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Mechanism of Action of Milk Lipoprotein Lipase at Substrate Interfaces: Effects of Apolipoproteins†

Richard L. Jackson,* Franc Pattus,‡ and Gerard de Haas

ABSTRACT: The mechanism of action of bovine milk lipoprotein lipase was studied by using a monomolecular film of 1,2-didecanoylglycerol. The apparent rate of hydrolysis of diglyceride increased with increasing surface pressures above 12 mN/m; the enzyme was inactive at pressures less than 12 mN/m. We have measured the effects of four plasma apolipoproteins (apoC-II, apoC-III, apoA-I, and apoE), bovine serum albumin, porcine pancreatic colipase, heparin, and NaCl on the kinetics of lipid hydrolysis. At a surface pressure of 15 mN/m, all of the proteins, with the exception of colipase, gave increased enzyme activity compared to lipase alone; apoC-II gave maximal activation. At 25 mN/m, apoC-II at concentrations of less than 0.25 μg/mL showed a specific activation, whereas the other proteins had no effect. Heparin activated at both high and low surface pressures; NaCl had little or no effect in this system. At a higher concentration of apoC-II (0.50 μg/mL), the apoprotein inhibited the enzyme. The addition of apoC-III, apoA-I, or apoE (final concentration 0.25 μg/mL), but not albumin or colipase, to apoC-II (0.25 μg/mL) caused an increase in surface pressure of 5-6 mN/m and an apparent rate which was less than half that found for lipase alone, suggesting that all of the apoproteins inhibit the apoC-II specific activation.

Lipoprotein lipase, glycerol ester hydrolase (EC 3.1.1.3), hydrolyzes plasma triglycerides which are transported in the circulation by chylomicrons and very low density lipoproteins (VLDL)[1]. The enzyme is located at the vascular endothelium and is rapidly released into plasma by intravenous injection of heparin (Korn, 1955). Lipoprotein lipase is found at high levels in adipose tissue, muscle, and lactating mammary gland. A number of investigators [for a review, see Fielding & Havel (1977)] have shown that apolipoprotein C-II (apoC-II) from human VLDL is required for maximal activity of the enzyme. The effects of the other apoC proteins (apoC-I and apoC-III) and the high density apolipoproteins apoA-I, apoA-II, and apoE on the activity of lipoprotein lipase are controversial (Östlund & Iverius, 1975; Ekman & Nilsson-Ehle, 1975; Brown & Baginsky, 1972; Baginsky & Brown, 1976; LaRosa et al., 1970; Havel et al., 1973; Quarfordt et al., 1977; Ganesan & Bass, 1976; Ganesan et al., 1976). Our understanding of the structure and the mechanism of action of lipoprotein lipase at the membrane surface and its activation by apoC-II is unknown. A major reason for the lack of information concerning the detailed mechanism has been the difficulty in

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[1] Abbreviations used: VLDL, very low density lipoproteins; apoC-I, apoC-II, and apoC-III, apoproteins of VLDL; HDL, high density lipoproteins; apoA-I and apoE, apoproteins of HDL; BSA, bovine serum albumin; FA, fatty acid; LpL, lipoprotein lipase.

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isolating pure enzyme from tissue or postheparin plasma. Because of the recent advances made in the preparation of homogeneous lipoprotein lipase from bovine milk (Kinnunen et al., 1976; Egelrud & Olivecrona, 1972; Iverius & Ostlund-Lindquist, 1973), we have initiated the present study in order to understand its mechanism of action. The mode of action of the enzyme was studied by using a monomolecular film of diglyceride. The major conclusion drawn from these studies is that the activity of lipoprotein lipase is dependent both on surface pressure and on specific proteins added to the system.

