J. Biochem. 126, 1074-1079 (1999)

Fatty Acid Alcohol Ester-Synthesizing Activity of Lipoprotein Lipase

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Received August 6, 1999; accepted September 28, 1999

The fatty acid alcohol ester-synthesizing activity of lipoprotein lipase (LPL) was characterized using bovine milk LPL. Synthesizing activities were determined in an aqueous medium using oleic acid or trioleylglycerol as the acyl donor and equimolar amounts of long-chain alcohols as the acyl acceptor. When oleic acid and hexadecanol emulsified with gum arabic were incubated with LPL, palmityl oleate was synthesized, in a time- and dose-dependent manner. Apo-very low density lipoprotein (apoVLDL) stimulated LPL-catalyzed palmityl oleate synthesis. The apparent equilibrium ratio of fatty acid alcohol ester/oleic acid was estimated using a high concentration of LPL and a long (20 h) incubation period. The equilibrium ratio was affected by the incubation pH and the alcohol chain length. When the incubation pH was below pH 7.0 and long chain fatty acyl alcohols were used as substrates, the fatty acid alcohol ester/free fatty acid equilibrium ratio favored ester formation, with an apparent equilibrium ratio of fatty acid alcohol ester/fatty acid of about 0.9/0.1. The equilibrium ratio decreased sharply at alkaline pH (above pH 8.0). The ratio also decreased when fatty alcohols with acyl chains shorter than dodecanol were used. When a trioleoylglycerol/fatty acyl alcohol emulsion was incubated with LPL, fatty acid alcohol esters were synthesized in a dose- and time-dependent fashion. Fatty acid alcohol esters were easily synthesized from trioleoylglycerol when fatty alcohols with acyl chains longer than dodecanol were used, but synthesis was decreased with fatty alcohols with acyl chain lengths shorter than decanol, and little synthesizing activity was detected with shorter-chain fatty alcohols such as butanol or ethanol.

Key words: acyltransferase, fatty acid alcohol ester, lipoprotein lipase.

Lipoprotein lipase (LPL) is widely distributed throughout the tissues of vertebrates and is the key enzyme responsible for the hydrolysis of triacylglycerols from triacylglycerol-rich lipoproteins such as chylomicrons and very low density lipoproteins in the capillary endothelium (*1*). As a member of the lipase family, LPL shares structural similarities with hepatic triglyceride lipase and pancreatic lipase, and their genes are derived from a common ancestral gene (*2*). The conservation of most of the disulfide bonds in LPL and pancreatic lipase suggests that LPL has a similar three-dimensional structure and a catalytic triad consisting of Asp-His-Ser. Rojas *et al.* (*3*) have suggested that the catalytic reaction of LPL proceeds through an acyl-enzyme intermediate, which is typical of serine proteases.

Previously, we reported that LPL forms fatty acid ethyl esters from fatty acids or triacylglycerols in an ethanol/water mixture (*4*). LPL catalyzes fatty acid ethyl ester formation through an acyl-enzyme intermediate, which is then deacylated by nucleophilic attack by ethanol. In this reaction, ethanol competes with water. Therefore, a high concentration of ethanol is needed to assay fatty acid ethyl ester production: the maximum concentration of ethanol is about 1 M and the ratio of acyl acceptor/acyl donor is about 2,500 (*4*). A question arises as to why such high concentrations of ethanol are required for fatty acid ethyl ester synthesis. Ethanol is water-soluble and would be present in the bulk water. In this study, using water-insoluble alcohols (long chain fatty alcohols) as substrates, we found that LPL has high ester-synthesizing activity in an aqueous medium. We focused on the acyl-transfer reaction at the surface of the substrate emulsion catalyzed by LPL. Therefore, we obtained LPL from bovine milk on a large scale and fully characterized the LPL-catalyzed acyl-transfer reaction.

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Abbreviations: LPL, lipoprotein lipase; BSA, bovine serum albumin; ApoC-II, apolipoprotein C-II; ApoVLDL, apo-very low density lipoprotein.

