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EFFECT OF MONOLAYER LIPID STRUCTURE AND COMPOSITION ON THE LIPOPROTEIN LIPASE-CATALYZED HYDROLYSIS OF TRIACYLGLYCEROL

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The effect of lipid composition and structure on the lipoprotein lipase-catalyzed hydrolysis of triacylglycerols was determined in a monolayer system consisting of purified bovine milk lipoprotein lipase and fatty acid-free albumin. In a monolayer of dioleoylphosphatidylcholine containing 1–6 mol% of either tri[^{14}C]oleoylglycerol or tri[^{14}C]palmitoylglycerol, lipoprotein lipase catalyzed the hydrolysis of the unsaturated triacylglycerol at a higher rate than the saturated lipid and in either the presence or absence of apolipoprotein C-II, the activator protein for the enzyme. For example, with 3 mol% triacylglycerol and in the presence of apolipoprotein C-II, the rate of the lipoprotein lipase-catalyzed hydrolysis of tri[^{14}C]oleoylglycerol was 27 μmol oleic acid produced/h per mg enzyme vs. 12 μmol for tri[^{14}C]palmitoylglycerol. The effect of phospholipid fatty acyl chain length and unsaturation/saturation, polar head group and surface density on the lipoprotein lipase-catalyzed hydrolysis of tri[^{14}C]oleoylglycerol was determined. The rate of enzyme hydrolysis of triacylglycerol was similar whether the phospholipid was a diester or diether lipid or the polar head group was ethanolamine or choline. In general, phospholipids with shorter and unsaturated fatty acyl chains gave higher rates of lipoprotein lipase hydrolysis of triacylglycerol than the corresponding longer and saturated lipids. However, with all phospholipids tested, the rate of enzyme hydrolysis decreased with increasing surface density. Lipoprotein lipase showed no activity toward triacylglycerol in a monolayer of sphingomyelin; addition of dioleoylphosphatidylcholine to the monolayer enhanced the rate of enzyme catalysis. Cholesterol (50 mol%) in a dipalmitoylphosphatidylcholine monolayer increased the rate of the lipoprotein lipase-catalyzed hydrolysis of tri[^{14}C]oleoylglycerol, whereas cholesterol decreased the rate in a dioleoylphosphatidylcholine monolayer. The effect of phospholipid structure and surface density on lipoprotein lipase activity could not be accounted for by the amount of apolipoprotein C-II which was present at the interface. Based on these findings and other reports in the literature, we suggest that the catalytic activity of lipoprotein lipase toward tri[^{14}C]oleylglycerol in various monolayers is dependent on the conformation or appropriate physical state of the triacylglycerol substrate at the lipid interface.

Introduction

Plasma triacylglycerols are transported in the circulation by chylomicrons and very-low-density lipoproteins. Lipoprotein-triacylglycerols are cleared from the circulation by the action of lipoprotein lipase located at the capillary endothelium in tissues such as heart, muscle and adipose tissue. For maximal activity, lipoprotein lipase requires apolipoprotein C-II, a low molecular weight pro-

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tein constituent of triacylglycerol-rich lipoproteins and high-density lipoproteins (see Refs. 1–5 for review). The interaction of apolipoprotein C-II and lipoprotein lipase and subsequent hydrolysis of triacylglycerols presumably occur at the monolayer surface of the lipoprotein particle which contains predominantly phosphatidylcholine, sphingomyelin, unesterified cholesterol and protein. Experimental evidence for the presence of small amounts of triacylglycerol in phospholipid monolayers has been provided by Gorrissen et al. [6] and Small and co-workers [7–9]. These investigators have shown that approx. 1–5 mol% triacylglycerols are solubilized by phosphatidylcholine liposomes. To investigate the interaction of lipoprotein lipase and its activator protein with lipid interfaces, we have utilized a monolayer system containing small amounts of triacylglycerol solubilized in a phospholipid monolayer [10]. We showed that lipoprotein lipase catalyzed the hydrolysis of tri[^{14}C]oleoylglycerol in an egg phosphatidylcholine monolayer in a manner which was dependent on triacylglycerol concentration, lipoprotein lipase and apolipoprotein C-II. In the present report, we have extended these studies and have determined the effects of monolayer lipid composition, structure and surface density on lipoprotein lipase catalysis of triacylglycerols.