Materials and Methods

Purification of Milk Lipoprotein Lipase. Lipoprotein lipase was isolated from fresh skimmed bovine milk by affinity chromatography on heparin–Sepharose as described by Kinnunen (1977). The isolated enzyme had a specific activity of 35 mmol of free fatty acid released per h per mg of protein by using a synthetic substrate of glyceryl tri[1-^{14}C]octanoate emulsified in 5% gum arabic (Ehnholm et al., 1975). By polyacrylamide gel electrophoresis in sodium dodecyl sulfate, the enzyme was homogeneous with a molecular weight of ~55 000. Lipoprotein lipase was iodinated by the iodine monochloride method as described by Roholt & Pressman (1972). Sodium [^{125}I]iodide (Amersham) was added to a stock solution of 3 mM iodine–9 mM potassium iodide to give 2.5 × 10^7 cpm/nmol of iodide. Iodination of the lipase (50 μg) was performed at −10 °C in 1 mL of 0.10 M Tris-HCl, pH 9.6, containing 20% glycerol; 1 mol equiv of iodine was added to the enzyme solution. After 30 min at −10 °C the enzyme was dialyzed overnight with several changes against 0.05 M Tris-HCl, pH 7.6, containing 10% glycerol and then stored at −20 °C in 50% glycerol. The iodinated enzyme contained 2.1 × 10^6 cpm/μg of lipase (0.45 iodide/lipase).

Isolation of Apoproteins. ApoC-II and apoC-III with two residues of sialic acid were isolated from human plasma VLDL by gel filtration and ion-exchange chromatography at 4 °C in 6 M urea as described previously (Jackson et al., 1977). ApoC-II and apoC-III were homogeneous by isoelectrofocusing between pH 4.0 and 6.0 (Catapano et al., 1978) and by polyacrylamide gel electrophoresis in urea (Davis, 1964). ApoA-I was prepared from human plasma HDL (Baker et al., 1973). ApoE was isolated as before (Roth et al., 1977) from VLDL and β-VLDL, which were obtained from the plasma of cholesterol-fed swine. ApoC-II was iodinated by the method of Roholt & Pressman (1972). Sodium [^{125}I]iodide (Amersham) was added to a stock solution of 3 mM iodine–9 mM potassium iodide to give 4.86 × 10^4 cpm/mmol of iodide. Iodination of apoC-II (1 mg) was performed in 1 mL of 0.05 M Tris-HCl, pH 9.6. One molar equivalent (as iodine) of the [^{125}I]-iodine–iodide solution was added to apoC-II at 0 °C. After 30 min at 0 °C, the protein was dialyzed (3500 molecular weight cutoff dialysis tubing) overnight against 0.05 M Tris-HCl, pH 7.6, with several changes of buffer. The modified protein contained 0.43 iodide/mol of apoC-II with an activity of 2.09 × 10^4 cpm/nmol of apoC-II.

Interfacial Measurement. Enzyme reaction rates were determined in a zero-order Teflon trough as described by Verger & de Haas (1973). The compartment containing the enzyme had a total volume of 210 mL and a total surface area of 91 cm^2. A description of the barostat and the special techniques for film transfer, film recovery, and radioactivity counting were the same as those described previously (Verger et al., 1973, 1976). Film transfers were performed in a three-compartment Teflon trough (Rietsch et al., 1977). The volume of each trough was 130 mL, and the surface area was 121.4 cm^2. All kinetic recordings and film transfers were carried out in a metallic box (Verger & de Haas, 1973) thermostated at 25 ± 0.5 °C. Reaction rates were determined graphically by using the maximal slope of the kinetic curves. In all assays the aqueous subphase contained 10 mM Tris–acetate–0.1 m NaCl, pH 7.5; doubly distilled water was used in all experiments. 1,2-Didecanoylglycerol was kindly supplied by Dr. R. Verger (Marseille).

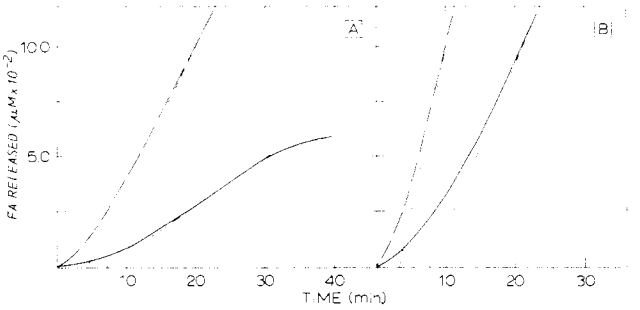

FIGURE 1: Kinetics of hydrolysis of 1,2-didecanoylglycerol monolayers by bovine milk lipoprotein lipase at 15 (A) or 25 mN/m (B). The kinetics were performed at 25 °C in a 210-mL "zero-order" trough at constant surface pressure as described under Materials and Methods. The reaction mixture contained lipoprotein lipase, 0.5 μg (—), or lipoprotein lipase, 0.5 μg, plus apoC-II, 50 μg (–·–), in 0.01 M Tris–acetate buffer containing 0.1 M NaCl, pH 7.5. The reaction rates (micromoles of FA hydrolyzed per minute per milligram of injected protein) were determined graphically as indicated by using the maximal slope of the kinetic curves.