MATERIALS AND METHODS

Materials—The following enzyme substrates and reagents were used. [1-^{14}C]Trioleoylglycerol (3.95 Gbq/mol), [1-^{14}C]oleic acid (2.1 Gbq/mmol), and [9,10-^{3}H]-oleic acid (273.8 GBq/mmol) were purchased from Dupont NEN (Boston, MA, USA). Trioleoylglycerol was purchased from Sigma (St. Louis, MO, USA), oleic acid, dioleoylglycerol, monooleoylglycerol, palmityl oleate, and fatty alcohols from Funakoshi (Tokyo), and heparin-Sepharose from Pharmacia LKB Biotechnology (Uppsala, Sweden). Bovine serum albumin (BSA) was obtained from Wako Pure Chemical Industries (Osaka), and was extracted by the method of Chen to remove free fatty acids (*5*).

Preparation of apoVLDL—Wistar strain rats, weighing

300-350 g, were starved overnight, then blood was collected in tubes containing 0.1% EDTA, and the plasma was separated by centrifugation at 1,000 × *g* for 15 min. Triacylglycerol-rich lipoproteins ($d < 1.02$ g/liter) were isolated by ultracentrifugation in KBr (22.257 g/liter) and delipidated with acetone/ethanol (1:1 by volume) and diethylether by the method of Jackson and Holdsworth (*6*). A fresh solution of 8 M urea was added to the delipidated protein, which was then stored at 4°C. The apoVLDL thus prepared, 33.4 μg/ml, stimulated the trioleoylglycerol-hydrolyzing activity of milk LPL by about 25-fold.

Purification of Milk LPL—LPL was purified from bovine skim milk using heparin-Sepharose by the method of Socorro and Jackson (*7*). The LPL fraction was adjusted to 50% with glycerol and stored at −80°C. Its specific trioleoylglycerol-hydrolyzing activity was 10.2 mmol/mg protein/h.

Preparation of Radiolabeled Palmityl Oleate—Palmityl [^{14}C]oleate and palmityl [^{3}H]oleate were synthesized by the pancreatic lipase-catalyzed esterification of [^{14}C]oleic acid or [^{3}H]oleic acid and isolated by preparative thin-layer chromatography. A suspension of 10 μmol oleic acid and 20 μmol hexadecanol in 1 ml 5% (w/v) gum arabic solution was sonicated for 5 min. Gum arabic is neutral and a good stabilizer for lipid emulsions. The emulsion was incubated with 500 μg pancreatic lipase at pH 6.5. After incubation for 1 h at 37°C, the lipids were extracted by adding 7.5 ml chloroform/methanol (1:2 by volume). The mixture was shaken for 15 s, then 2.5 ml chloroform and 3.75 ml H_2O were added, and the mixture was shaken again for 5 s, and then centrifuged at 1,000 × *g* for 10 min. The lower phase was dried with a stream of nitrogen, solubilized in 250 μl chloroform, and separated by thin-layer chromatography (Whatman silica gel K-5). The plates were developed with hexane/diethylether/ acetic acid (85:15:1 by volume), and the lipid spots were scraped off and extracted with chloroform/methanol (2:1 by volume).

Enzyme Assay—Fatty acid alcohol ester synthesis from oleic acid was determined using [^{14}C]oleic acid. A suspension of 30 μmol [^{14}C]oleic acid (5,000,000 dpm) and 30 μmol fatty alcohol in 2 ml 5% (w/v) gum arabic solution was sonicated for 5 min. The assay mixture consisted of 0.2 ml 100 mM Tris-HCl buffer (pH 8.0) containing 0.75 μmol [^{14}C]oleic acid (125,000 dpm), 0.75 μmol fatty alcohol, 20 μmol NaCl, 2.5 mg gum arabic, 6.7 μg apoVLDL, and enzyme solution. Incubation was carried out at 37°C and the reaction was stopped by adding 1.5 ml chloroform/methanol (1:2 by volume) containing standard lipids [10 nmol fatty alcohol, [^{3}H]palmityl oleate (50,000 dpm), and oleic acid]. The mixture was shaken for 15 s, then 0.5 ml chloroform and 0.75 ml H_2O were added, and the mixture was shaken again for 5 s, then centrifuged at 1,000 × *g* for 10 min. The lower phase was dried with a stream of nitrogen, solubilized in 50 μl chloroform, and chromatographed on silica plastic sheets (Whatman silica gel PE SIL G). The plates were developed with hexane/diethylether/ acetic acid (85:15:1 by volume). The lipids were located with iodine vapor, and spots containing each of the lipids were cut out in order to measure their radioactivity. These values were adjusted for yield, determined from the recovery of ^{3}H. The substrate-product distribution in the radiolabeled lipid spots was also determined directly using a Bio-Imaging Analyzer, BAS 1000 (Fuji Film, Tokyo), to analyze the substrate (oleic acid) and product (palmityl oleate) ratio.

