Effects of albumin and apolipoprotein C-II on the acyl-chain specificity of lipoprotein lipase catalysis

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Abstract In this study we used monoacid triacylglycerols of various acyl-chain lengths as substrates for probing the active-site structure and substrate specificity of lipoprotein lipase (LPL). An unexpected finding was that the albumin ligand binding site is accessible not only to long-chain fatty acids for its recognized functional role as a fatty acid acceptor, but also to short- and medium-chain monoacid triacylglycerol substrates. The observed striking inhibitory effect (99%) of albumin on the LPL-catalyzed hydrolysis of trihexanoylglycerol is probably the result of the high affinity interaction of albumin with this substrate. Spectrophotometric analyses indicated that there is one high affinity binding site per albumin molecule (apparent K_D = 1.8 ± 0.9 μM) for the interaction with trihexanoylglycerol. Despite LPL acyl-chain specificity being obscured by the substrate binding effect of albumin, a systematic study of the lipolysis reaction under various assay conditions demonstrated that tributyroylglycerol represents the best substrate for LPL, and the preferential order of LPL catalysis for both the basal and apoC-II-activated activities is: $C_4 > C_6 > C_8 > C_{10} > C_{12} > C_{18:1}$. In some assay conditions, the presence of albumin affects the above-mentioned order, which can be attributed to substrate binding by albumin, rather than an alteration in the specificity of LPL. The synergistic effect of apoC-II and albumin resulted in the preferential activation of LPL for the hydrolysis of long-chain triacylglycerols. Even with optimal assay conditions for the hydrolysis of long chain triacylglycerols, there is still a preferential reactivity of LPL with short- and medium-chain triacylglycerols.—**Wang, C-S., H. Bass, R. Whitmer, and W. J. McConathy.** Effects of albumin and apolipoprotein C-II in the acyl-chain specificity of lipoprotein lipase catalysis. *J. Lipid Res.* 1993. **34:** 2091–2098.

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Lipoprotein lipase (LPL), an acylglycerol hydrolase (EC3.1.1.34), is localized in the capillary endothelium and is widely distributed in extrahepatic tissues, including heart, skeletal muscle, and adipose tissue (1–3). The function of LPL is to direct the influx of plasma triacylglycerol in the form of fatty acids into the peripheral tissues for storage, and to provide fuel for energy requirements. Because of the importance of LPL, we have continued our studies on the functional properties of this physiologically important enzyme.

The requirement for serum albumin in the LPL-catalyzed reaction has been well documented (4–7). In LPL catalysis in the absence of albumin, the cleaved long-chain fatty acid product is released very slowly into the assay medium and is poorly displaced by a new substrate molecule, thus preventing continuation of the catalytic cycle. However, in the presence of albumin, the rate of lipolysis is no longer dependent on this step, which implies that the rate of transfer of fatty acid to albumin is much faster than the chemical cleavage step of catalysis (4, 5).

Our initial goal in the present study was to determine the minimal fatty acid chain length with which albumin becomes the necessary cofactor for the product removal of LPL catalysis. However, to our surprise, we observed that the albumin ligand binding site is accessible not only for binding with long-chain fatty acids, but also for binding with short- and medium-chain triacylglycerols. Because this inhibitory effect of albumin obscured the conclusions regarding the acyl-chain specificity of the enzyme, we have examined in detail the effect of albumin on the substrate specificity of both basal and apoC-II-activated LPL activities. By taking into account the inhibitory effect of albumin in LPL catalysis, we conclude that both the basal form and apoC-II-activated LPL have a preferential reactivity with short- and medium-chain triacylglycerols. The poor reactivity of LPL with long-chain triacylglycerol, despite its being the physiological substrate, can be attributed, at least in part, to the steric hindrance effects of bulky long-chain triacylglycerols at the LPL-active site.

Abbreviations: LPL, lipoprotein lipase; apoC-II, apolipoprotein C-II; GLC, gas-liquid chromatography.

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MATERIALS AND METHODS

Materials

Unless otherwise stated, all chemicals were purchased from Sigma Chemical Co., St. Louis, MO. Fatty acid-free bovine serum albumin was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). Apolipoprotein C-II was prepared as described by Curry et al. (8).