Other Materials and Methods. Phospholipid phosphorus was determined by the method of Bartlett (1959). ^{125}I radioactivity was determined in a Packard Auto Gamma scintillation spectrometer. Protein was determined as described by Schacterle & Pollack (1973). Bovine serum albumin (BSA) was fatty acid free. Heparin was obtained from Vitrum, Stockholm, Sweden. Porcine pancreatic colipase was a generous gift from Dr. R. Verger (Marseille).

Results

Hydrolysis Rates of Substrate Monolayers. To determine the effects of surface pressure on the lipase-catalyzed hydrolysis of monomolecular films of substrate, we have utilized the zero-order trough as described by Verger & de Haas (1973). In Figure 1 the kinetics of hydrolysis by lipoprotein lipase (0.5 μg) of 1,2-didecanylglycerol films at 15 and 25 mN/m in the absence and presence of apoC-II is shown. At 15 nM/m the kinetics of hydrolysis gave a sigmoidal curve with an initial lag period indicative of a pre-steady-state condition and a steady-state condition, and then the reaction progressively slowed down, suggesting enzyme inactivation. The addition of apoC-II (50 μg) to the subphase had a threefold effect on the kinetics of hydrolysis at 15 mN/m (Figure 1); the apoprotein made the kinetics more linear, decreased the pre-steady-state condition, and increased lipase activity. The effects were observed either when apoC-II was added first to the subphase and then lipase was added or when apoC-II and lipase were premixed and then added to the subphase. At the concentration (0.25 μg/mL) of apoprotein used in these experiments, apoC-II alone gave a surface pressure increase of only 0.5 mN/m. Thus, the increased kinetics of hydrolysis of substrate by lipase in the presence of apoC-II was not due to changes in surface pressure. At 25 mN/m the kinetics of hydrolysis after the initial lag period was linear both in the absence and in the presence of apoC-II (Figure 1). The addition of apoC-II to the subphase enhanced product formation and reduced the lag time. Furthermore, the rates of hydrolysis at 25 mN/m in either the absence or the presence of apoC-II

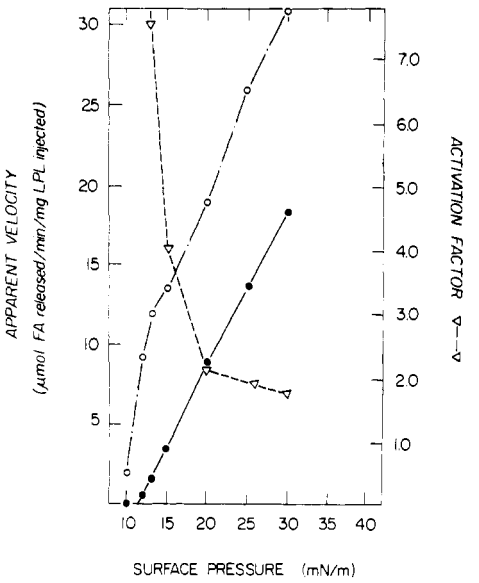

FIGURE 2: Effects of surface pressure and apoC-II on the kinetics of hydrolysis of 1,2-didecanoylglycerol monolayers by bovine milk lipoprotein lipase. The kinetics were performed as described for Figure 1. The reaction mixture contained lipoprotein lipase, 0.5 μg (●), or lipoprotein lipase, 0.5 μg, plus apoC-II, 50 μg (○). Activation factor (▽) = (V in presence of apoC-II)/(V in absence of apoC-II).

were greater than that at 15 mN/m.

Figure 2 shows the variation in the apparent rate of hydrolysis at steady-state kinetics of 1,2-didecanoylglycerol vs. surface pressure. In the absence of apoC-II, the apparent reaction velocity was nearly zero at 10–11 mN/m. At surface pressures greater than 11 mN/m, the reaction rates increased linearly to the collapse pressure of the lipid. The addition of apoC-II increased the apparent reaction velocity at all surface pressures. At the lower surface pressures, the lipase was active in the presence of apoC-II; the activation factor was >10. At higher surface pressures the activation factor was ~2. The induction or lag time (τ) observed during pre-steady-state conditions was independent of surface pressure but did decrease when apoC-II was added to the subphase. With 1,2-didecanoylglycerol the lag time was 7.6 min in the absence and 4.2 min in the presence of apoC-II at 25 mN/m.

