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Action of Lipoprotein Lipase on Phospholipid Monolayers. Activation by Apolipoprotein C-II†

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ABSTRACT: Action of lipoprotein lipase and its activation by apolipoprotein C-II (apoC-II) were studied with monomolecular films of 1,2-didodecanoyl-*sn*-glycero-3-phosphoglycerol as a substrate. The enzyme velocity and the specific activity of the interface-bound enzyme show a bell-shaped curve as a function of lipid packing, both in the presence and absence of apoC-II. Above critical surface pressure of 20 dyn cm^{-1}, lipoprotein lipase alone is no longer able to hydrolyze a monolayer of 1,2-didodecanoyl-*sn*-glycero-3-phosphoglycerol. However, lipoprotein lipase readily penetrates into the phospholipid interface up to surface pressures exceeding 40 dyn cm^{-1}, without any effect by apoC-II. Activation of lipoprotein lipase by apoC-II can be assigned to be due to two specific effects. Below the critical surface pressure of 20 dyn cm^{-1}, apoC-II merely increases the turnover number of lipoprotein lipase 4-fold. The minimal sequence region required to produce this effect is contained in the carboxyl-terminal residues 55–78 of the activator, as determined with synthetic peptide fragments apoC-II(43–78), -(50–78), -(55–78), -(60–78), and -(66–78). Above the surface pressure of 20 dyn cm^{-1}, apoC-II activates in an all-or-none manner a noncatalytic enzyme already bound to the substrate interface, causing the critical surface pressure to increase from 20 to 25 dyn cm^{-1}. The presence of the phospholipid-associating residues 43–50 in the carboxyl-terminal synthetic activator peptide is mandatory for the latter effect. The main conclusions are that the expression of the catalytic activity of lipoprotein lipase can be regulated by the physical state of the substrate interface and that apoC-II can affect this regulation.

Lipoprotein lipase (EC 3.1.1.34) is a triacylglycerol hydrolase located at the capillary endothelium in peripheral tissues such as heart, muscle, and adipose tissue [for reviews, see Smith et al. (1978) and Kinnunen et al. (1983)]. Its action appears to be the rate-limiting step in the removal of circulating plasma triacylglycerol transported in chylomicrons and very low density lipoproteins (Garfinkel et al., 1967; Kompiang et al., 1976; Bensadoun & Kompiang, 1979). For maximal activity lipoprotein lipase requires the presence of a small protein cofactor, apolipoprotein C-II, which is a component of the surface film of substrate lipoproteins, chylomicrons and VLDL[1] (La Rosa et al., 1970; Havel et al., 1973). Direct protein–protein interaction between lipoprotein lipase and apoC-II occurring at 1:1 stoichiometry has been established (Smith et al., 1982; Miller & Smith, 1973; Chung & Scanu, 1977; Fielding & Fielding, 1977). Extensive studies with native and synthetic peptide fragments have identified functionally distinct regions in the activator (Kinnunen et al., 1977; Musliner et al., 1977; Catapano et al., 1979; Smith et al., 1980). We have recently proposed the apoC-II-induced in-

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[1] Abbreviations: VLDL, very low density lipoprotein; apoC-II, apolipoprotein C-II; $diC_{12}PG$, 1,2-didodecanoyl-*sn*-glycero-3-phosphoglycerol; π, surface pressure; v, enzyme velocity; Tris, tris(hydroxymethyl)aminomethane.

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crease in the catalytic rate constant of lipoprotein lipase to be due to apoC-II functioning as a specific acyl-enzyme hydrolase (Vainio et al., 1982, 1983; Kinnunen et al., 1982, 1983).