Preparation of LPL

Bovine milk LPL was used in this study. LPL was first purified from bovine skim milk by flotation of the enzyme with added Intralipid, followed by removal of fat from the isolated fat cake with acetone–ether treatment, as described previously (9). Acetone–ether powder represents a readily available and enriched source of bovine LPL and was used for the purification of LPL. Acetone–ether powder was suspended (5 mg/ml) in 50 mM NH_4OH–HCl buffer, pH 8.5, for 10 min in an ice bath. Aliquots (4 ml) of the suspension were then applied to a small heparin-Sepharose column calibrated to contain 1 ml of the wet gel. The column was eluted with 4 ml 0.3 M NaCl and 0.72 M NaCl. The purified enzyme was eluted with 3 ml 2 M NaCl. The NaCl solutions were prepared with 50 mM NH_4OH–HCl, pH 8.5. LPL eluted with 2 M NaCl was stable for several hours at 0°C. We prepared the purified enzyme on the day of the kinetic experiments.

Lipolysis of triacylglycerols

The stock substrate solution was prepared by emulsifying a single molecular species or a mixture of monoacid triacylglycerols in 50 mM NH_4OH–HCl buffer with dioleoylphosphatidylglycerol as the emulsifying reagent. The concentration of each substrate was adjusted to 1.25 mM, which represents a 5-fold concentrated solution. The molar ratio of substrate concentration to dioleoylphosphatidylglycerol was fixed at 10:1. The mixture was emulsified using a W-380 sonicator (Heat Systems-Ultrasonics, Inc.) at setting of 5 (50% of maximum output), for 30 sec in an ice bath, and after cooling was further sonicated for an additional 30 sec.

The assay mixture (final volume 5 ml) contained a final concentration of 0.25 mM of each molecular species of substrate and 0.10 ml of the purified bovine milk LPL as eluted with 2 M NaCl from the heparin-Sepharose column. The experiments, which also included the addition of albumin (60 mg/ml) and apoC-II (10 μg/ml), are indicated in the figure legend. After incubation of the assay mixture in a shaking water bath at 37°C for 2.5, 5, 10, 15, 20, 40, and 60 min, duplicate samples of 250 μl were taken and transferred to tubes containing 4 ml of n-heptane–isopropanol 7:3 (v/v) for termination of the enzyme reaction. The solvent mixture also contained 50 μg cholesteryl butyrate as the internal standard. Each extract was acidified with 5.0 ml 0.33 N H_2SO_4, mixed for 30 sec, and allowed to stand overnight. The upper phase was transferred to a 2-ml conical autosampler vial, followed by evaporation of the solvent under nitrogen. The residue was redissolved in 200 μl hexane. A 5-μl aliquot was then injected into the gas–liquid chromatograph.

Gas–liquid chromatography analysis

The GLC analyses were performed using a Varian 3700 gas–liquid chromatograph equipped with a series 8000 autosampler and a Spectrophysics SP4270 integrator. The separation was performed using 3% OV-1 on 100–120 Supercoport (Supelco, Inc.) packed in a 50-cm glass column with an inner diameter of 2 mm. The initial column temperature was 120°C and temperature was programmed to increase at 15°/min. When the temperature reached 347°C, the column was held at this temperature for 7 min. This completed one cycle of the injection. Nitrogen was used as the carrier gas at a flow rate of 20 ml/min. A flame ionization detector was used for analysis of the effluents. The retention time of the molecular species of triacylglycerols and internal standards was as follows: tributyroylglycerol, 2.27 min; trihexanoylglycerol, 5.32 min; trioctanoylglycerol, 7.94 min; cholesteryl butyrate (internal standard), 9.79 min; tridecanoylglycerol, 10.20 min; trilaurylglycerol, 12.31 min; and trioleoylglycerol, 17.37 min. Because triacetylglycerol and tripropionylglycerol cannot be adequately separated from the solvent-front, we could not include these two short-chain triacylglycerols in our kinetic studies.

Spectrophotometric analyses

Fluorescence measurements were made at 25°C with the Aminoco-Bowman Fluorescence Spectrometer. Albumin tryptophanyl fluorescence was recorded at 340 nm with excitation wavelength at 280 nm. The band-width of excitation and emission were both set at 2 nm. The sensitivity of the instrument was set at 1000 volts of the detector high voltage. Absorbance measurements were made at 25°C using a Hewlett-Packard Diode Array Spectrophotometer. The turbidity of the trihexanoylglycerol emulsions was measured at 450 nm. Because trihexanoylglycerol can be readily emulsified in the absence of emulsifying reagent, for simplifying the interpretation of the results of the interaction between trioctanoylglycerol and albumin, we did not include dioleoylglycerolphosphatidylcholine for emulsifying trihexanoylglycerol in aqueous solution in this binding study. The mixture was incubated at room temperature for 2 h to allow it to reach equilibrium.

Analysis of results

Non-linear regression analyses for curve-fitting were performed using the SAS computer program (SAS Institute, Inc., Cary, NC). The derivative-free algorithm as described by Ralston and Jennrich (10) was used for the non-linear least-square curve-fitting.