One of the limitations of the monolayer technique is that the amount of enzyme which is actually involved in hydrolysis of the substrate is unknown. To determine the amount of enzyme at the interface, we prepared ^{125}I-labeled lipoprotein lipase; the labeled enzyme had the same specific activity as the unlabeled enzyme and was activated to the same extent by apoC-II. Figure 3A shows the dependence of the specific activity of lipoprotein lipase on surface pressure. In these experiments, hydrolysis was allowed to continue to steady-state kinetics; the film was then collected, and the interfacial excess of lipase was determined by radioactive counting. As seen in Figure 3A, there is a continuously increasing specific activity between 15 mN/m and the collapse pressure of the lipid without any optimal value in activity. The addition of apoC-II (50 μg) to the subphase enhanced the specific activity of the lipase to the same extent over all surface pressures investigated.

With ^{125}I-labeled lipoprotein lipase it was also possible to determine the amount of enzyme per unit area at the interface. As shown in Figure 3B, the amount of lipase in the monolayer decreased with increasing surface pressure. Thus, although the amount of enzyme at the interface is less at higher pressures, the specific activity is greater. The addition of apoC-II to the subphase caused even less enzyme to be present at the interface with a corresponding increase in specific activity.

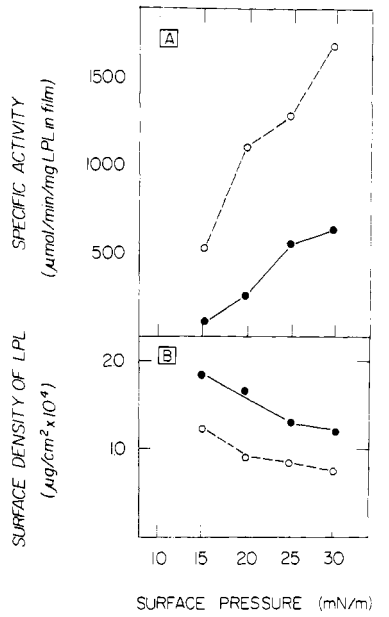

FIGURE 3: Effects of surface pressure and apoC-II on the specific activity (A) and surface density (B) of bovine milk lipoprotein lipase. Reaction rates were determined by using monolayers of 1,2-didecanoylglycerol. The reaction mixture contained ^{125}I-labeled lipoprotein lipase (0.5 μg, 7.2 × 10^4 cpm/μg) (●) or ^{125}I-labeled lipoprotein lipase plus apoC-II (50 μg) (○). The specific activity of lipoprotein lipase was calculated from the measured rate constant and the amount of lipoprotein lipase in the monolayer. The surface density of lipoprotein lipase was calculated from the amount of enzyme recovered from the film and the dimensions of the reaction compartment (91 cm^2).

Effects of ApoC-II on Lipase Activity. The effects of apoC-II on the apparent velocity and lag time of lipoprotein lipase using 1,2-didecanoylglycerol at 25 mN/m are shown in Figure 4A. In these experiments apoC-II was first injected underneath a monolayer of substrate at 25 mN/m. After 5 min, lipoprotein lipase (0.5 μg) was added to the subphase and the apparent velocity was determined at steady-state kinetics. As seen in Figure 4A, the apparent velocity increased with increasing amounts of apoC-II added to the subphase; the velocity was optimal with 40 μg of apoC-II. In addition, the lag time decreased from 8 min with no apoC-II to 3 min with 40 μg of apoC-II. To determine the surface density of apoC-II, we prepared ^{125}I-labeled apoprotein. As shown in Figure 4A, [^{125}I]apoC-II activated lipoprotein lipase to the same extent as unlabeled apoC-II.

In the next experiment, the apparent velocity vs. the amount of apoC-II at the interface was determined. In these experiments, increasing amounts of [^{125}I]apoC-II were added underneath a monolayer of 1,2-didecanoylglycerol at either 25 or 13 mN/m. After 5 min lipoprotein lipase was added to the subphase. After the kinetics of hydrolysis reached steady-state conditions, the film was collected and the surface density was calculated from the amount of [^{125}I]apoC-II in the film and the dimensions of the reaction compartment. Figure 4B shows a plot of the apparent velocities as a function of the amount of apoC-II at the interface.

