedures

Electrophoresis of chylomicron tetramethylurea-soluble apoproteins was as described by Lukens & Borensztajn (1978a). Gels were scanned with a Beckman R-112 densitometer. Collection of rat heart perfusate as a source of lipoprotein lipase, perfusion of the hearts and measurement of their $^{14}\text{CO}_2$ production, the lipoprotein lipase assay system, and protein and triacylglycerol determinations were as described by Lukens & Borensztajn (1978a,b).

Results and Discussion

Apoprotein binding to chylomicrons

Fig. 1 shows the apoprotein patterns obtained by tetramethylurea/polyacrylamide-gel electrophoresis of chylomicrons. The overall pattern was similar to that described by Mjøs *et al.* (1975). In the control chylomicrons apoproteins C-III-0 and C-II, which were not clearly separated from each other, accounted for about 4% of the total stainable material on the gel and apoprotein C-III-3 accounted for about 2.3%.

Fig. 1. Tetramethylurea/polyacrylamide-gel electrophoresis (a) and relative concentration of chylomicron apoproteins (b).

Chylomicrons were incubated with different amounts of apoprotein C-III-0 (a₁) or C-III-3 (a₂), as indicated, for 20 min at room temperature. This was followed by the removal of unbound apoproteins by gel chromatography as described in the Methods section. The material obtained from the delipidation of 8 mg of chylomicrons (as determined from the triacylglycerol content) was applied to each gel. Photodensitometric scanning (at 600 nm) and measurement of the relative concentration (b₁ and b₂) of each band were carried out with a Beckman R-112 densitometer. The amount of apoprotein C-III added to the chylomicrons (μg/mg of triacylglycerol) was: A, 0; B, 2; C, 5; D, 10.

Apoprotein E accounted for most (70%) of the tetramethylurea-soluble proteins. In the present study, the chylomicrons were exposed to rat serum before being washed (see the Methods section) and had, therefore, a full apoprotein complement (Havel *et al.*, 1973b). Consequently, specific binding of added apoprotein could occur only by displacement of other apoproteins already on the surface. In Fig. 1 the relative amounts of apoprotein in each gel are shown. It is apparent that added apoproteins C-III-0 and C-III-3 did in fact bind to the chylomicrons. In the case of apoprotein C-III-0, the increased binding occurred mainly at the expense of apoprotein E, which was displaced. The relative amounts of apoproteins A-I and C-III remained virtually unaltered. Displacement of apoprotein E also occurred when apoprotein C-III-3 was added to the chylomicrons. However, at a concentration of 10 μg of apoprotein C-III-3, apoprotein A-I was displaced as well as apoprotein E. Similar results were obtained when the gels were

prepared with one-half the chylomicron material of those in Fig. 1 (T. J. Kotlar & J. Borensztajn, unpublished work). The gel band for apoprotein C-II could not be quantified because of its proximity to the band for apoprotein C-III-0. There is no reason, however, to suppose its displacement from the chylomicron by the addition of apoproteins C-III-0 or C-III-3. Indeed, as seen in Fig. 1, the addition of 2, 5 or 10 μg of apoprotein C-III-3/mg of chylomicron triacylglycerol left the combined contribution of apoproteins C-III-0 and C-II virtually unchanged

(4–5%). Furthermore, the addition of as much as 10 μg of apoprotein/mg of triacylglycerol of either of the C-III apoproteins to the chylomicrons did not decrease the activity of the endothelium-bound lipoprotein lipase (see below). Such a decrease would have resulted, if apoprotein C-II had been displaced (Lukens & Borensztajn, 1978b). Thus although the electrophoretic method used did not allow the quantitative determination of apoprotein C-II, the results illustrated in Fig. 1 clearly demonstrate that the addition of increased amounts of apoproteins C-III-0 and C-III-3 to chylomicrons resulted in marked decreases in the apoprotein C-II/C-III ratio on the lipoprotein surface.