RESULTS

In order to ascertain whether the presence of albumin is necessary for LPL catalysis with short-chain and medium-chain triacylglycerol substrates, as is the case for hydrolysis of long-chain triacylglycerols, we initiated our study by comparing the hydrolytic reactions of tributyroylglycerol and trihexanoylglycerol with LPL, in both the presence and the absence of albumin. The kinetic patterns of these reactions determined by GLC analyses are shown in **Fig. 1.** The most unexpected finding from the observed kinetic patterns was that albumin inhibits hydrolysis of trihexanoylglycerol (Fig. 1A), rather than showing the anticipated activation effect. By using the pseudo-first order rate constant of the substrate disappearance in LPL catalysis as the expression of the reaction rate, we concluded that there was about 99% reduction in the rate of lipolysis of trihexanoylglycerol (**Table 1**) due to the presence of albumin in the assay mixture. There was also a striking, but smaller, inhibitory effect of albumin (85%) for LPL catalysis in the hydrolysis of tributyroylglycerol (Fig. 1B, Table 1). A progressive reduction of inhibitory effect of albumin was observed when trioctanoylglycerol (35%) and tridecanoylglycerol (29%) were used as substrates in the lipolytic reactions. With the further increase of acyl-chain length of the monoacid triacylglycerols, we started to observe the activation effect of albumin, as can be seen in the LPL-catalyzed hydrolysis of trilaurylglycerol and trioleoylglycerol (Table 1). However, even with the activation effect of albumin, the activity of LPL with trioleoylglycerol and trilaurylglycerol was still very low. The apparent k_1 values for these various substrates as derived in the absence and presence of albumin are shown in Table 1. From the data, we can deduce that in the absence of albumin there is an inverse relationship between the basal reactivity of LPL and the increased acyl-chain length of the substrate: $C_4 > C_6 > C_8 > C_{10} > C_{12} > C_{18:1}$. From Table 1 it becomes obvious that the preferential effect of albumin in inhibiting the rate of hydrolysis of short chain triacylglycerol caused the change of the preferential acyl-chain reactivity to: $C_8 > C_{10} > C_4 > C_{12} > C_{18:1} > C_6$.

TABLE 1. Apparent k_1 values of LPL in hydrolyzing monoacid triacylglycerols[a]

	Without Albumin (Basal Activity)	With Albumin	% Inhibition (Activation)
	min^{-1}		
C_4	0.41 ± 0.04[b]	0.06 ± 0.02	85
C_6	0.25 ± 0.01	0.0035 ± 0.0004	99
C_8	0.20 ± 0.05	0.13 ± 0.05	35
C_{10}	0.130 ± 0.003	0.09 ± 0.01	28
C_{12}	0.008 ± 0.005	0.018 ± 0.006	(130)[c]
$C_{18:1}$	0.0033 ± 0.0004	0.010 ± 0.006	(200)

[a] Each of the substrates was incubated with LPL individually. The enzyme concentration was 1.2 μg/ml. The initial substrate concentration of each of the substrates was 0.25 mM.

[b] Mean ± SD (n = 3).

[c] The numbers in parentheses indicate activation rather than inhibition.

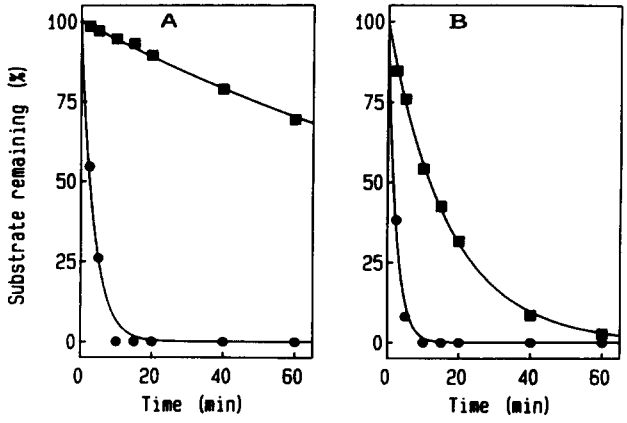

Fig. 1. The kinetics of the hydrolysis of (A) trihexanoylglycerol and (B) tributyroylglycerol with the basal form (absence of apoC-II) of LPL. The symbols used are: (●), in the absence, and (■) in the presence of albumin (60 mg/ml), and both in the absence of apoC-II. The initial substrate concentration was 0.25 mM. The LPL concentration was 1.2 μg/ml in the assay mixture.