Effects of Other Proteins on Hydrolysis Rates. In Table I are shown the effects on the kinetics of lipid hydrolysis by three other apolipoproteins (apoC-III, apoA-I, and apoE) and two nonlipoprotein proteins (BSA and porcine pancreatic colipase). At a surface pressure of 15 mN/m, the addition of each apoprotein or BSA to the subphase made the kinetics more linear and increased the rate of hydrolysis without changing the lag time; colipase had no effect on these parameters. Although the apparent reaction velocity was 1.5-fold

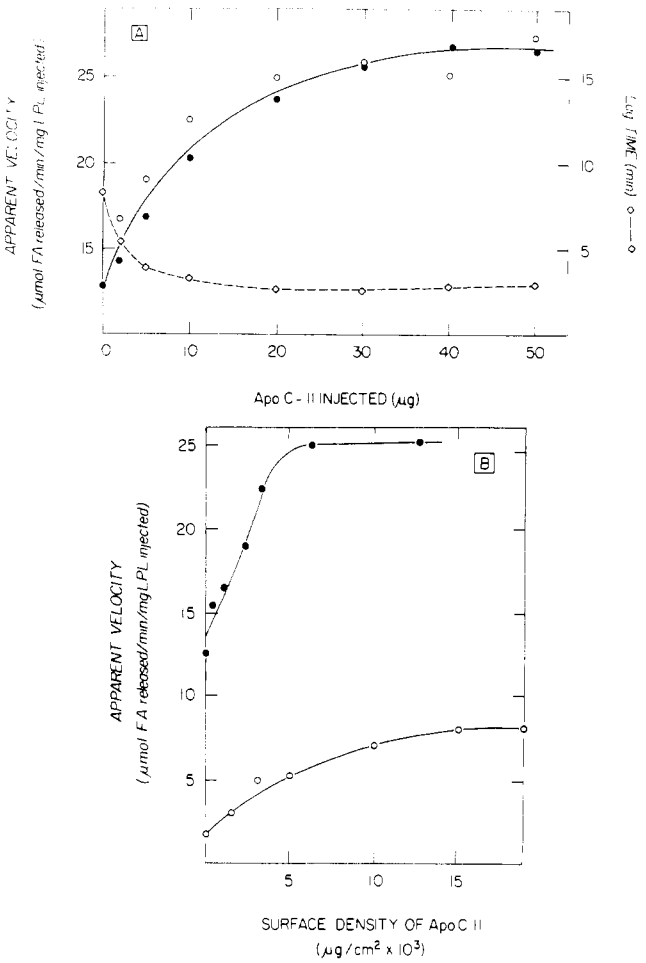

FIGURE 4: (A) Effects of apoC-II concentration on the apparent velocity and lag time of bovine milk lipoprotein lipase. The kinetics were determined by using monolayers of 1,2-didecanoylglycerol at 25 mN/m. The reaction mixtures contained lipoprotein lipase (0.5 μg) and the indicated amounts of apoC-II (●) or ^{125}I-labeled apoC-II (○) (0.43 mol/mol of apoC-II, 2.09 × 10^4 cpm/nmol of apoC-II). The lag times were determined by extrapolation of the maximal slope of the kinetic curve to the time axis (◇). (B) Apparent velocity of bovine milk lipoprotein lipase as a function of the surface density of apoC-II. The monolayer consisted of 1,2-didecanoylglycerol at 13 (○) or 25 mN/m (●). The reaction mixture contained bovine milk lipoprotein lipase, 0.5 μg, and the indicated amount of ^{125}I-labeled apoC-II (0.43 mol/mol of apoC-II, 2.09 × 10^4 cpm/nmol of apoC-II). The film was collected after the kinetics of hydrolysis was in steady state. The surface densities were calculated from the amount of ^{125}I-labeled apoC-II in the film and the dimensions of the reaction compartment (91 cm²) and were plotted against the apparent velocities.

greater than that observed with lipase alone, apoC-III, apoA-I, apoE, and BSA were not as effective as apoC-II in enhancing lipase activity or decreasing the lag time. At 25 mN/m each of the apoproteins, BSA, and colipase had no effect on the kinetics of hydrolysis; at this pressure the addition of apoC-II gave a 1.6-fold increase in activity. At 15 mN/m, apoproteins and BSA accumulate at the interface[2] while colipase is not tensioactive at this pressure (Verger et al., 1977).