The lipoprotein lipase reaction takes place at the monomolecular phospholipid surface film of plasma lipoproteins, which appears to contain small amounts (2–5 mol %) of triacylglycerol available for transport and metabolism (Miller & Small, 1980; Hamilton & Small, 1981; Pieroni & Verger, 1979). Generation of polar reaction products by lipoprotein lipase at the site of the reaction changes the quality of the substrate interface, which may in turn regulate the action of the enzyme. In the present study, the monolayer technique was used to provide a model system where the quality of the interface can be controlled (Verger & de Haas, 1973; Verger & Pattus, 1982). Monomolecular films of phosphatidylglycerol bear negative charges on the face in contact with water, plausibly mimicking the more complicated situation of the presence of polar lipolytic products at the phospholipid surface film of lipoproteins. Since $diC_{12}PG$ monolayers in addition proved to be excellent phospholipid substrates for lipoprotein lipase, they were used as model membranes.

The aim of this paper is to describe how the physical state of the interface regulates lipoprotein lipase action and its enhancement by apoC-II. Synthetic peptide fragments of the activator were used to identify in the primary sequence the regions responsible for the activating effect under different interfacial conditions. On the basis of this experimental approach, a novel concept is developed for the lipoprotein lipase activation by apoC-II.

Materials and Methods

Proteins. Lipoprotein lipase was purified from bovine milk (Kinnunen, 1977) and iodinated with sodium [^{125}I]iodide (14.9 mCi/μg of iodine, Amersham International) as described previously (Slotboom et al., 1978; Jackson et al., 1980). The enzyme was of the same quality as in a previous study (Vainio et al., 1982). The iodinated derivative contained 0.48 molecule of iodide per lipase molecule and had the same specific activity as the unlabeled enzyme. Apolipoprotein C-II was isolated from human very low density lipoprotein as reported (Brown et al., 1970; Shore & Shore, 1969). The peptides apoC-II-(43–78), -(50–78), -(55–78), -(60–78), and -(66–78) were prepared by solid-phase synthesis (Kinnunen et al., 1977; Edelstein et al., 1981). Peptides were isolated by reversed-phase chromatography (Hancock et al., 1981). Protein was determined by automatic amino acid analysis with a Biotronic LC 7000 analyzer.

Lipids. 1,2-Didodecanoyl-*sn*-glycero-3-phosphoglycerol was synthesized by Dr. J. F. Tocanne (Centre de Recherche de Biochimie et de Génétique Cellulaire, Toulouse, France). Egg yolk phosphatidylcholine was from Sigma and further purified by silicic acid column chromatography.

Monolayer Experiments. The surface barostat method was used to determine the rate of hydrolysis of lipid monolayers at a constant surface pressure (Verger & de Haas, 1973, 1976). The techniques for film recovery and radioactivity counting were as used earlier (Rietsch et al., 1977). The key-type zero-order trough (Lairon et al., 1980) that was used to follow enzyme kinetics consisted of a reaction compartment (total volume 31 mL with a total surface of 45.5 cm^2) and a reservoir (2 × 20 cm). Standard buffer used throughout the study was of the following composition: 20 mM Tris-HCl, pH 7.4, 0.2 M NaCl, and 10 mM $CaCl_2$. Deionized water was distilled from alkaline sodium permanganate in an all-glass apparatus. The aqueous subphase of the reaction compartment was thermostated to 25 ± 0.5 °C with an immersed glass coil and agitated with a magnetic stirrer turning at 250 rpm. Before each experiment, the Teflon trough was carefully cleaned with ethanol and rinsed thoroughly with tap water and finally with distilled water. Reactions were started with the injection of the indicated amount of lipoprotein lipase into the subphase of the reaction compartment. When apoC-II or any of the synthetic peptides were used, they were injected into the reaction compartment 2 min before the addition of the enzyme. The penetration experiment was carried out in small cylindrical Teflon troughs (total volume 17 mL, total surface 12.6 cm^2). A detailed description of the equipment and technique for the film-transfer experiment has been published by Rietsch et al. (1977).