Among the possible mechanisms of the inhibitory effect of albumin we favor the hypothesis that there is a direct interaction of albumin with short-chain triacylglycerols. It is possible that the ligand binding site of albumin is sterically accessible to the short-chain, but not to the bulky long-chain triacylglycerols, and therefore albumin displays an opposing effect on LPL catalysis in response to the substrate acyl-chain length. The substrate binding effect of albumin can result in reduced "effective" substrate concentration for LPL catalysis and, consequently, the observed inhibitory effect. If this is the case, the direct interaction of albumin with the short-chain triacylglycerol should result in the "solubilization" of the substrate molecule, and consequent reduction of the turbidity of the substrate emulsion. To test the validity of this hypothesis, we examined the interaction of albumin with trihexanoylglycerol at various concentrations of trihexanoylglycerol emulsion (**Fig. 2A**). Absorbance measurements at 450 nm indicated that there is an apparent clearance of the turbidity with trihexanoylglycerol concentrations below or at the equivalent concentration of albumin (0.4 mM), allowing the conclusion of a 1:1 stoichiometry. Despite the observation of the clearance of the emulsion, the absorbance of the mixture was slightly higher than that of albumin alone. We attribute the increased absorbance to the presence of unbound trihexanoylglycerol. Thus, the concentration of the unbound trihexanoylglycerol in the reaction mixture was deduced from the difference of absorbance at 450 nm of trihexanoylglycerol + albumin from that of albumin alone, permitting determination of the

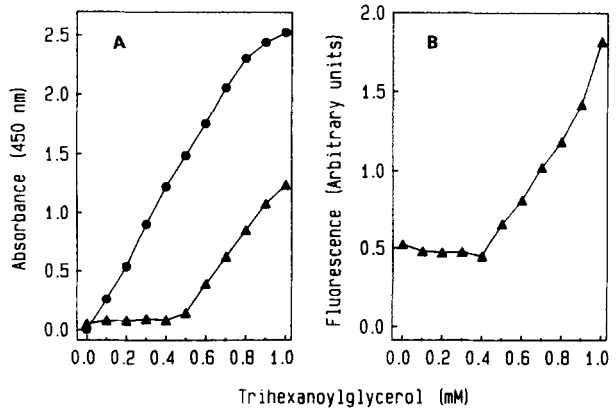

Fig. 2. Spectrophotometric analysis of the interaction of trihexanoylglycerol with bovine serum albumin. The experiment was performed in 50 mM NH_4OH-HCl buffer, pH 8.5. (A) Absorbance at 450 nm for trihexanoylglycerol emulsion in the absence (●) and presence of 0.4 mM (▲) bovine serum albumin. (B) Effect of increased concentration of trihexanoylglycerol on the tryptophanyl fluorescence of bovine serum albumin (0.4 mM). The wavelength excitation was 280 nm; the emission was recorded at 340 nm.

dissociation constant for the high affinity interaction of albumin with trihexanoylglycerol (apparent K_D = 1.8 ± 0.9 μM; mean ± SD; n = 4).

Examination of the intrinsic tryptophanyl fluorescence properties of albumin upon its interaction with trihexanoyl indicated that there is minimum change of fluorescence (Fig. 2B) when trihexanoylglycerol concentration is below or at the equivalent concentration of albumin (0.4 mM). However, further increase in the concentration of trihexanoylglycerol resulted in enhanced tryptophanyl fluorescence (Fig. 2B), while the absorbance measurements (Fig. 2A) indicated a significantly high amount of unbound trihexanoylglycerol in the incubation mixture. Thus, the binding between trihexanoylglycerol and albumin associated with enhanced fluorescence must be due to a low affinity interaction at secondary site(s).

Because of the availability of the GLC technique, which allows the assay of various substrates in a single competitive assay mixture, we also examined the effect of albumin and apoC-II on LPL acyl-chain specificity. This analytical technique greatly reduces the number of assays required for deduction of the preferential LPL reactivity with various molecular species of triacylglycerols under different treatments. Because these molecular species of substrates were present in the same assay mixture, they competed with each other for binding with the enzyme at the active site, and also competed for interaction with albumin at the ligand binding site. Therefore, the derived kinetic rate constants were useful for reflecting the effect of the competitive phenomenon. The derived rate constants are expected to be different from those obtained in Table 1, in which the kinetic data were derived from the assay of each substrate individually. The GLC patterns for LPL catalysis at various time intervals during the lipolytic reaction with an equimolar starting substrate mixture (0.25 mM each) consisting of tributyroyl-, trihexanoyl-, trioctanoyl-, tridecanoyl-, and trioleoylglycerol in the absence of albumin are shown in **Fig. 3.** From the GLC patterns we can readily conclude that the peaks of each molecular species of triacylglycerols are well separated. Also, the lipolytic reaction of the long-chain triacylglycerol substrates did not generate products with retention time similar to that of the shorter chain triacylglycerol substrates, and therefore the lipolytic reaction did not affect the substrate concentration determination by GLC.