Materials and Methods

Materials

Dilauroylphosphatidylcholine (di12:0PC), dimyristoylphosphatidylcholine (di14:0PC), dipalmitoylphosphatidylcholine (di16:0PC), dioleoylphosphatidylcholine (di18:1PC), dilinoleoylphosphatidylcholine (di18:2PC), dilinolenoleoylphosphatidylcholine (di18:3PC), diarachidonoylphosphatidylcholine (di20:4PC), dilinoleoylphosphatidylethanolamine (di18:2PE), dilinolenoleoylphosphatidylethanolamine (di18:3PE), and diarachidonoylphosphatidylethanolamine (di20:4PE) were synthesized according to published procedures [11]. Synthetic palmitoyl sphingomyelin was a generous gift from Dr. Shapiro (Rehovet, Israel). Didocosanoylphosphatidylcholine (di22:0PC) was obtained by hydrogenation of didocosenoylphosphatidylcholine (di22:1PC) [12]. Di-O-myristoylphosphatidylcholine (di14:0PC, diether) was a gift from Dr. deHaas (Utrecht, The Netherlands). Di-1-*O*-hexadecyl-2-*O*-(1-octadec-1, 9-enyl) (16:0,18:1PC, diether) was obtained from Calbiochem-Behring Corp. Tri[*carboxyl*-1-^{14}C]oleoylglycerol (90 mCi/mmol) was purchased from New England Nuclear. Tri[*carboxyl*-1-^{14}C]palmitoylglycerol (50 mCi/mmol) was from Amersham. The purity of each lipid was established by thin-layer chromatography in petroleum ether/diethyl ether/formic acid (60 : 40 : 1.5, v/v) or in chloroform/methanol/acetic acid/water (90 : 30 : 8 : 2.8, v/v). All lipids were prepared as stock solutions in chloroform. Fatty acid-free bovine serum albumin (fraction V) and heparin (porcine mucosal, 169.9 units/mg) were purchased from Sigma.

Apolipoprotein C-II was isolated from triacylglycerol-rich lipoproteins of fasting subjects with familial endogenous hypertriglyceridemia with fasting chylomicronemia (type V hyperlipoproteinemia) as described previously [13]. Lipoprotein lipase was isolated from bovine skimmed milk by affinity chromatography on heparin-Sepharose 4B as described by Kinnunen [14]. The purified enzyme had a specific activity of 36 mmol oleic acid released/h per mg in an assay system of tri[*carboxyl*-1-^{14}C]oleoylglycerol emulsified with Triton X-100 [15]. Lipoprotein lipase was stored at −70°C in 50% glycerol. Dilutions of lipoprotein lipase were prepared daily in 10 mM Tris-HCl (pH 8.4)/0.9% NaCl containing 50% glycerol.

Analytic procedures

Protein concentrations were determined by the method of Lowry et al. [16] using bovine serum albumin as standard. Phospholipid phosphorus was determined by the method of Bartlett [17]. Interfacial/monolayer studies were performed as described previously [10]. In a typical experiment, lipids were first spread from chloroform solutions to the desired surface pressure. After the recording of surface radioactivity and pressure had stabilized (5–10 min), 25 μl heparin (1 mg/ml), 100 μl fatty acid-free bovine serum albumin (1 mg/ml) and various amounts of apolipoprotein C-II (1 mg/ml in 6 M guanidine), as indicated, were added to the subphase at 1-min intervals. Lipoprotein lipase, as indicated, was then added to the subphase. The

initial rate of release of [^{14}C]oleic acid from the interface was determined graphically by measuring the slope of the kinetic curves. All experiments were carried out at 33°C in 10 mM Tris-HCl (pH 8.4)/0.9% NaCl.

Apolipoprotein C-II was iodinated with sodium [^{125}I]iodide according to the method of Bilheimer et al. [18]; the ^{125}I-labelled protein had a specific radioactivity of 214 dpm/ng. ^{125}I-labelled apolipoprotein C-II enhanced the activity of lipoprotein lipase to the same extent as the unlabelled apoprotein. ^{125}I-labelled apolipoprotein C-II was collected from the interface by aspiration into a counting vial and the amount of radioactivity determined after correcting for the amount of subphase collected.