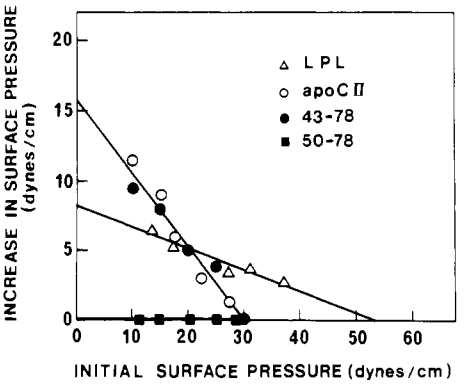

FIGURE 1: Penetration of a phospholipid monolayer by lipoprotein lipase and apoC-II. The observed increase in surface pressure when a protein is injected beneath a monolayer of egg yolk phosphatidylcholine is given as a function of the initial surface tension. Experiments were done in small cylindrical Teflon troughs (total volume 17 mL, total surface 12.6 cm^2). One microgram of lipoprotein lipase (LPL), apoC-II(43–78), and apoC-II(50–78) or 2 μg of apoC-II was injected into the aqueous subphase, and the increase in surface pressure was recorded for 15 min.

Results

Lipoprotein Lipase and ApoC-II Penetration of a Phosphatidylcholine Monolayer. Penetration of a protein molecule into a lipid–water interface is reflected as an increase in the surface pressure of the lipid monolayer (Macritchie, 1978). We first determined the ability of lipoprotein lipase and apoC-II to penetrate into a phospholipid monolayer. The maximum increase of surface pressure was determined as a function of initial surface pressure (Figure 1). The critical pressure for penetration may thus be defined by extrapolation to the initial surface pressure above which there is no increase in surface pressure. Lipoprotein lipase was found to be an extremely tensioactive protein. It could readily bind to an egg yolk phosphatidylcholine monolayer at surface pressure as high as 40 dyn cm^{-1}. Critical pressure for penetration was estimated to be above 50 dyn cm^{-1} (Figure 1).[2] These surface-pressure increases were observed at considerable low protein concentrations, comparable to those of highly tensioactive cardiotoxins (Bougis et al., 1981). In contrast to this, apoC-II was able to penetrate into the monolayer only up to a critical pressure of 30 dyn cm^{-1} (Figure 1). Identical behavior was observed with synthetic fragment apoC-II(43–78), but the depletion of seven amino acid residues to produce the fragment apoC-II-

[2] No evidence of either short-chain didodecanoylphosphatidylcholine or long-chain egg yolk phosphatidylcholine hydrolysis by lipoprotein lipase was observed, with use of up to 20 times higher concentration of lipoprotein lipase and apoC-II than normally used by us with phosphatidylglycerol monolayers.