Effects of NaCl and Heparin on Hydrolysis Rates. The effect of NaCl on the apoC-II-stimulated lipase activity was of interest since inhibition by 1.0 M salt has been considered characteristic for lipoprotein lipase. However, NaCl (1 M) had little effect on enzyme activity with 1,2-didecanoylglycerol when assayed at 25 mN/m; the relative activity compared to 0.1 M salt was 0.8 in either the absence or the presence of apoC-II.

[2] R. L. Jackson, F. Pattus, and R. A. Demel (unpublished experiments); R. Verger (personal communication).

Table I: Kinetics of Lipoprotein Lipase Hydrolysis of 1,2-Didecanoylglycerol Monolayers

addn[a]	lag time (min)[b] 15 mN/m	25 mN/m	act.[c] 15 mN/m	25 mN/m
none	8.1	7.6	3.5 (1.0)	12.2 (1.0)
apoC-II	4.2	4.2	12.2 (3.5)	20.2 (1.6)
apoC-III	6.8	6.5	5.9 (1.7)	12.9 (1.0)
apoA-I	10.6	7.0	5.2 (1.5)	13.5 (1.1)
apoE	7.2	7.6	5.2 (1.5)	13.5 (1.1)
BSA	6.5	7.0	5.5 (1.6)	13.9 (1.1)
colipase	10.0	8.3	3.5 (1.0)	12.5 (1.0)
heparin	7.5	7.6	4.5 (1.3)	17.0 (1.4)

[a] Kinetics were performed as described under Materials and Methods in a zero-order trough containing a reaction compartment of 91 cm² (210 mL). After the film of lipid was formed at either 15 or 25 mN/m, the various proteins and heparin (50 μg each) were added underneath the monolayer. After 10 min, lipoprotein lipase (0.5 μg) was added and the kinetics were recorded. [b] Lag times were determined by extrapolation of the steady-state kinetics. [c] Activity is expressed as micromoles of free fatty acid released per minute per milligram of lipase injected into the subphase. The numbers in parentheses are the relative activities.

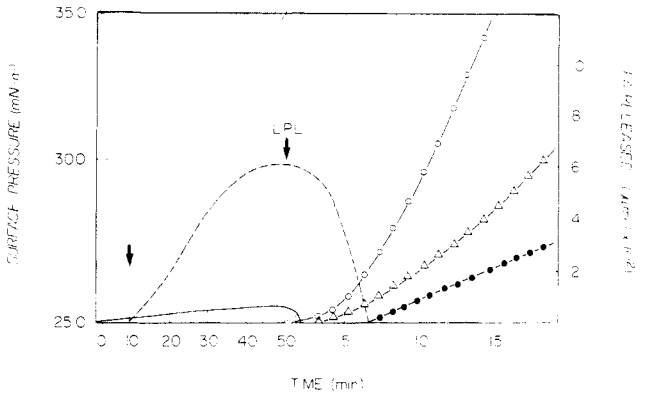

FIGURE 5: Effects of apoproteins on surface pressure of a monolayer of 1,2-didecanoylglycerol and on the kinetics of lipid hydrolysis by lipoprotein lipase (LpL) at 25 mN/m. The experiments were performed at 25 °C in a 210-mL zero-order trough as described under Materials and Methods. In experiment 1 (—; ○), apoC-II (50 μg) was injected underneath a film of lipid at an initial pressure of 25 mN/m. After 50 min there was an increase in surface pressure to 25.5 mN/m. At 50 min LpL was injected. When the pressure reached 25 mN/m, it was held constant at 25 mN/m and the kinetics of lipid hydrolysis recorded (○). The curve △ represents the kinetics of lipid hydrolysis for lipase alone at 25 mN/m. In experiment 2 (---; ●), apoC-II (50 μg) was injected and then 10 min later (↓) apoC-III (50 μg) was injected. At 50 min LpL was added. After the surface pressure reached 25 mN/m, the kinetics of hydrolysis was again recorded at a constant pressure of 25 mN/m.