Results

Effect of triacylglycerol composition

The effect of monolayer content of triacylglycerol on the lipoprotein lipase-catalyzed hydrolysis of tri[^{14}C]oleoylglycerol and tri[^{14}C]palmitoylglycerol is shown in Fig. 1. Consistent with previous findings [10], the rate of lipoprotein lipase catalysis of tri[^{14}C]oleoylglycerol in the absence of apolipoprotein C-II was barely detectable at substrate concentrations between 1 and 3 mol% tri[^{14}C]oleoylglycerol in a monolayer of di18:1PC; at 5 and 6 mol% tri[^{14}C]oleoylglycerol, the rates of catalysis were 14 and 24 μmol [^{14}C]oleate released/h per mg lipoprotein lipase, respectively. In the presence of apolipoprotein C-II, the rates of catalysis were markedly enhanced at all substrate concentrations. With tri[^{14}C]palmitoylglycerol monolayers, the rates of catalysis in the absence of apolipoprotein C-II were measurable only with 6 mol% triacylglycerol; the rates of hydrolysis were enhanced in the presence of apolipoprotein C-II but were less than one-half the rates for the lipoprotein lipase-catalyzed hydrolysis of tri[^{14}C] oleoylglycerol.

Effect of phospholipid composition

The effect of various diacyl and diether phosphatidylcholines on the lipoprotein lipase-catalyzed hydrolysis of monolayers containing 5 mol% tri[^{14}C]oleylglycerol was next examined. As is shown in Fig. 2, the rates of enzyme hydrolysis of

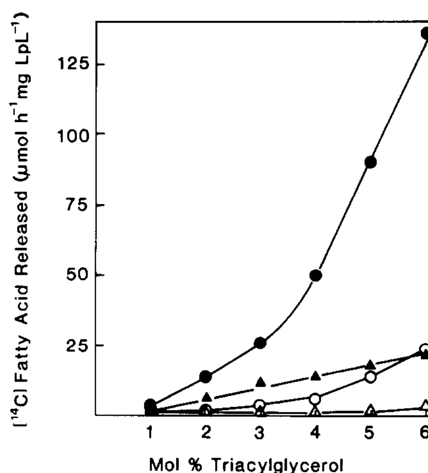

Fig. 1. Effect of monolayer content of triacylglycerol and apolipoprotein C-II on the rate of the lipoprotein lipase (LpL)-catalyzed hydrolysis of tri[^{14}C]oleoylglycerol and tri[^{14}C]-palmitoylglycerol. The monolayer (8 nmol total lipid) contained di18:1PC and various mol%, as indicated, of tri[^{14}C]oleoylglycerol (○, ●) or tri[^{14}C]palmitoylglycerol (△, ▲) at an initial surface pressure of 23 mN/m. After the surface film had stabilized, usually 5 min, heparin (25 μg), fatty acid-free bovine serum albumin (100 μg) and apolipoprotein C-II (100 ng, closed symbols) were added to the subphase. After an additional 10 min, lipoprotein lipase (10 ng) was added and the rate of lipoprotein lipase hydrolysis was determined as described in Methods. The rates were determined in the presence (closed symbols) and absence (open symbols) of apolipoprotein C-II.

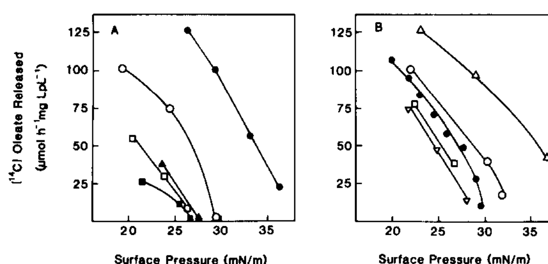

Fig. 2. Effect of monolayer phosphatidylcholine composition on the lipoprotein lipase-catalyzed hydrolysis of tri[^{14}C]-oleoylglycerol. The monolayer contained the indicated phosphatidylcholine plus 5 mol% tri[^{14}C]oleoylglycerol at the indicated surface pressure. After the monolayer had stabilized, heparin (25 μg), fatty acid-free bovine serum albumin (100 μg) and apolipoprotein C-II (100 ng) were added to the subphase. After an additional 10 min, lipoprotein lipase was added to the subphase and the rate of hydrolysis was determined as described in Methods. A: ■, di16:0; □, di14:0; ▲, di14:0 ether; ○, di12:0; ●, di22:0. B: ▽ di18:2, □, di16:0/18:1 ether; ●, di18:1; ○, di18:3; △, di20:4.