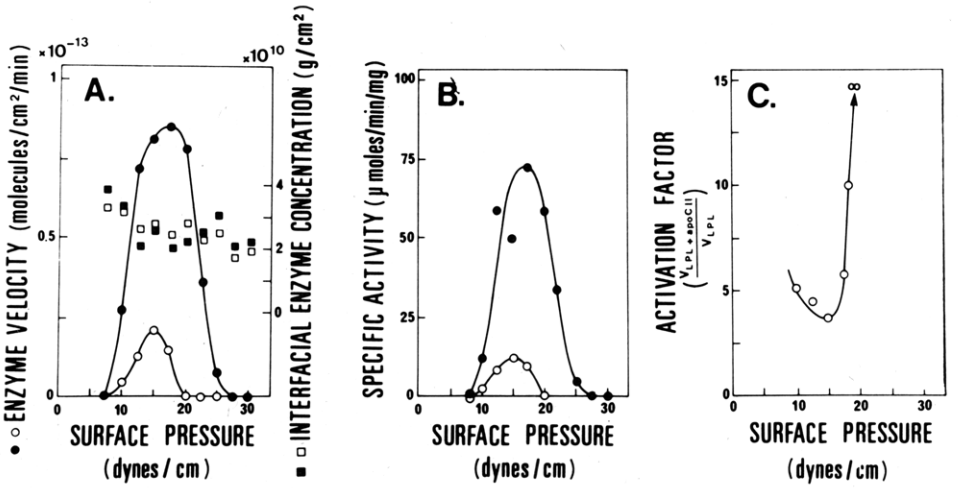

FIGURE 2: Hydrolysis of monolayers of 1,2-didodecanoyl-*sn*-glycero-3-phosphoglycerol by ^{125}I-labeled lipoprotein lipase. (A) Rate of hydrolysis of diC_{12}PG monolayers by ^{125}I-labeled lipoprotein lipase (0.55 μg) as a function of surface pressure: (○) no apoC-II added; (●) 1 μg of apoC-II injected 2 min before the addition of the enzyme. Interfacial enzyme concentration in the presence (■) and absence (□) of apoC-II. (B) Specific activity of lipoprotein lipase as a function of surface pressure in the presence of (●) and absence (○) of apoC-II. (C) Activation of lipoprotein lipase by apoC-II as a function of surface pressure. Activation factor is defined as the ratio of the rate of hydrolysis of diC_{12}PG in the presence and absence of 1 μg of apoC-II. Values above 20 dyn cm^{-1} are infinite.