Fatty acid alcohol ester hydrolysis was determined using palmityl[^{14}C]oleate as a substrate. A suspension of 30 μmol [^{14}C]palmityl oleate (5,000,000 dpm) in 2 ml 5% (w/v) gum arabic solution was sonicated for 5 min. The assay mixture consisted of 0.2 ml 100 mM Tris-HCl buffer (pH 8.0) containing 0.75 μmol [^{14}C]palmityl oleate (125,000 dpm), 20 μmol NaCl, 2.5 mg gum arabic, and 6.7 μg apoVLDL. Incubation was carried out at 37°C. Free oleic acid was extracted and determined by the method of Belfrage and Vaughan (*8*). The substrate-product distribution in the radiolabeled lipid spots was also determined directly using the BAS 1000 to analyze the substrate (palmityl oleate) and product (oleic acid) ratio. The lipids were extracted by adding 1.5 ml chloroform/methanol (1:2 by volume) containing standard lipids (10 nmol hexadecanol, palmityl oleate, and oleic acid). The mixture was shaken for 15 s, then 0.5 ml chloroform and 0.75 ml H_2O were added and the mixture was shaken again for 5 s, and then centrifuged at 1,000 × *g* for 10 min. The lower phase was dried with a stream of nitrogen, solubilized in 50 μl chloroform and separated by thin-layer chromatography (Whatman silica gel K-5) developed with hexane/diethylether/acetic acid (85:15:1 by volume). The radiolabeled lipid spots were analyzed using the BAS 1000.

Fatty acid alcohol ester synthesis from trioleoylglycerol was determined using [^{14}C]trioleoylglycerol. A suspension of 30 μmol [^{14}C]trioleoylglycerol (5,000,000 dpm) and 30 μmol fatty alcohol in 2 ml 5% (w/v) gum arabic solution was sonicated for 5 min. The assay mixture consisted of 0.2 ml 100 mM Tris-HCl buffer (pH 8.0) containing 0.75 μmol [^{14}C]trioleoylglycerol (125,000 dpm), 0.75 μmol fatty alcohol, 20 μmol NaCl, 2.5 mg gum arabic, 6.7 μg apoVLDL, and 5 mg BSA. Incubation was carried out for 1 h at 37°C. The lipids were extracted as described above and separated by thin-layer chromatography (Whatman silica gel K-5). The plates were developed first with hexane/diethylether/acetic acid (60:40:2 by volume) and then with hexane/diethylether/acetic acid (95:5:1 by volume). The separated radioactive lipid spots were analyzed using the BAS 1000. The radioactivity in the lipids was also determined using a liquid scintillation counter after scraping off the lipid spots.

RESULTS

When emulsified oleic acid and hexadecanol were incubated with LPL purified from bovine milk, a fatty acid alcohol ester (palmityl oleate) was synthesized in a dose- and time-dependent manner. Figure 1 illustrates the LPL concentration dependence of fatty acid alcohol ester synthesis. The ester formation increased linearly with the concentration of LPL up to 79 μg/ml when no apoVLDL was added. Upon the addition of apoVLDL, the synthesizing activity increased at each LPL concentration. Figure 2 shows the time dependence of fatty acid alcohol ester synthesis. The synthesis of palmityl oleate increased with increasing incubation time, becoming saturated after 2 h of incubation using a high concentration of LPL (158 μg/ml). At this point, the ratio of palmityl oleate/free oleic acid reached 0.6/0.4. This ratio did not change during long-term incubation for 20 h. LPL was also able to hydrolyze palmityl

oleate, and the apparent equilibrium ratio of palmityl oleate/oleic acid produced by this hydrolysis reached 0.72/0.28. ApoVLDL stimulated both the fatty acid alcohol ester-hydrolyzing and -synthesizing activities of LPL (Fig. 3): apoVLDL (33.4 μg/ml) stimulated hydrolysis by about 2.8-fold and synthesis by 1.9-fold. The specific palmityl oleate hydrolyzing activity was about 30 times greater than the synthesizing activity.