The deduced pseudo-first order rate constants for each of the substrate molecular species are indicated in **Fig. 4,** and were derived under experimental conditions including: (A) in the absence of albumin; (B) in the presence of albumin; (C) in the presence of apoC-II; and (D) in the presence of both albumin and apoC-II. From the data shown in Fig. 4A, we can readily see that the basal form of LPL has a preferential reactivity with tributyroylglycerol and the preferential reactivity of LPL with these various substrates follows the order: $C_4 > C_6 > C_8 > C_{10} > C_{18:1}$. This trend is the same as that observed in Table 1 for the basal activity of LPL. However, there is also an interesting quantitative relationship of the rate constants derived from the two methods. Despite the presence of the competitive substrates in the assay mixture, the derived k_1 value (Fig. 4A) for tributyroylglycerol was similar to that derived from Table 1. As the LPL concentration in these two assays is the same, an immediate conclusion of this finding is that the longer chain triacylglycerols are very poor competitive substrate inhibitors for the LPL reaction in the hydrolysis of shorter chain triacylglycerols. In contrast, the k_1 value for longer chain triacylglycerols was reduced due to the presence of the shorter chain triacylglycerol competitive substrates. For example, the k_1 value for tridecanoylglycerol in Fig. 4A (0.023 min^{-1}) was about 1/6 of that found in Table 1 (0.13 min^{-1}). These findings would suggest that there is a molecular sieving effect of the LPL active site for competitive interaction with various triacylglycerol molecular species.

By comparing the rate constants shown in Figs. 4A and 4B to those shown in Table 1, we have come to the conclusion that albumin exhibits less inhibitory effect on the LPL catalysis when the reaction is performed under competitive assay conditions. While there is less inhibitory effect of albumin on the hydrolysis of tributyroylglycerol and trihexanoylglycerol than that shown in Table 1, the inhibitory effect of albumin on the hydrolysis of trioctanoylglycerol and tridecanoylglycerol is no longer observed (Fig. 4). The obvious conclusion of this finding is that the different molecular species of short-chain and medium-chain triacylglycerols can compete with each