tri[^{14}C]oleoylglycerol were dependent on the fatty acyl chain length, the degree of saturation/unsaturation and the surface packing of the lipids. With monolayers of di12:0PC and di22:0PC, rates of hydrolysis were markedly higher than with di16:0PC or di14:0PC; diester or diether myristoylphosphatidylcholine gave nearly identical rates of hydrolysis, indicating that phospholipid hydrolysis is not a prerequisite for the lipoprotein lipase-catalyzed hydrolysis of triacylglycerol. At surface pressures greater than 26 mN/m, there was little or no hydrolysis with di14:0PC or di16:0PC as the bulk lipid. The effect of fatty acyl chain unsaturation on enzyme activity is shown in Fig. 2B. Di18:1PC, di18:2PC and di18:3PC monolayers all gave higher rates of lipoprotein lipase hydrolysis of tri[^{14}C]oleoylglycerol than did the saturated lipids di14:0PC and di16:0PC; the diether phospholipid (16:0/18:1PC) had an effect on lipoprotein lipase activity similar to that of di18:2PC.

The effect of substituting ethanolamine for choline in the polar head group of the phospholipid on the lipoprotein lipase-catalyzed hydrolysis of tri[^{14}C]oleoylglycerol is shown in Fig. 3A. Lipoprotein lipase activities toward tri[^{14}C]oleoylglycerol with monolayers of di18:2PE, di18:3PE and di20:4PE were nearly identical to those for the corresponding phosphatidylcholine lipids (Fig. 2).

Effect of sphingomyelin and cholesterol

Lipoprotein lipase activity toward tri[^{14}C]oleoylglycerol contained in a monolayer of palmitoyl sphingomyelin was negligible and is consistent with previous findings using bovine brain sphingomyelin [10]. Furthermore, the low

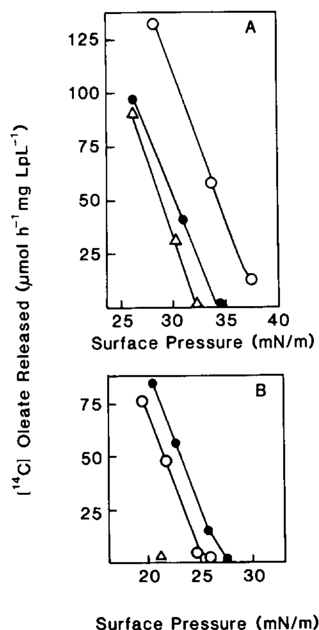

Fig. 3. Effect of monolayer phosphatidylethanolamine and sphingomyelin composition on the lipoprotein lipase-catalyzed hydrolysis of tri[^{14}C]oleoylglycerol. A. The monolayer contained the indicated phosphatidylethanolamine plus 5 mol% tri[^{14}C]oleoylglycerol at the indicated surface pressure. △, di18:3; ●, di18:2; ○, di20:4. B. The monolayer contained 5 mol% tri[^{14}C]oleoylglycerol plus palmitoyl sphingomyelin (△) or plus sphingomyelin:di18:1PC (1:1, mol/mol, ○) or plus sphingomyelin:di18:1PC (1:4, mol/mol, ●). After the monolayers had stabilized, heparin (25 μg), fatty acid-free bovine serum albumin (100 μg) and apolipoprotein C-II (100 ng) were added to the subphase. After an additional 10 min, lipoprotein lipase (10 ng) was added to the subphase and the rate of hydrolysis was determined as described in Methods.