Figure 4 shows the effect of the alcohol fatty acyl chain length on fatty acid alcohol ester synthesis. High ester-synthesizing activities were observed with decanol, dodecanol, and tetradecanol. This activity decreased when fatty alcohols with acyl chains shorter than decanol were used, and little wax-ester synthesizing activity was detected when ethanol was used as a substrate. ApoVLDL stimulated synthesizing activity when fatty alcohols with acyl chains longer than decanol were used. However, no such stimulatory effect was observed when fatty alcohols with acyl chains shorter than octanol were used. Using a high concentration of LPL (158 μg/ml) and a long incubation period (20 h), the apparent equilibrium ratios of fatty acid alcohol ester/oleic acid were estimated at pH 6.8 and pH 8.0 (Fig. 5). The apparent equilibrium ratio was lower at pH 8.0 than at pH 6.8, being around 0.9/0.1 at pH 6.8 and 0.6/0.4 at pH 8.0 when hexadecanol was used as the

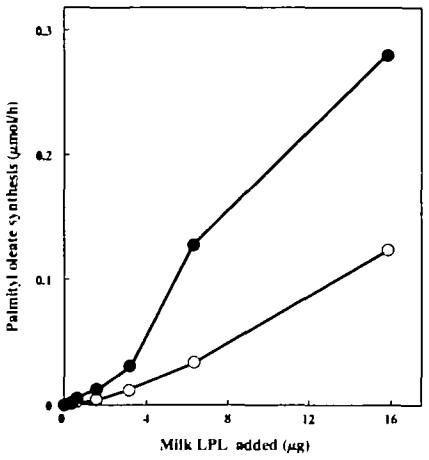

Fig. 1. **Effect of milk lipoprotein lipase concentration on the synthesis of palmityl oleate.** Palmityl oleate synthesis from [^{14}C]-oleic acid (3.75 mM) and hexadecanol (3.75 mM) was determined with (●) or without (○) apoVLDL (33.4 μg/ml). After incubation for 1 h at 37°C and pH 8.0, lipids were extracted and determined as described in "MATERIALS AND METHODS." Values are means for three separate assays.

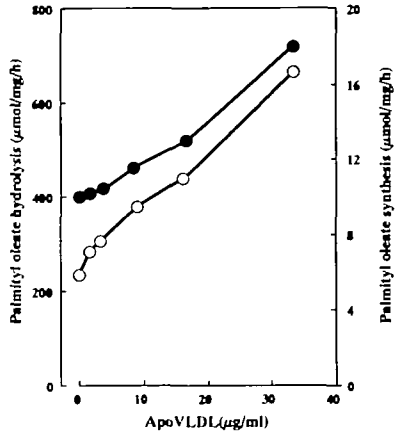

Fig. 3. **Effect of apoVLDL on the synthesis and hydrolysis of palmityl oleate.** The hydrolysis of palmityl oleate (○) was determined using [^{14}C]palmityl oleate (3.75 mM) as substrate. The synthesis of palmityl oleate (●) was determined using [^{14}C]oleic acid (3.75 mM) and hexadecanol (3.75 mM) as substrates. After incubation at 37°C and pH 8.0, lipids was extracted and the synthesized palmityl oleate or released oleic acid were determined. Values are means for three separate assays.

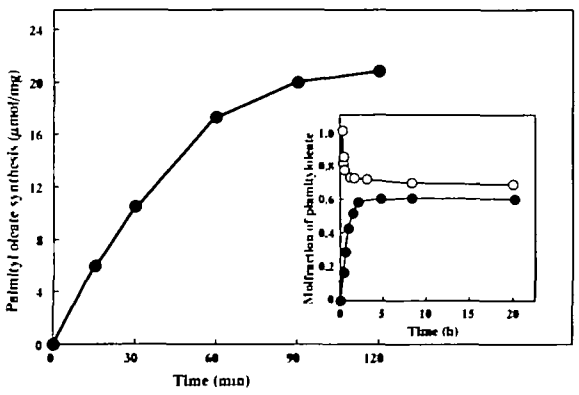

Fig. 2. **Effect of incubation time on the synthesis of palmityl oleate.** The synthesis of palmityl oleate was determined using [^{14}C]-oleic acid (3.75 mM) and hexadecanol (3.75 mM) as substrates with apoVLDL (33 μg/ml). After incubation at 37°C and pH 8.0, lipids were extracted and determined as described in "MATERIALS AND METHODS." (Insert) The synthesis (●) and hydrolysis (○) of palmityl oleate was determined after long-term incubation. The hydrolysis of palmityl oleate was determined using [^{14}C]palmityl oleate (3.75 mM) as substrate with apoVLDL (33.4 μg/ml). Milk LPL was 158 μg/ml. Values are means for three separate assays.