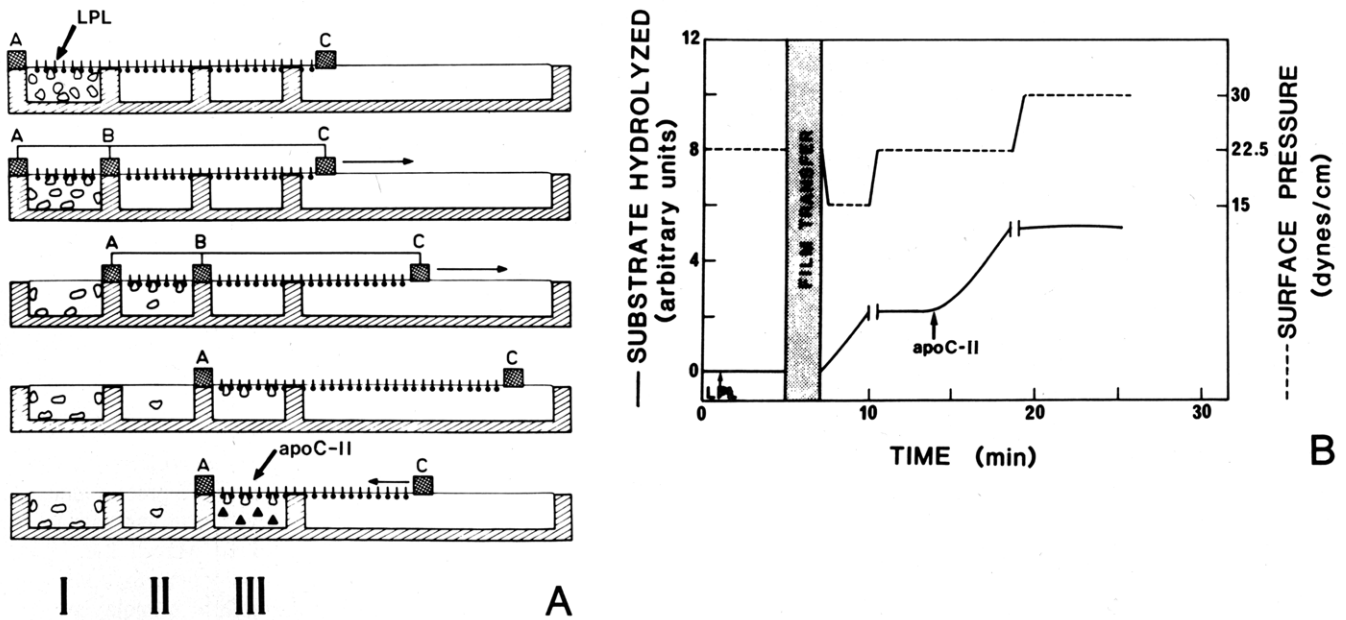

FIGURE 3: (A) Schematic illustration of technique for film transfer [see Rietsch et al. (1977) for further technical details]. Roman numerals are used for the numbering of the compartments. The small crosshatched squares A, B, and C represent the barriers. Barrier C is mobile and controlled by the surface balance when the reaction is recorded. The corresponding kinetic recording is reproduced in panel B. *The top section of the scheme* is related to recording the reaction after addition of the enzyme to reaction compartment I (arrow). *The three sections in the middle* illustrate the film transfer. The portion of the film situated over compartment I is separated by barrier B, and the three barriers A, B, and C are linked and translated together to the right. The film is rinsed over compartment II and further translated to reaction compartment III where barrier B is removed. *The bottom section of the scheme* corresponds to recording the reaction after the film transfer, including the addition of the cofactor to compartment III (arrow). (B) Catalytic and noncatalytic binding of lipoprotein lipase to a monolayer of 1,2-didodecanoyl-*sn*-glycero-3-phosphoglycerol. Effect of surface pressure and apoC-II. A detailed description of the experiment is given under Results; see also panel A for a corresponding schematic illustration of the technique. At the end of the experiment, the surface film above reaction compartment III was collected, and samples from the aqueous subphases of all three reaction compartments were taken for determination of the amount of ^{125}I-labeled lipoprotein lipase. 64, 2.5, and 0% of the original radioactivity were recovered in the aqueous phases of reaction compartments I, II, and III, respectively. 1.7% of the ^{125}I-labeled lipoprotein lipase injected into the subphase of compartment I was recovered in the surface film of reaction compartment III. As determined in control experiments, 2.1% of ^{125}I-labeled lipoprotein lipase adsorbed to the interface when injected under a diC_{12}PG monolayer spread under a surface pressure of 22.5 dyn cm^{-1}. Thus it can be estimated that approximately 81% of the enzyme adsorbed to the surface film above reaction compartment I was transferred with the film to reaction compartment III. The specific activity of the enzyme before and after transfer was the same (data not shown). All values given are means of duplicate determinations.

(50–78) resulted in a complete loss of the ability of the peptide to penetrate into the interface, thus confirming that the α-helical segment between residues 43–50 contains the essential determinants for the lipid association of apoC-II (Sparrow & Gotto, 1978; Catapano et al., 1979).

Hydrolysis of Monomolecular Films of 1,2-Didodecanoyl-sn-glycero-3-phosphoglycerol by Lipoprotein Lipase. We measured the enzymatic hydrolysis of diC_{12}PG monolayers by lipoprotein lipase at different constant surface pressures with the surface barostat method and simultaneously determined the amount of ^{125}I-labeled lipoprotein lipase adsorbed to the interface. Lipoprotein lipase penetrated into interface up to a surface pressure exceeding 30 dyn cm^{-1} (Figure 2A). The interfacial enzyme concentration remained essentially

constant irrespective of lipid packing (i.e., lipid density per area). ApoC-II had no effect on the binding of the enzyme to the interface. The enzyme velocity and the specific activity showed a bell-shaped curve as a function of lipid packing (Figure 2A,B). Above a critical surface pressure of 20 dyn cm^{-1}, lipoprotein lipase alone was no longer able to hydrolyze a $diC_{12}PG$ monolayer although it was still able to adsorb to it.

Activation of lipoprotein lipase by apoC-II appeared to be due to two specific effects. Below the critical surface pressure of 20 dyn cm^{-1}, apoC-II merely increased the turnover number of lipoprotein lipase reflected as an equivalent increase in enzyme velocity (Figure 2). Above this critical surface pressure, apoC-II appeared to activate in an all-or-none manner a noncatalytic enzyme already bound to the interface. Accordingly, the values for the activation factor are infinite above 20 dyn cm^{-1} (Figure 2C).[3]

In order to verify the existence of the latter phenomenon, we performed the film-transfer experiment in a specially designed four-compartment trough, as illustrated in Figure 3 [see Rietsch et al. (1977) for further details]. A monolayer of $diC_{12}PG$ was spread to an initial surface pressure of 22.5 dyn cm^{-1}, i.e., above the critical packing density of lipoprotein lipase action. ^{125}I-Labeled lipoprotein lipase (3 μg) was injected into the stirred subphase of reaction compartment I (arrow). No activity was observed. After 5 min, the film above compartment I was transferred to compartment II for rinsing and then further to compartment III. During the procedure surface pressure was kept constant at 22.5 dyn cm^{-1}. No activity was observed after the transfer. Surface pressure was then rapidly decreased to 15 dyn cm^{-1} by relaxing the film. Enzymatic hydrolysis of the monolayer started immediately without any observable lag period. After the enzyme velocity was recorded for 5 min, the surface pressure was raised back to the initial value of 22.5 dyn cm^{-1} by compression of the film. Consequently, enzyme action ceased but was restored by the addition of apoC-II (1 μg) to compartment III (arrow). A typical lag time of 2 min was observed, most likely reflecting penetration of apoC-II into the film. The specific activity of the enzyme after the transfer was the same as before (see legend to Figure 3B for details). After the second kinetic was recorded for 5 min, the surface pressure was further raised to 30 dyn cm^{-1}, which abolished enzyme action.