The addition of heparin to the subphase of a monolayer trough containing 1,2-didecanoylglycerol increased lipoprotein lipase activity at both 15 and 25 mN/m (Table I). However, heparin had no effect on the lag time of lipase action at either 15 or 25 mN/m.

Effects of Apoproteins on the Kinetics of Activation of ApoC-II. In Figure 5 the effect of apoC-III on the activation of lipoprotein lipase by apoC-II is shown. In these experiments, apoC-II (50 μg) was added to the subphase of a trough containing a monolayer of 1,2-didecanoylglycerol at 25 mN/m. The surface pressure was then monitored as shown by the *solid line* (Figure 5); the surface pressure increased 0.5 mN/m to a constant value of 25.5 mN/m. After 50 min, lipoprotein lipase (0.5 μg) was added. When the pressure decreased to 25 mN/m, the pressure was maintained constant at 25 mN/m

and the kinetics of lipid hydrolysis was recorded (open circles, Figure 5). The curve obtained when lipase alone was added at zero time is shown for comparison (Figure 5, triangles). In the next experiment, apoC-III (50 μg) was added to the trough 10 min after the addition of apoC-II (50 μg). As shown in Figure 5, the addition of apoC-III caused a marked increase in surface pressure to ~30.2 mN/m (broken line). After constant pressure was maintained, lipase (0.5 μg) was added to the trough. After 2 min, there was a precipitous drop in surface pressure due to hydrolysis of the lipid. When the surface pressure reached 25 mN/m, the pressure was again maintained constant and the kinetics of hydrolysis recorded. Because of the surface pressure changes after the addition of lipase, it was not possible to measure the kinetics of hydrolysis during this period; viz., activity is dependent on surface pressure. However, after the surface pressure reached 25 mN/m and the pressure remained constant, the kinetics of hydrolysis was remarkably different from that found for lipase plus apoC-II; the rate of hydrolysis was linear but interestingly was considerably less than that with lipase alone or with apoC-II (50 μg). The effects observed by the addition of apoC-III (50 μg) to apoC-II (50 μg) were also found for apoA-I (50 μg) or apoE (50 μg); BSA (50 μg) did not inhibit the activation of lipase by apoC-II when the surface pressure was 25 mN/m. The latter finding is consistent with the fact that BSA is only tensioactive at pressures less than 18 mN/m. From these results, we conclude that the inhibition of apoC-II-stimulated bovine milk lipoprotein lipase activity by other apolipoproteins is not specific. In fact, when 100 μg of apoC-II (vs. 50 μg) was added to the subphase, it too was inhibitory.

Discussion

A constant pressure monolayer technique as first applied by Dervichian (1971) was used to study the effects of surface pressure and of various apolipoproteins on the kinetics of hydrolysis of lipid by milk lipoprotein lipase. With 1,2-didecanoylglycerol as substrate, the apparent enzyme velocity increased continuously at surface pressures greater than 10 mN/m with no optimum reaction rate. The lack of any surface pressure optimum has also been reported for other lipolytic enzymes (Verger et al., 1976; Miller & Ruysschaert, 1971; Brockman et al., 1976). An interesting finding in the present study was that the amount of enzyme at the interface decreased at higher surface pressures, thus giving greater specific activities. Verger et al. (1976) and Pattus et al. (1979) also found for pancreatic phospholipase A_2 that the amount of enzyme present at the interface decreased with increasing surface pressure. The present results and other recent studies (Barque & Dervichian, 1979; Dervichian & Barque, 1979) show that the quantity of enzyme at the interface and its specific activity are strongly dependent on the surface pressure of the substrate monolayer. At a surface pressure of 10 mN/m, no substrate was hydrolyzed. The absence of activity at this pressure cannot be explained on the basis of the lack of penetration of the lipase to the interface (Figure 3). In addition, using a three-compartment trough (Rietsch et al., 1977) we were able to show that ^{125}I-labeled lipoprotein lipase, which collected at the interface at 10 mN/m, remained inactive even when transferred to a lipid film maintained at 20 mN/m. Although further experiments are needed to fully understand the inactivation of lipoprotein lipase at low surface pressures, we suggest that the enzyme undergoes surface denaturation which causes irreversible adsorption of the enzyme at the interface. All of the proteins used in the present study which were tensioactive at 11 mN/m protected the enzyme against inactivation at low surface pressures. The denaturation of lipolytic enzymes caused by interfacial forces has been clearly demonstrated by Rietsch et al. (1977) in the same monolayer system. Momsen & Brockman (1976) have shown that colipase and taurodeoxylate protect against surface inactivation of pancreatic lipase when added to glass beads covered with tripropionin. Brockerhoff (1971) also reported that bile salts or albumin prevents irreversible interfaces.