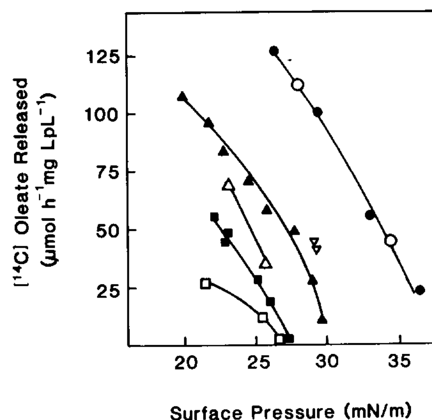

Fig. 4. Effect of monolayer content of cholesterol on the lipoprotein lipase-catalyzed hydrolysis of tri[^{14}C]oleoylglycerol. The monolayer contained the indicated phosphatidylcholine in either the presence (open symbols) or absence (closed symbols) of 50 mol% cholesterol; all monolayers contained 5 mol% tri[^{14}C]oleoylglycerol. A pure cholesterol monolayer containing 5 mol% tri[^{14}C]oleoylglycerol is indicated as ▽. Each monolayer was formed at the indicated surface pressure. After the monolayer had stabilized, heparin (25 μg), fatty acid-free bovine serum albumin (100 μg) and apolipoprotein C-II (100 ng) were added to the subphase. After an additional 10 min, lipoprotein lipase (10 ng) was added to the subphase and the rate of hydrolysis determined as described in Methods. ■, □, di16:0; ▲, △, di18:1; ●, ○, di22:0.

activity was apparent between 33 and 42°C (data not shown), a temperature range in which sphingomyelin is in the liquid phase. Mixing sphingomyelin with di18:1PC at mol ratios of 1:1 or 1:4 (sphingomyelin:di 18:1PC) markedly enhanced the rates of tri[^{14}C]oleoylglycerol hydrolysis (Fig. 3B). However, the rates of enzyme catalysis were less than those for a di18:1PC monolayer (Fig. 2B).

The effects of cholesterol on the lipoprotein lipase-catalyzed hydrolysis of tri[^{14}C]oleoylglycerol in phospholipid monolayers was next examined. As is shown in Fig. 4, 50 mol% cholesterol in di16:0PC caused an increase in lipoprotein lipase activity toward tri[^{14}C]oleoylglycerol, whereas 50 mol% cholesterol decreased lipoprotein lipase activity in a phospholipid monolayer of di18:1PC; cholesterol had no effect on the rate of hydrolysis of tri[^{14}C]oleoylglycerol in a monolayer of di22:0PC. Lipoprotein lipase catalyzed the hydrolysis of tri[^{14}C]oleoylglycerol even in a film of cholesterol with no phospholipid present (Fig. 4), again showing that phospholipid is not a prerequisite for catalysis.

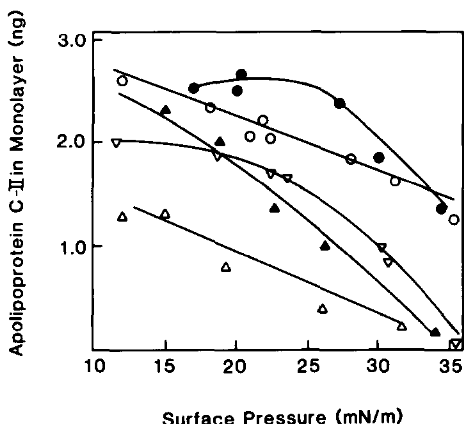

Fig. 5. Effect of monolayer lipid composition and surface pressure on the accumulation of ^{125}I-labelled apolipoprotein C-II at the interface. The monolayers contained the indicated phosphatidylcholine or palmitoyl sphingomyelin (△) and 5 mol% tri[^{14}C]oleoylglycerol. After the monolayers had stabilized, heparin (25 μg), fatty acid-free bovine serum albumin (100 μg) and ^{125}I-labelled apolipoprotein C-II (70 ng, 15000 cpm) were added to the subphase. After 10 min, the monolayer film was collected and ^{125}I radioactivity determined; an equivalent volume was removed from the subphase, counted and subtracted from the values in the film. ▲, di12:0; ▽, di16:0; ○, di18:1; ●, di18:2.