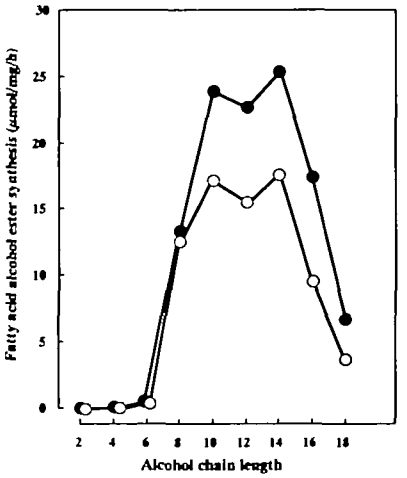

Fig. 4. **Effect of fatty alcohol acyl chain length on the synthesis of fatty acid alcohol ester.** The synthesis of fatty acid alcohol esters was determined using [^{14}C]oleic acid (3.75 mM) and alcohols of various chain lengths (3.75 mM) as substrates with (●) or without (○) apoVLDL (33.4 μg/ml). After incubation at 37°C and pH 8.0 for 1 h, lipids were extracted and determined as described in "MATERIALS AND METHODS." Values are means for three separate assays.

substrate. The equilibrium ratio was decreased when fatty alcohols with acyl chains shorter than tetradecanol were used, and no fatty acid alcohol ester (ethyl oleate) production was observed when ethanol was used as a substrate. The effects of pH on the apparent equilibrium ratios of palmityl oleate/oleic acid during hydrolysis and synthesis are shown in Fig. 6. Above pH 5.5, both equilibrium curves are superimposable; between pH 5.5 and 6.5, the apparent equilibrium ratios of palmityl oleate/free oleic acid during hydrolysis/synthesis are around 0.9/0.1, but the apparent equilibrium ratios of palmityl oleate/free oleic acid decrease sharply above pH 8.0.

Figure 7 shows the effect of the LPL concentration on palmityl oleate formation from trioleylglycerol with 2.5% BSA. When an equimolar [^{14}C]trioleylglycerol/hexadecanol emulsion was incubated with LPL, the formation of palmityl oleate and oleic acid increased with increasing LPL concentration. At an LPL concentration of 158 μg/ml, most of the trioleoylglycerol was degraded and the ratio of palmityl oleate:oleic acid was about 1:2. Figure 8 shows the effect of incubation time on palmityl oleate formation from

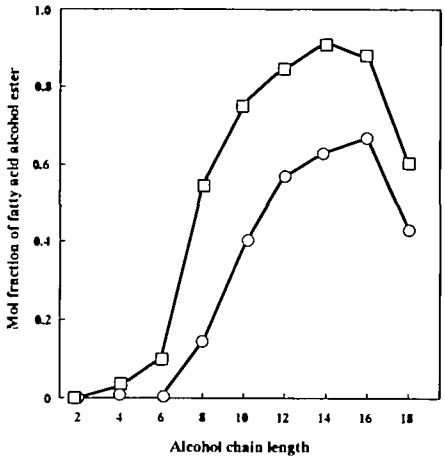

Fig. 5. **Effect of fatty alcohol acyl chain length on the synthesis of fatty acid alcohol ester.** The synthesis of fatty acid alcohol esters was determined using [^{14}C]oleic acid (3.75 mM) and alcohols of various chain lengths (3.75 mM) as substrates with apoVLDL (33.4 μg/ml) and milk LPL (158 μg/ml) at pH 6.8 (□) or pH 8.0 (○). After incubation for 20 h at 37°C, lipids were extracted and the apparent equilibilium molar fraction of fatty acid alcohol ester was determined. Values are means for three separate assays.