Activation of Lipoprotein Lipase by Synthetic Peptide Fragments of ApoC-II. Synthetic peptide fragments of apoC-II corresponding to the COOH-terminal residues 43–78, 55–78, 60–78, and 66–78 were utilized to localize in the primary sequence the regions responsible for the two aforementioned activating effects (Figure 4). At a surface pressure of 15 dyne cm^{-1} apoC-II(66–78) was unable to activate lipoprotein lipase (Figure 4A). ApoC-II(60–78) produced a 50% activation compared to native apoC-II, and the addition of five more residues to produce apoC-II(55–78) resulted in an activating ability similar to that of apoC-II. Addition of further residues to introduce the lipid binding segment to the amino terminus of the fragment did not increase the activation. These results confirm several earlier studies where residues 55–78 have been established to contain the minimal sequence region

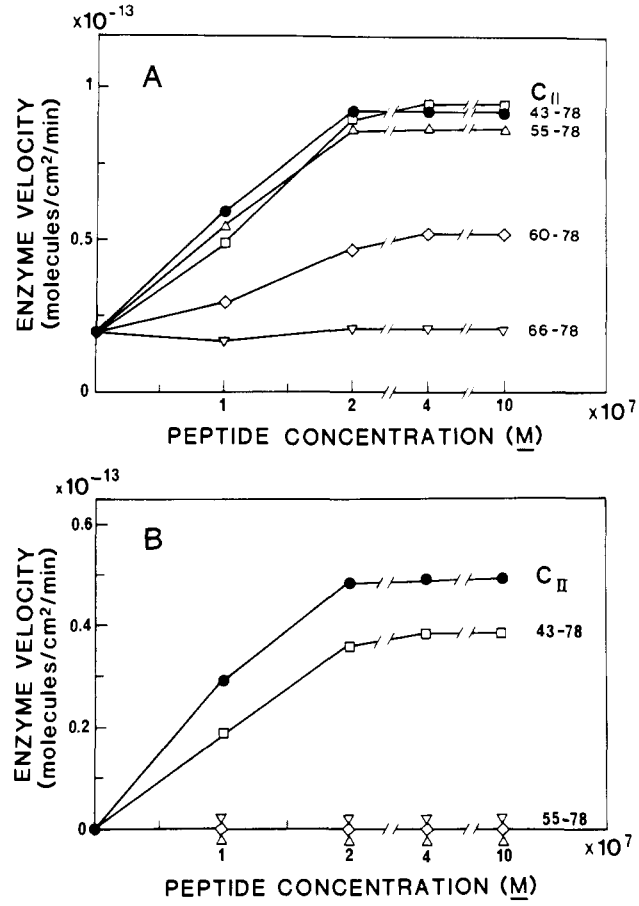

FIGURE 4: Activation of lipoprotein lipase by synthetic fragments of apoC-II. Experimental details are as in the legend to Figure 2. (A) Surface pressure 15 dyn cm^{-1}. (B) Surface pressure 22.5 dyn cm^{-1}. Peptides apoC-II(60–78) and -(66–78) did not activate lipoprotein lipase; the explanations of their symbols are not repeated on the face of the chart.

of apoC-II required to produce maximal enhancement of lipoprotein lipase activity (Kinnunen et al., 1977; Catapano et al., 1979; Smith et al., 1980). In contrast to this, the inclusion of the phospholipid binding region between residues 43 and 50 of apoC-II was found to be an absolute requirement for the activation to occur at a high surface pressure of 22.5 dyn cm^{-1}, i.e., above the critical surface pressure of 20 dyn cm^{-1} for lipoprotein lipase action (Figure 4B). Thus only apoC-II(43–78) could activate lipoprotein lipase. ApoC-II(55–78) or any of the shorter peptides were without effect on the enzyme activity under these conditions. At both surface pressures, maximal activation was observed at an apoC-II concentration corresponding to 1 mol of apoC-II added per mol of lipoprotein lipase.