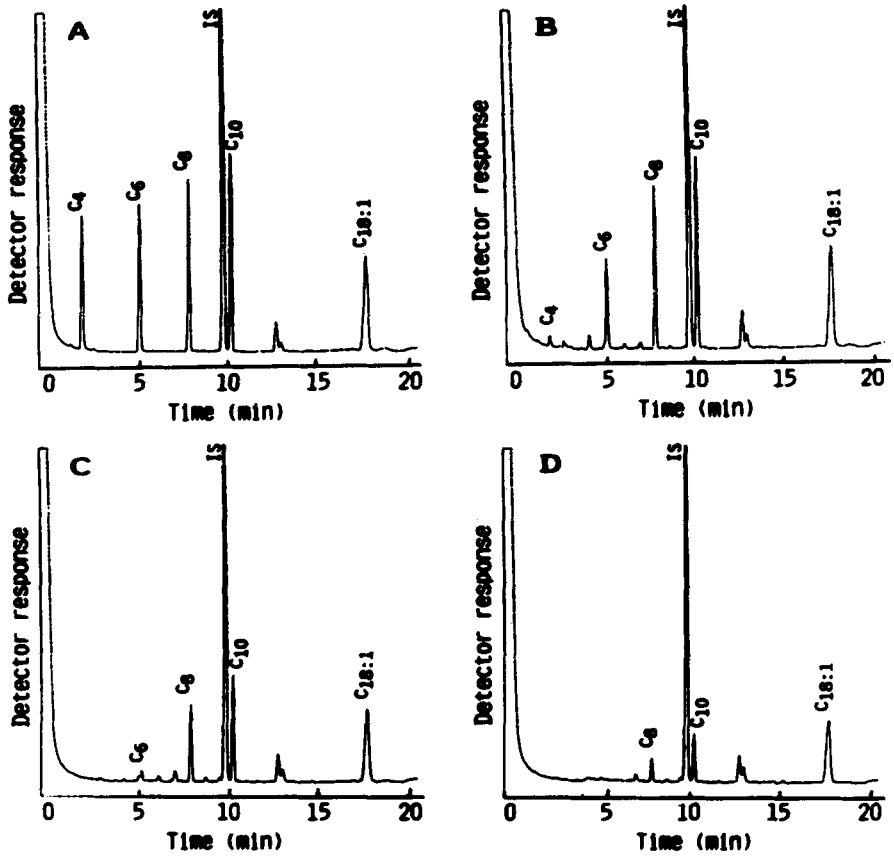

Fig. 3. The gas-liquid chromatography pattern of the hydrolysis of monoacid triacylglycerol with the basal form of LPL (in the absence of albumin and apoCII). (A) Control assay mixture in the absence of LPL. (B, C, D) The chromatography patterns of the assay mixture after 5, 10, and 60 min incubation with LPL. The peaks identified in the figures are: C_4, tributyroylglycerol; C_6, trihexanoylglycerol; C_8, trioctanoylglycerol; IS, internal standard, cholesterol butyrate; C_{10}, tridecanoylglycerol; $C_{18:1}$, trioleoylglycerol. The initial concentration of each molecular species of the substrates was 0.25 mM. The LPL concentration in the assay mixture was 1.2 μg/ml.

other for interaction with albumin. It is probably for this reason that, despite the preferential inhibitory effect of albumin on the rate of hydrolysis of short-chain triacylglycerols, this inhibitory effect was not sufficient to change the order of $C_4 > C_6 > C_8 > C_{10} > C_{18:1}$ (Fig. 4B), as is seen for the basal activity of LPL (Table 1).

For the expression of its full catalytic potential, LPL requires the presence of the activator apoC-II. Therefore, in this study we performed the experiment indicated in Fig. 4C to examine the effect of apoC-II on the substrate specificity of LPL. The kinetic study indicated that apoC-II caused reduced reactivity of tributyroylglycerol, while all the other longer chain triacylglycerols were found to have higher reactivity than that of the basal form of LPL. Among the triacylglycerols, apoC-II exhibited the largest activation effect in the hydrolysis of trioctanoylglycerol (Fig. 4C vs. 4A).

Under the experimental conditions of Fig. 4D, with both apoC-II and albumin present in the assay mixture, we conclude that the hydrolysis of long-chain triacylglycerol by LPL requires the synergistic effect of these cofactors. By calculating the relative activities of each substrate in the presence of these cofactors to that of basal activity of LPL (Fig. 4D vs. 4A), we further conclude that the combined effects of the two cofactors result in the preferential activation of the enzyme for hydrolysis of long-chain triacylglycerol (fold of activation: C_4, 0.7; C_6, 1.6; C_8, 7.0; C_{10}, 7.7; $C_{18:1}$, 15.1). However, even under these assay conditions, which are unfavorable for the hydrolysis of tributyroylglycerol, it still represents the best substrate among the examined monoacid triacylglycerols (Fig. 4D). Under these assay conditions, the rate of hydrolysis of trihexanoylglycerol did not follow the trend of inverse relationship of reactivity and the acyl-chain length of the substrates, with its reactivity being slightly lower than that of trioctanoylglycerol. By taking into consideration the overall kinetic patterns of LPL catalysis, the reverse of the preferential reactivity of the two substrates is probably the result of the preferential inhibitory effect of albumin with trihexanoylglycerol, rather than the change of acyl-chain specificity of LPL.

The other unexpected finding of the synergistic effect of

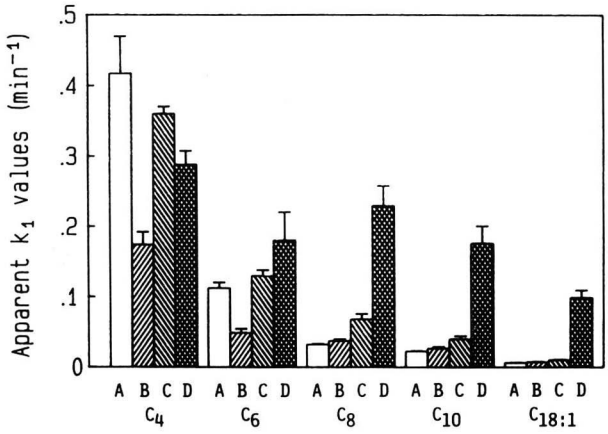

Fig. 4. The derived pseudo-first order rate constants of the hydrolysis of an equimolar mixture of monoacid triacylglycerol (0.25 mM each) with LPL. The substrates were tributyroylglycerol (C_4), trihexanoylglycerol (C_6), trioctanoylglycerol (C_8), tridecanoylglycerol (C_{10}), and trioleoylglycerol ($C_{18:1}$), the same as described in Fig. 2. The assay was performed (A) without albumin and apoC-II; (B) with albumin but without apoC-II; (C) with apoC-II but without albumin; and (D) with both apoC-II and albumin. The LPL in the assay mixture was 1.2 μg/ml. Albumin concentration was 60 mg/ml. ApoC-II concentration was 10 μg/ml. The error bars are standard errors derived from computer nonlinear curve-fitting.