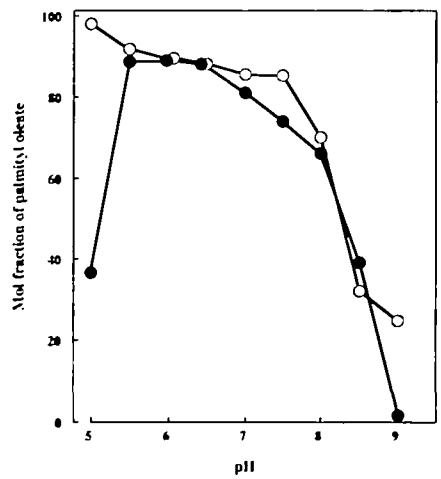

Fig. 6. **Effect of pH on the synthesis/hydrolysis of palmityl oleate.** The hydrolysis (○) was determined using palmityl [^{14}C]oleate (3.75 mM) as a substrate with apoVLDL (33.4 μg/ml) and milk LPL (158 μg/ml). The synthesis (●) was determined using [^{14}C]oleic acid (3.75 mM) and hexadecanol (3.75 mM) as substrates with apoVLDL (33.4 μg/ml) and milk LPL (158 μg/ml). After incubation for 20 h at 37°C, lipids were extracted and the molar fraction of palmityl oleate was determined. Values are means for three separate assays. Acetate buffer (pH 5.0 and 5.5), phosphate buffer (pH 6.0-8.0), and Tris buffer (pH 8.0-9.0) were used. The ionic strength was 0.1.

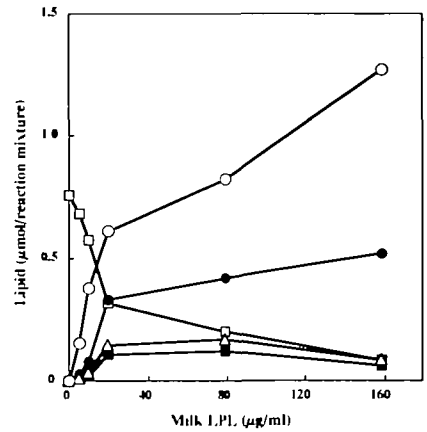

Fig. 7. **Effect of milk LPL concentration on the synthesis of palmityl oleate from trioleylglycerol.** [^{14}C]Trioleylglycerol (3.75 mM) and hexadecanol (3.75 mM) were incubated with apoVLDL (33.4 μg/ml) and BSA (25 mg/ml). After incubation for 2 h at 37°C and pH 8.0, lipids were extracted and the molar concentrations of palmityl oleate (●), trioleylglycerol (□), oleic acid (○), dioleoylglycerol (■), and monooleylglycerol (△) were determined. Values are means for three separate assays.

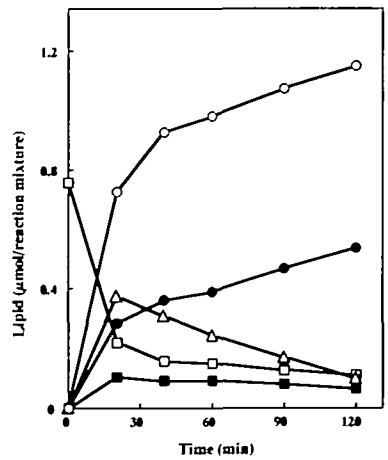

Fig. 8. **Effect of incubation time on the synthesis of palmityl oleate from trioleylglycerol.** [^{14}C]Trioleylglycerol (3.75 mM) and hexadecanol (3.75 mM) were incubated with apoVLDL (33.4 μg/ml), milk LPL (158 μg/ml), and BSA (25 mg/ml). After incubation at 37°C and pH 8.0, lipids were extracted and the molar concentrations of palmityl oleate (●), trioleylglycerol (¯), oleic acid (○), dioleoylglycerol (■), and monooleylglycerol (△) were determined. Values are means for three separate assays.

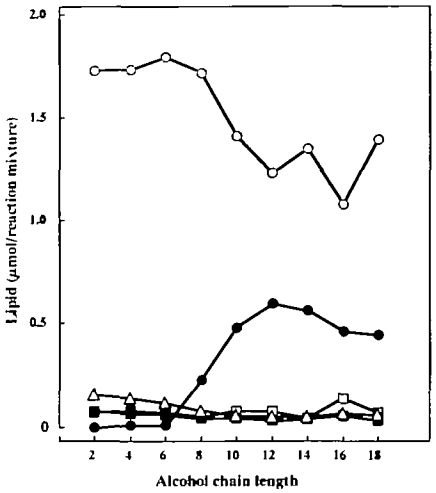

Fig. 9. **Effect of fatty alcohol acyl chain length on the synthesis of fatty acid alcohol ester from trioleoylglycerol.** Fatty acid alcohol ester synthesis was determined using [^{14}C]trioleoylglycerol (3.75 mM) and alcohols with various chain lengths (3.75 mM) as substrates with apoVLDL (33.4 μg/ml), milk LPL (158 μg/ml), and BSA (25 mg/ml). After incubation for 2 h at 37°C and pH 8.0, lipids were extracted and the molar concentrations of fatty acid alcohol ester (●), trioleylglycerol (□), oleic acid (○), dioleoylglycerol (■), and monooleylglycerol (△) were determined. Values are means for three separate assays.