It is well established that apoC-II is an activator of lipoprotein lipase. However, with a short-chained diglyceride apoC-II was not an absolute requirement for hydrolysis. At high surface pressures apoC-II produced a twofold increase in the rate of hydrolysis. This degree of enhancement is similar to that found by Chung & Scanu (1977), also using trioctanoyl monolayers. The activation is, however, significantly less than that found when the triglyceride is in emulsion form; rat heart lipoprotein lipase is activated 30-fold with trioctanoylglycerol emulsions (Chung & Scanu, 1977) as substrate. ApoC-II not only increased the apparent velocity of the lipase but also caused a reduction in the lag time (τ). The stoichiometry of the complex formed between apoC-II and the lipase was determined by the concentration of apoC-II required to produce a maximum stimulation of activity. As shown in Figure 4, at 25 mN/m, 40 μg of apoC-II was required to maximally activate 0.5 μg of lipoprotein lipase. These concentrations correspond to a molar ratio of 1:200 (lipase/apoC-II) and represent very weak interaction. This result is clearly different from that reported by Chung & Scanu (1977), who found that apoC-II activates lipoprotein lipase in a 1:1 molar ratio. An exact comparison of these results is not possible since the latter authors used rat heart lipoprotein lipase. In addition, surface pressure was not maintained constant in the Chung & Scanu (1977) study. Miller & Smith (1973) showed that there was interaction between milk lipoprotein lipase and apoC-II in a monolayer system. However, the stoichiometry of this complex was not determined.

The stimulatory effect of apoC-II at low surface pressures was not specific for apoC-II; all tensioactive proteins activated. However, colipase, which is not tensioactive at 15 mN/m (Verger et al., 1976), had no effect. The stimulatory effects of low concentrations of apoC-I and apoC-III on lipase activity with lipid emulsions as the substrates have also been reported by a number of investigators (LaRosa et al., 1970; Havel et al., 1970, 1973). One possible mechanism which might account for this apparent stimulation of lipase activity at low surface pressure is that any tensioactive protein protects the enzyme from surface denaturation at the lipid interface. At a surface pressure of 25 mN/m and at concentrations of apoprotein of <0.25 μg/mL, only apoC-II showed a stimulatory effect. At 0.5 μg/mL, apoC-II inhibited the enzyme. A similar finding has been reported by Östlund & Iverius (1975) using a triglyceride–phospholipid emulsion as substrate and milk lipoprotein lipase. Addition of apoC-III, apoA-I, or apoE to a final concentration of 0.25 μg/mL to a trough containing apoC-II (0.25 μg/mL) also inhibited the lipase. Based on the data shown in Figure 5, we suggest that all of the apoproteins at high concentrations inhibited lipoprotein lipase by changing the surface density of the lipid. Whether this alteration results in a change in the quantity or specific activity of the lipase at the interface or in the ability of apoC-II to interact with the enzyme is unknown.

In the usual assay systems for milk lipoprotein lipase, the enzyme is inhibited by NaCl at concentrations above 0.5 M (Fielding & Fielding, 1976; Bengtsson & Olivecrona, 1977). However, in the monolayer system with 1,2-didecanoylglycerol, a short-chain diglyceride, as substrate, NaCl had virtually no

effect. Rapp & Olivecrona (1978) have also shown that NaCl had no effect on the hydrolysis of tributyrin. Addition of increasing amounts of heparin to the reaction trough resulted in an increase of lipase activity at both 15 and 25 mN/m. However, heparin did not change the pre-steady-state kinetics of the reaction.

In summary, our results show that lipoprotein lipase is surface active and penetrates a surface monolayer of diglyceride in the absence of apoC-II. In the presence of apoC-II there is both a decrease in the pre-steady-state kinetics and a specific activation. The mechanism by which apoC-II increases the specific activity of the enzyme and the specific region of apoC-II which is responsible for this activation are currently under study.

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