Effect of apolipoprotein C-II on lipoprotein lipase activity

One possibility that might account for decreased lipoprotein lipase activity toward tri[^{14}C]-oleoylglycerol at higher surface pressures (Figs. 2 and 3) is that the accumulation of apolipoprotein C-II at the interface is dependent on lipid packing. The results shown in Fig. 5 appear to eliminate a relationship between the amount of activator protein at the interface and lipoprotein lipase activity (Figs. 2 and 3) in various phosphatidylcholine monolayers. In these experiments, 70 ng of ^{125}I-labelled apolipoprotein C-II were injected under a monolayer of lipid at the indicated surface density. With these conditions, 10 min was sufficient for the maximal amount of apolipoprotein C-II to accumulate at the interface (data not shown). After 10 min, the lipid films were collected by aspiration and the amount of ^{125}I-labelled apolipoprotein C-II in the monolayer was determined as described in Methods. As is shown in Fig. 5, the accumulation of apolipoprotein C-II at the lipid interface decreased with increasing surface pressure, particularly with the saturated lipids palmitoyl sphingomyelin, di12:0PC and di16:0PC. However, there was no correlation between the amount of apolipoprotein C-II at the interface and lipoprotein lipase activity (Fig. 2). For example, lipoprotein lipase was inactive toward tri[^{14}C]oleoylglycerol at 27 mN/m in a monolayer of di16:0PC. In contrast, the amount of apolipoprotein C-II that accumulated in a di16:0PC monolayer was greater than that for a di12:0PC monolayer: lipoprotein lipase activity for tri[^{14}C]oleoylglycerol in a di12:0PC film was greater than that in di16:0PC. Similarly, at a surface pressure of 22 mN/m, lipoprotein lipase showed no activity toward tri[^{14}C]oleoylglycerol in a sphingomyelin monolayer (Fig. 3), even though apolipoprotein C-II accumulates at the interface.

In the next experiment, a constant amount of ^{125}I-labelled apolipoprotein C-II (70 ng) was injected under a monolayer of di18:1PC at 23 mN/m and the amount of lipoprotein lipase added to the subphase was increased from no lipoprotein lipase to a mol ratio of lipoprotein lipase to apolipoprotein C-II of 8. As is shown in Fig. 6, the amount of lipoprotein lipase injected under the monolayer did not change the amount of ^{125}I-

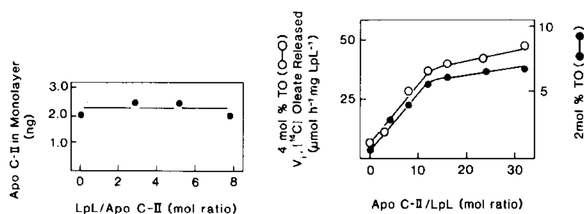

Fig. 6. (left-hand figure) Effect of the molar ratio of lipoprotein lipase to apolipoprotein C-II on the accumulation of ^{125}I-labelled apolipoprotein C-II at a lipid interface. The monolayer contained dioleoylphosphatidylcholine plus 5 mol% tri[^{14}C]-oleoylglycerol at a surface pressure of 23 mN/m. After the monolayer had stabilized, heparin (25 μg), fatty acid-free bovine serum albumin (100 μg) and ^{125}I-labelled apolipoprotein C-II (70 ng, 15000 cpm) were added to the subphase; lipoprotein lipase was then added to the subphase at the indicated molar ratios. After 10 min, the monolayer film was collected and the amount of apolipoprotein C-II at the interface determined as described in Methods.

Fig. 7. Dependence of apolipoprotein C-II and triacylglycerol content on the rate of the lipoprotein lipase-catalyzed hydrolysis of tri[^{14}C]oleoylglycerol. The monolayer contained dioleoylphosphatidylcholine plus either 2 mol% (●) or 4 mol% tri[^{14}C]oleoylglycerol (O) at a final surface pressure of 23 mN/m. After the monolayer had stabilized, heparin (25 μg), fatty acid-free bovine serum albumin (100 μg) and apolipoprotein C-II, as indicated, were added to the subphase. After 10 min, lipoprotein lipases (10 ng) was added to the subphase and the rate of enzyme hydrolysis was determined as described in Methods.

labelled apolipoprotein C-II which accumulated at the interface.

It is also possible that the dependence of lipoprotein lipase activity on apolipoprotein C-II is a function of the triacylglycerol concentration in the monolayer. To test this possibility, enzyme activity toward tri[^{14}C]oleoylglycerol was determined at various mol ratios of apolipoprotein C-II to lipoprotein lipase. As is shown in Fig. 7, maximal enzyme activity occurred at a mol ratio of apolipoprotein C-II to lipoprotein lipase of approx. 12, whether the monolayer contained 2 or 4 mol% tri[^{14}C]oleoylglycerol.