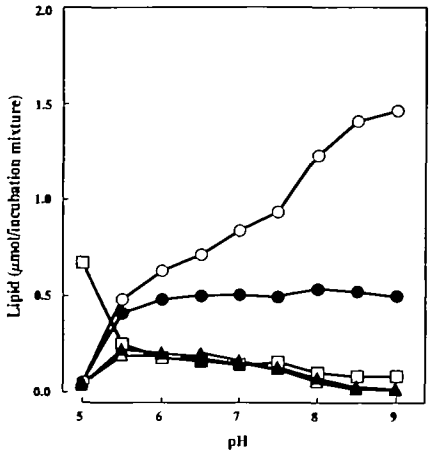

Fig. 10. **Effect of pH on palmityl oleate synthesis from trioleylglycerol.** Palmityl oleate synthesis was determined using [^{14}C]-trioleoylglycerol (3.75 mM) and hexadecanol (3.75 mM) as substrates with apoVLDL (33.4 μg/ml), milk LPL (158 μg/ml), and BSA (25 mg/ml). After incubation for 2 h at 37°C, lipids were extracted and the molar concentrations of palmityl oleate (●), trioleylglycerol (□), oleic acid (○), dioleoylglycerol (■), and monooleylglycerol (△) were determined. Values are means for three separate assays.

trioleylglycerol. The formation of palmityl oleate and oleic acid increases with increasing incubation time up to 2 h of incubation, when most of the trioleoylglycerol is degraded and the ratio of palmityl oleate:oleic acid is about 1:2. The effect of the fatty alcohol acyl chain length on fatty acid alcohol ester formation from trioleoylglycerol is shown in Fig. 9. Most of the trioleoylglycerol was degraded by a high concentration of LPL (158 μg/ml). No fatty acid alcohol ester formation was detected when fatty alcohols with acyl chains shorter than hexanol were used. Free oleic acid formation decreased as the acyl chain length of the fatty alcohol increased; conversely, fatty acid alcohol ester formation increased. Figure 10 shows the effect of pH on palmityl oleate synthesis from triacylglycerol. Trioleoylglycerol degradation increased with increasing pH to 9.0. Palmityl oleate formation was almost constant at pH values over 6.0, whereas free oleic acid formation increased with rising pH up to 9.0. The ratio of oleic acid/palmityl oleate was 1.2 at pH 5.5, whereas it was 2.9 at pH 9.0.

DISCUSSION

In the present study, we demonstrate that LPL can catalyze ester synthesis at low concentrations of long-chain alcohols such as hexadecanol: the hexadecanol concentration was 3.75 mM and the ratio of acyl acceptor/acyl donor was 1. When an equimolar amount of ethanol was used as an acyl acceptor, no fatty acid ethyl ester was detected (Figs. 4 and 9). One explanation for this phenomenon is that ethanol is water-soluble while hexadecanol is water-insoluble. The lipase-catalyzed hydrolysis and synthesis of insoluble lipids occurs at the lipid-water interface in the presence of bulk water (*9*). A long-chain fatty alcohol would be a constituent of the substrate at the lipid-water interface, whereas ethanol would not, since it would be present in the bulk water. Therefore, a long-chain fatty alcohol is a more effective acyl acceptor than ethanol. However, high concentrations of long-chain fatty alcohols inhibit lipase activity by covering the substrate surface, thus blocking the interaction with lipase (*10, 11*).