Discussion

Lipoprotein lipase was found to be a highly surface-active protein readily penetrating into a phospholipid monolayer up to high surface pressures exceeding 40 dyn cm^{-1} (Figures 1 and 2). Only small changes were observed in the adsorption of lipoprotein lipase to the interface as a function of surface pressure. Accordingly, the bell-shaped v vs. π profile could not be explained by variation in the amount of enzyme present at the surface film. Further, the results clearly demonstrate that apoC-II did not affect the interfacial enzyme concentration (Figure 2). Adsorption of lipoprotein lipase to the substrate interface thus appears to occur independently of apoC-II.[4]

[3] The apparent increase in the activation factor at low surface pressure below 10 dyn cm^{-1} (i.e., high interfacial energy) is due to protection of lipoprotein lipase by apoC-II from irreversible surface denaturation. It is observed with other added proteins, such as serum albumin and apolipoproteins, as well as with apoC-II, and can thus be considered as a relatively nonspecific phenomenon [data not shown, see Jackson et al. (1980)].

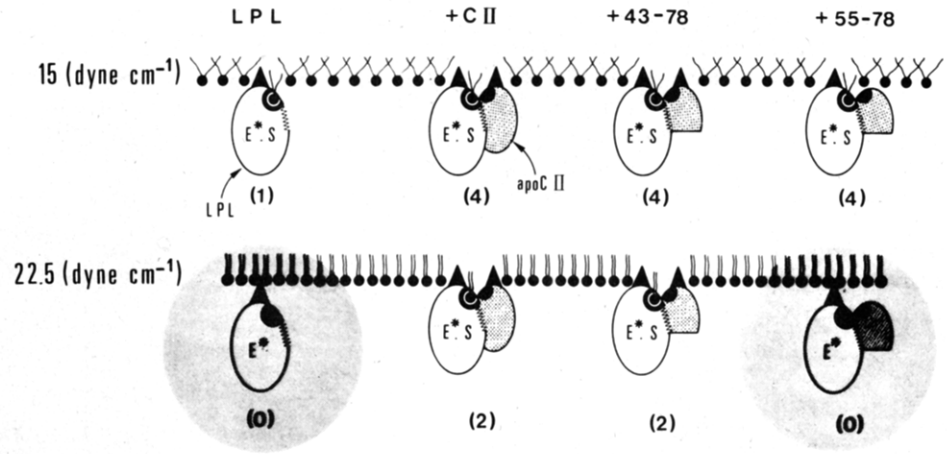

FIGURE 5: Mechanisms of activation of lipoprotein lipase by apoC-II: increased turnover and assisted penetration. See Discussion for details. (Upper panel) $DiC_{12}PG$ monolayer at a surface pressure of 15 dyn cm^{-1}; (lower panel) $diC_{12}PG$ monolayer at a surface pressure of 22.5 dyn cm^{-1}. Symbols for lipoprotein lipase and apoC-II are indicated with arrows. The black triangles illustrate the lipid binding regions in the protein molecules, black circles within the symbols represent the suggested catalytic sites, and the zig-zag line between the symbols depicts protein–protein interaction. In those insets where synthetic fragments of apoC-II are illustrated, the symbol for apoC-II is cut into half. E* and E*·S are symbols for the penetrated enzyme and penetrated enzyme–substrate complex, respectively, as originally defined by Verger et al. (1973). The numbers in parentheses are relative values for the catalytic activity of the complex. The special cases of a noncatalytic enzyme bound to the substrate interface are encircled with shaded background.