Discussion

Monolayer lipid films containing various fatty acyl glycerols have been used as substrates for studying lipoprotein lipase action. For example, a diacylglycerol, 1,2-didecanoylglycerol, was used in a previous report from this laboratory [19]. Smith and coworkers [20–22] and Chung and Scanu [23] carried out studies on monomolecular films of trioctanoylglycerol. Vainio et al. [24] also used trioctanoylglycerol as a substrate, but in the presence of a short-chain phosphatidylcholine, didodecanoylphosphatidylcholine. Although the short-chain lipids offer the advantage that the products are water-soluble and consequently leave the interface, they are not the physiological, long-chain substrates present in lipoproteins. Scow et al. [25] investigated the lipoprotein lipase-catalyzed hydrolysis of a long-chain lipid, trioleoylglycerol. However, the amount of triacylglycerol in the monolayer trough was 14-times that needed to cover the air/water surface. Thus, the trioleoylglycerol formed lenses resembling the core of a lipoprotein particle and not that of the outer monolayer interface.

The outer monolayer of triacylglycerol-rich lipoproteins consists mainly of phosphatidylcholine, sphingomyelin, unesterified cholesterol and protein, including apolipoprotein C-II [26]. It is assumed that lipoprotein lipase catalyzes the hydrolysis of triacylglycerols contained within the monolayer surface. Based on model studies with sonicated emulsions of phosphatidylcholine and trioleoylglycerol, Small and co-workers [7–9] have suggested that triacylglycerols are located in the lipoprotein monolayer with their glycerol backbone and primary carbonyls at the aqueous interface and with their fatty acyl chains parallel to those of the phospholipids. A recently described [10] monolayer system which mimics the lipoprotein interface allowed us to assess the role of lipid composition, structure and surface density on lipoprotein lipase activity toward triacylglycerols contained within the monolayer.

Although lipoprotein lipase has phospholipase A_1 activity [1], phospholipid was not hydrolyzed with the experimental conditions used in this study. Furthermore, lipoprotein lipase activity toward tri[^{14}C]oleoylglycerol occurred in the presence of diether phosphatidylcholines, lipids which are not hydrolyzed. Additional evidence that phosphatidylcholine hydrolysis is not a prerequisite for the lipoprotein lipase-catalyzed hydrolysis of triacylglycerol was the finding that catalysis occurred in a cholesterol/triacylglycerol monolayer in the absence of phospholipid.

Lipoprotein lipase activity was dependent on both the fatty acyl composition of the triacylglycerol substrate and bulk phospholipid. The saturated lipid, tri[^{14}C]palmitoylglycerol, was less effective as a substrate for lipoprotein lipase than was tri[^{14}C]oleoylglycerol. This difference in lipoprotein lipase activity may be explained by the apparent solubility of tripalmitoylglycerol in phosphatidylcholine. Gorrissen et al. [6] reported that the incorporation of tripalmitoylglycerol into egg phosphatidylcholine bilayers at 25°C was less than 0.1 mol%, while trioleoylglycerol was soluble to 2.5 mol%. Thus, decreased lipoprotein lipase activity with the saturated triacylglycerol may be due to a nonhomogeneous distribution of substrate at the water-lipid interface.

The dependence of fatty acyl composition of the bulk phospholipid on lipoprotein lipase activity toward tri[^{14}C]oleoylglycerol was a property of both the chain length of the lipid and saturation/unsaturation. In general, the unsaturated longer-chain phospholipids gave higher rates of lipoprotein lipase hydrolysis of triacylglycerol than the corresponding saturated lipids. An exception was di22:0PC, a phospholipid which has nearly the same molecular area as di16:0PC, but is a better bulk lipid for the lipoprotein lipase-catalyzed hydrolysis of tri[^{14}C]oleoylglycerol than di16:0PC. This difference in enzyme activity may be related to the accessibility of the glycerol backbone to the aqueous interface. Also, since the fatty acyl chain length of di22:0PC is greater than that of trioleoylglycerol, the mobility of the triacylglycerol within the monolayer may be restricted.