When the hydrolysis/synthesis of palmityl oleate by LPL was studied, the specific activity for palmityl oleate hydrolysis was about 30-fold higher than that for synthesis (Fig. 3). However, the equilibrium favored ester formation: the apparent equilibrium ratio of palmityl oleate/free oleic acid was about 0.6/0.4 at pH 8.0 and 09/0.1 at pH 7.0 (Fig. 6). Over pH 6.0, a short chain alcohol ester (ethyl oleate) and trioleoylglycerol were completely degraded by LPL (data not shown). The equilibrium ratio was dependent on the incubation pH and the chain length of the alcohol: when short-chain alcohols or an alkaline pH were used, the ratio favored free fatty acid formation (Figs. 5 and 6). These results suggest that the equilibrium ratio might depend on the water solubility of the substrate. The water solubility of fatty acids increases at alkaline pH, and that of alcohols increases with a decrease in their chain length.

It has been suggested that the enzymatically catalyzed acylation of long-chain fatty alcohols proceeds in three way (*12*). First, free fatty acids can be directly incorporated into fatty acid alcohol esters (*13*). Second, fatty acyl-CoA can act as an acyl donor for the esterification of alcohols (*14*). Third, acyl moieties can be transferred from phospholipids to alcohols (*15*). The fatty acid alcohol ester-synthesizing activity of LPL from oleic acid is strongly influenced by albumin: albumin at a concentration of 10 mg/ml or more causes complete inhibition (data not shown). This result suggests that albumin-bound fatty acids do not act as substrates for fatty acid alcohol ester synthesis by LPL. However, fatty acid alcohol ester synthesis from trioleoylglycerol is not influenced by high concentrations of albumin

(25 mg/ml) (Figs. 7 and 8). These results suggest that LPL might catalyze acyltransfer from triacylglycerol to alcohol. Therefore, LPL catalyzes the synthesis of fatty acid alcohol esters in two ways: direct esterification of free fatty acids and acyltransfer from triacylglycerol to alcohol.

It is well known that apolipoprotein C-II (apoC-II) enhances LPL activity (*16*). This activation is observed at lipid-water interfaces such as the surface of a long-chain triacylglycerol emulsion. In this study, we investigated the effect of apoC-II on the synthesis and hydrolysis of fatty acid alcohol esters using apoVLDL as a source of apoC-II. ApoC-II stimulates not only fatty acid alcohol ester-hydrolysis but also fatty acid alcohol ester-synthesis (Fig. 3). However, the stimulatory effect disappeares when fatty alcohols with acyl chains shorter than octanol are used; the octanoyl oleate-synthesizing activity is not accelerated by apoC-II (Fig. 4). Previously, we reported that LPL-catalyzed ethyl oleate synthesis is not stimulated by apoC-II (*4*). Similar results have been observed using other soluble substrates such as tributyroylglycerol and *p*-nitrophenylbutyrate. ApoC-II does not accelerate the hydrolysis of tributyroylglycerol (*17*), and rather than producing activation, it inhibits *p*-nitrophenylbutyrate-hydrolysis (*18*). Using a homologous series of saturated phosphatidylcholines as substrates, Shinomiya *et al.* reported that the activation by apoC-II is a linear function of the number of carbon atoms of a single fatty acyl chain of the substrate (*19*).

The primary structure of LPL contains Gly-Xaa-Ser-Xaa-Gly, which is the common active site sequence of a serine protease, and its catalytic mechanism resembles the mechanism of serine proteinase catalysis (*20*). These data suggest that the enzyme reactions catalyzed by LPL proceed *via* an acyl-enzyme intermediate. Therefore, the mechanism of fatty acid alcohol ester formation by LPL can be explained as follows. With a fatty acid as substrate, an acyl-enzyme intermediate is formed through an enzyme-substrate complex, and nucleophilic displacement by water or fatty alcohol occurs. With fatty alcohol as an acceptor fatty acid, alcohol ester synthesis occurs. When a triacylglycerol is used as substrate, an acyl-enzyme intermediate is also formed, and the acyl-enzyme intermediate is deacylated by the nucleophilic attack of water or fatty alcohol. With water as an acceptor for fatty acids, "hydrolysis" occurs, but with fatty alcohol as an acceptor, "fatty acid alcohol ester synthesis" occurs.

In this study we focused on the acyl-transfer reaction catalyzed by LPL, and demonstrate that this enzyme catalyzes the formation of fatty acid alcohol esters from oleic acid or triacylglycerol in an aqueous medium. LPL is widely distributed in extra-hepatic tissues. Therefore, these finding can be applied to understanding the mechanism of some water insoluble ester formations by LPL at various sites. Further experiments are needed to confirm this hypothesis.

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