Fig. 2. Effect of decreased apoprotein C-II/C-III ratio on the oxidation of ^{14}C -labelled chylomicron triacylglycerol fatty acid by the perfused rat heart

^{14}C -labelled chylomicrons were incubated with different amounts of apoprotein C-III-0 or C-III-3, as indicated on the abscissa (values are in $\mu\text{g}/\text{mg}$ of triacylglycerol), for 20 min at room temperature. After the removal of unbound apoprotein by gel chromatography, as described in the Methods section, chylomicrons were diluted to a concentration of 0.27 $\mu\text{-equiv.}$ of triacylglycerol fatty acid/ml in the perfusion medium. Hearts from rats starved for 18–24 h were perfused for 10 min with the various ^{14}C -labelled chylomicron preparations, and the $^{14}\text{CO}_2$ formed was measured as described by Lukens & Borensztajn (1978a). The results are expressed as means $\pm$ s.d. (indicated by the bars), and have been corrected for $^{14}\text{CO}_2$ that could have been derived from the oxidation of the ^{14}C -labelled non-esterified fatty acid present in the chylomicron preparations (Lukens & Borensztajn, 1978a). The number of hearts perfused in each group is shown in parentheses.

Hydrolysis of chylomicron triacylglycerol by endothelium-bound lipoprotein lipase

Fig. 2 shows the results obtained when ^{14}C -labelled chylomicrons with increased amounts of surface apoproteins C-III-0 and C-III-3 were perfused through isolated rat hearts. The $^{14}\text{CO}_2$ formed as a result of the uptake and oxidation of ^{14}C -labelled chylomicron triacylglycerol fatty acid was used as an indicator of the capacity of the heart endothelium-bound lipoprotein lipase to hydrolyse the triacylglycerol (Lukens & Borensztajn, 1978a). The results show that increasing the amount of surface apoprotein C-III-0 or C-III-3 produced no significant difference in the lipoprotein lipase activity of the perfused heart. In addition, the maintenance of constant lipoprotein lipase activity in the perfused heart in the face of marked decreases of apoprotein C-II/C-III ratios on the surface of the chylomicrons demonstrates that this ratio is of no apparent significance in the process of triacylglycerol-rich lipoprotein removal from circulation. It is noteworthy that the increase in surface apoprotein C-III occurred at the expense of apoproteins E and A-I (Fig. 1) without affecting the hydrolysis of chylomicron triacylglycerol. This observation confirms previous findings with trypsin-treated chylomicrons (Lukens & Borensztajn, 1978b) that of the surface proteins apoprotein C-II alone affects the activity of the endothelium-bound lipoprotein lipase.

Hydrolysis of chylomicron triacylglycerol by solubilized lipoprotein lipase

Fig. 3 shows the results obtained when the hydrolysis of chylomicron triacylglycerol was measured *in vitro* by using solubilized heart lipoprotein lipase. In agreement with previous observations (Brown & Baginsky, 1972; Havel *et al.*, 1973a; Bensadoun *et al.*, 1974; Lukens & Borensztajn, 1978b) there was a progressive inhibition of the enzyme activity when chylomicrons with increased amounts of apoproteins C-III-0 and C-III-3 were used as substrate. We had

Fig. 3. Effect of decreased apoprotein C-II/C-III ratio on the hydrolysis of ^{14}C -labelled chylomicron triacylglycerol by lipoprotein lipase in vitro

The ^{14}C -labelled chylomicrons used as substrate for lipoprotein lipase were prepared as described in the legend to Fig. 2. The conditions for the lipoprotein lipase assay were as previously described (Lukens & Borensztajn, 1978b). Results for chylomicron triacylglycerol hydrolysis are expressed as a percentage of the value for control chylomicrons (100%), and each point represents the average of assays carried out in duplicate. Symbols: ○, apoprotein C-III-0; ▲, apoprotein C-III-3.

previously suggested (Lukens & Borensztajn, 1978b) that apoprotein C-III may inhibit solubilized lipoprotein lipase by binding to the enzyme in solution, thus preventing its interaction with the substrate. The present results indicate that this explanation is not valid, since apoproteins C-III-0 and C-III-3 were bound to the substrate. The mechanism whereby apoprotein C-III can inhibit solubilized lipoprotein lipase remains to be explained. It is clear, however, from the results obtained with endothelium-bound

enzyme (Fig. 2) that this inhibition *in vitro* is of no physiological significance.

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