The observed bell-shaped specific activity vs. π profile (Figure 2) indicates that the catalytic site of lipoprotein lipase is adapted to perform its function at a certain optimal lipid density per area. The lack of enzyme activity above the critical surface pressure of 20 dyn cm^{-1} is evidently due to the presence of a noncatalytic enzyme at the interface, as demonstrated by the results of the film-transfer experiment (Figure 3). It clearly shows how the catalytic activity of lipoprotein lipase can be regulated by the physical state of the interface and how the protein cofactor can have an effect on this regulation.

Mechanisms of Activation of Lipoprotein Lipase by ApoC-II. Enhancement of lipoprotein lipase catalysis by apoC-II appeared to be due to two specific effects. Depending on the surface pressure, apoC-II either merely increased the turnover number of lipoprotein lipase or activated in an all-or-none manner an already interface-bound enzyme. The minimal sequence regions required to produce these effects were different (Figure 4).

In order to relate these two effects, we constructed a schematic model of the lipoprotein lipase activation by apoC-II, illustrated in Figure 5. At low lipid packing (Figure 5, upper panel), lipoprotein lipase readily penetrates into the phospholipid monolayer. The susceptible ester bond of the substrate is brought into close enough contact with the active site of the enzyme for the hydrolysis to occur. Lipid binding of apoC-II is not needed for the activating effect. Instead, protein–protein interaction between the carboxyl-terminal residues 62–74 of apoC-II and a complementary site in lipoprotein lipase produces correct spatial alignment of the enzyme and the cofactor at the interface (Smith et al., 1982), resulting in an increased catalytic rate constant of the enzyme.

When the lipid packing is increased above the critical surface pressure of 20 dyn cm^{-1} (Figure 5, lower panel), the catalytic site of lipoprotein lipase is prevented from reaching the substrate *sn*-1 ester bond, although the lipase is still able to adsorb to the interface. This special case of a noncatalytic enzyme bound to the substrate interface is encircled with shaded background in Figure 5. In the presence of either native apoC-II or the synthetic fragment apoC-II(43–78), which both contain the phospholipid binding region between residues 43 and 50, lipoprotein lipase assumes correct alignment with the substrate monolayer. The absence of the lipid-associating residues in the synthetic peptide apoC–II(55–78) makes it unable to stimulate lipoprotein lipase under these conditions.

We suggest that the activation of the noncatalytic enzyme at the interface takes place by assisted penetration of the enzyme by the cofactor. The alternatives that could explain the requirement for the presence of the phospholipid-associating residues in apoC-II would be that the activator either alters the lipid structure or changes the orientation of the enzyme at the interface. The activating effect saturates at an apoC-II concentration corresponding to 1 mol of apoC-II added per mol of lipoprotein lipase, which strongly suggests the effect to involve direct enzyme–activator interaction. Further, it is reasonable to assume that if apoC-II would change the lipid structure, the presence of the lipid binding region between residues 43 and 50 would affect lipoprotein lipase activation also at low lipid packing. In contrast to this, apoC-II(55–78) and apoC-II(43–78) produced equal activation at a surface pressure of 15 dyn cm^{-1}. An effect by apoC-II on lipoprotein lipase orientation cannot be excluded.

The two proposed activation mechanisms—increased turnover and assisted penetration—are by no means mutually exclusive. Under certain conditions, they may well be working simultaneously (Posner, 1980), the latter effect being a prerequisite for the first one to occur. The presence of two cooperative activation mechanisms of this kind should secure the catalytic efficiency of lipoprotein lipase under changing interfacial conditions during the hydrolysis of triacylglycerol-rich plasma lipoproteins.

[4] A distinctly different conclusion was reached when activation of a closely related enzyme, pancreatic lipase, was studied in a similar system (Verger et al., 1977). There it was shown that pancreatic lipase is able to penetrate into the interface only to a certain critical surface pressure, above which only the presence of the protein cofactor, colipase, resulted in the anchoring of the enzyme to the interface, thereby shifting the critical surface pressure toward higher values.

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Registry No. Lipoprotein lipase, 9004-02-8; $diC_{12}PG$, 80164-19-8.

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