apoC-II and albumin is that, in the presence of apoC-II and the competitive substrates, albumin was found to also have an activation effect on the rate of hydrolysis of the short chain trihexanoylglycerol substrate (Fig. 4D vs. 4A). However, when the assay was performed with trihexanoylglycerol alone, i.e., in the absence of competitive substrates, the presence of apoC-II did not result in overcoming the inhibition effect of albumin (**Fig. 5D vs. 5A**).

DISCUSSION

Our initial goal in this study was to define the minimal acyl-chain length with which LPL requires albumin as a fatty acid acceptor. However, the results of the present kinetic studies indicated that albumin can act not only as fatty acid acceptor, but also as an inhibitor by its direct interaction with short- and medium-chain triacylglycerols. Absorbance and fluorescence measurements (Fig. 2) indicated that there is only one high affinity ligand binding site per albumin molecule (apparent K_D = 1.8 ± 0.9 μM), which is in contrast to the presence of five or six high affinity binding sites on albumin for the interaction with fatty acids (11–14). The determined K_D is of similar magnitude to that of the high affinity interaction between long chain fatty acid and albumin (12). Because of the unexpected finding of the substrate binding effect of albumin, we have reexamined the albumin effect in more detail, not only on the basal LPL, but also on apoC-II-activated LPL activities.

When the substrate concentration is much below the apparent Michaelis-Menten constant, K_m, in an enzyme reaction, the derived pseudo-first order rate constant for the progressive curve of the substrate concentration in the assay mixture will approach the ratio of V_{max}/K_m, which is also the substrate specificity constant (15). The magnitude of the ratio of these kinetic parameters is related not only to the relative affinity of the substrate to the enzyme, but is also affected by the magnitude of the chemical step of the enzyme catalysis with each substrate. Thus, the derived pseudo-first order rate constants are good representations of the relative reactivity of each of the molecular species of the substrate with the enzyme.

From the data shown in the acyl-chain specificity studies, we can readily conclude that tributyroylglycerol represents the best substrate for LPL, and the LPL preferential reactivity can be described to follow the order of: $C_4 > C_6 > C_8 > C_{10} > C_{12} > C_{18:1}$.

Because of the inhibitory effect of albumin on the hydrolysis of short- and medium-chain triacylglycerols, and particularly on the hydrolysis of trihexanoylglycerol, the change of the above order in the results of some assays can be attributed to the inhibitory effect of albumin rather than to the change of the acyl-chain specificity of LPL. In the presence of apoC-II and albumin, there is a preferential activation effect of LPL for the hydrolysis of long-chain triacylglycerols. Under this assay condition, which is not the optimum for the LPL-catalyzed hydrolysis of tributyroylglycerol, it still has a higher reactivity than that of longer chain triacylglycerols. However, the finding that the rate of hydrolysis of trihexanoylglycerol is slightly lower than that of trioctanoylglycerol (Fig. 4D), can be ex-

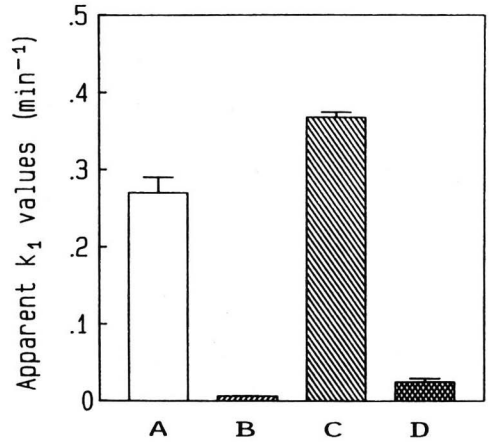

Fig. 5. The apparent k_1 values for the hydrolysis of trihexanoylglycerol (0.25 mM) with LPL. The experimental condition was the same as that described in Fig. 4 except that the reaction was performed with this substrate alone, i.e., in the absence of the competitive substrates. The assay was performed (A) without albumin and apoC-II; (B) with albumin but without apoC-II; (C) with apoC-II but without albumin; and (D) with both apoC-II and albumin. The concentration of LPL was 1.2 μg/ml. ApoC-II concentration was 10 μg/ml. Albumin concentration was 60 mg/ml. The error bars are standard errors derived from computer nonlinear curve-fitting.