Consistent with our previous findings [10], lipoprotein lipase did not catalyze the hydrolysis of tri[^{14}C]oleoylglycerol contained within a pure sphingomyelin monolayer. Dilution of the sphingomyelin monolayer with di18 : 1PC markedly enhanced the rate of enzyme hydrolysis of the triacylglycerol. Unesterified cholesterol increased lipoprotein lipase activity in a di16:0PC monolayer and decreased it in di18:1PC. These results are consistent with the well-known fluidizing effect of cholesterol on saturated lipids and condensing effect on unsaturated ones [27]. Unesterified cholesterol had little effect on lipoprotein lipase activity in a di22:0PC monolayer. This result is consistent with only a small fluidizing effect of cholesterol on di22:0PC (R. Demel, unpublished results) and is probably due to the difference in chain length between the two lipids.

It is evident from the data shown in Figs. 2 and 3 that lipoprotein lipase activity toward triacylglycerol is dependent on the packing density of the bulk lipid. There are several possibilities that might explain this decreased enzyme activity. One possibility is that the adsorption of the enzyme to the interface is dependent on surface pressure. Although the amount of lipoprotein lipase at the lipid interface was not determined, a previous report from this laboratory [19] and a recent study by Vainio et al. [28] show that lipoprotein lipase is surface active and accumulates in an active form at lipid monolayers at surface pressures up to 40 mN/m. Thus, it seems unlikely that the dependence of lipoprotein lipase activity on surface pressure can be explained by the fact that lipoprotein lipase does not penetrate the lipid interface.

Another possibility to explain the effect of lipid packing on lipoprotein lipase activity is that the rate of catalysis is dependent on the amount of apolipoprotein C-II which accumulates at the interface. We previously showed [29], and others recently confirmed [28,30], that apolipoprotein C-II penetrates into lipid monolayers and causes an increase in pressure up to a critical value of approx. 30 mN/m. The results in Fig. 5 show that apolipoprotein C-II accumulates at the interface at even higher pressures than 30 mN/m. Furthermore, there appears to be no correlation between the amount of apolipoprotein C-II which accumulated at the interface and the dependence of surface pressure on lipoprotein lipase activity.

A third possibility to explain the surface pressure dependence on enzyme activity is that apolipoprotein C-II and lipoprotein lipase are both present at the interface at high surface pressures, but that the lateral mobility and/or conformation of the proteins, and possibly of the triacylglycerol, might be altered so as to reduce enzyme activity. We have no information as to the protein conformational requirements for catalysis. With respect to triacylglycerol, it seems unlikely that the concentration of tri[^{14}C]oleoylglycerol within the bulk lipid is reduced at high lipid density. We showed previously [10] that trioleoylglycerol and di18:1PC

are totally miscible in each other up to 5 mol% triacylglycerol; phase separation occurs between 5 and 10 mol% trioleoylglycerol. Although the lipids are miscible, this does not rule out the possibility that the conformation of the triacylglycerol is dependent on surface pressure. In this regard, other investigators [31–34] have proposed that monolayer lipid structure affects the activity of pancreatic lipase at an air-water interface. Momsen et al. [31,32] have suggested that the diacylglycerol, 1,3-didecanoylglycerol, assumes two discrete conformations with cross-sectional areas of 75 Å^2 (expanded state) and 38Å^2 (condensed state), respectively: compression of the lipid results in a transition of molecules between the two conformers. Although we have no direct evidence, we suggest that triacylglycerols also assume various conformations which are dependent on lipid packing. We further suggest that in the condensed state the glycerol backbone is oriented in a conformation at the lipid-water interface so that the primary acyl bond is less available for enzyme catalysis. This hypothesis implies that the structure and composition of the bulk lipid in the lipid monolayer is a determinant in the lipoprotein lipase-catalyzed hydrolysis of triacylglycerols.

Finally, the conclusion of this study that lipoprotein lipase activity toward triacylglycerols is dependent on the bulk lipid may have some physiological consequences. It is well-known (Ref. 35, for review) that diets rich in polyunsaturated fat decrease the plasma levels of triacylglycerols. One possible mechanism for this decrease is that the phospholipid fatty acyl chain composition of the lipoprotein becomes more unsaturated. As a result, the triacylglycerols within the outer monolayer of the lipoprotein particle assume a conformation which is more accessible to lipoprotein lipase catalysis. Experiments are currently in progress to test this possibility using reconstituted lipoproteins of known composition.

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