plained based on the preferential inhibitory effect of albumin on the LPL-catalyzed hydrolysis of trihexanoylglycerol. Similarly, we have shown in a previous report (16) that there is a higher reactivity of human LPL with trioctanoylglycerol than with trihexanoylglycerol, which led to the conclusion that C_8 is the optimum acyl-chain length of the substrate for interaction with LPL. Because the assay was performed in the presence of a high concentration of albumin, and with serum as the source of the activator apoC-II, it is likely that the higher reactivity of LPL with trioctanoylglycerol than with trihexanoylglycerol can also be attributed to the preferential inhibitory effect of albumin on the hydrolysis of trihexanoylglycerol. The finding of the direct binding of albumin with short-chain and medium-chain triacylglycerols raises the possibility that albumin might also participate in mediating the cellular uptake of these triacylglycerols without prior hydrolysis by lipoprotein lipase or hepatic lipase.

The mutation of apoC-II structure genes has been shown to lead to hyperchylomicronemia, as has been found in the cases of LPL gene mutations (1). ApoC-II deficiency has shown physiologically that apoC-II is necessary for the optimum functioning of LPL. As the physiological substrates of LPL are long-chain triacylglycerols, it is interesting to observe that the synergistic effect of apoC-II and albumin leads to the optimum activation of the enzyme for the hydrolysis of long-chain triacylglycerols. McLean et al. (17) have also studied the fatty acyl-chain specificity of the phospholipase A_1 activity of bovine LPL and reported the activity of the enzyme following the trend of *sn*-1 acyl-chain length with $C_{14} > C_{16} > C_{18}$. However, the activation factor in apoC-II was reported to be dependent on the *sn*-2 acyl-chain length with the factor of 29.2 for C_{16} versus 14.8 for C_{14}. In contrast to the optimum activation of LPL-catalyzed hydrolysis of long-chain triacylglycerols with the synergistic effect of apoC-II and albumin, the effect of apoC-II alone (without added albumin) can lead to the optimum activation of the enzyme for hydrolysis of trioctanoylglycerol (Fig. 4C vs. 4A). This difference can be related to the product inhibition effect by long-chain fatty acid as the alternative rate-limiting step of LPL catalysis when albumin is absent in the assay mixture. The synergistic effect of apoC-II and albumin indicates that the role of apoC-II in activating the enzyme and the role of albumin in accepting the long-chain fatty acids are important for optimum LPL catalysis in the hydrolysis of long-chain triacylglycerols.

As revealed from this study, although the physiological substrates of lipases are long-chain triacylglycerols, the ability of long-chain acylglycerols to form a lipid interface is not in itself advantageous for the most efficient catalysis by lipases. In fact, the increased acyl-chain length can lead to a poorer water solubility which can lead to a lower "effective" substrate concentration and may, in part, contribute to lower reactivity with LPL. Further understanding of the mechanism that leads to the substrate preference of LPL requires detailed knowledge of the active site structural features of the enzyme. At present, the crystal structure of LPL is not available for correlation of structure and function features of the enzyme. However, as there is a high degree of sequence homology between LPL and pancreatic lipase (1), the structural information on pancreatic lipase can provide a good model for understanding the preferential reactivity of LPL with tributyroylglycerol. Examination of the active-site structure of human pancreatic lipase (18) reveals that there is a chain segment or flap that can block access to the active site. When this loop is moved due to the interaction with lipid interface, it is still difficult for a substrate as bulky as a long-chain triacylglycerol to reach the active-site serine for catalysis. Thus, the steric hinderance effect probably plays an important role in the acyl-chain specificity of pancreatic lipase. Early studies of Entressangles et al. (19) have already shown that, among the monoacid triacylglycerols, tributyroylglycerol represents the optimum substrate for porcine pancreatic lipase and has the highest V_{max}. As the parameter V_{max} is proportional to k_{cat}, the rate constant of the chemical step of catalysis, the preference for tributyroylglycerol is probably due not only to the ready accessibility of the substrate to the active-site, but also a higher rate of chemical step of the catalysis with this substrate. In view of the structural homology between pancreatic lipase and LPL, the structural basis for the substrate preference of pancreatic lipase is likely also applicable to the present finding for LPL.

In conclusion, because of the substrate binding effect of albumin, the assessment of the acyl-chain specificity study of LPL is obscured by the albumin inhibition effect. Despite this, we still reached the conclusion that tributyroylglycerol is the best substrate among the monoacid triacylglycerols used in this kinetic study. The steric accessibility and an optimum enzymic catalysis in the chemical step of the hydrolysis of butyrate ester probably contributed to the observed preferential hydrolysis of this substrate.

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