The hydrolysis of ester substrates in mixed monolayers with 1-palmitoyl-2-oleoylphosphatidylcholine by pancreatic carboxylester lipase (CEL) shows an abrupt increase from <10 to 100% when substrate abundance exceeds a critical value (Tsujita, T., Muderhwa, J. M., and Brockman, H. L. (1989) J. Biol. Chem. 264, 8612–8618). Adsorption of native CEL to these mixed lipid films under the same conditions shows a monotonic increase with substrate abundance to a maximum of ~4 pmol/cm². In the range of low substrate hydrolysis, bound enzyme reaches ~0.6 pmol/cm² or about one-sixth of an enzyme monolayer. Given the high turnover number of the enzyme, absence of bound catalyst cannot explain the lack of substrate hydrolysis. Similarities of the adsorption data obtained with different substrates suggest a common, area-based mechanism with an excluded area for the phospholipid. Quantitative analysis confirms this and gives an excluded area of \(43.5 \pm 1.0 \, \text{Å}²/\text{molecule}\).

Comparable studies with the hydrolysis product, oleic acid, replacing each substrate show markedly different results. Up to oleic acid mol fractions of 0.5, bound CEL levels are essentially constant at 0.215 pmol/cm². This behavior relative to substrate-containing films cannot be ascribed to differences in surface pressure, surface potential, monolayer compressibility, nonexcluded area, CEL denaturation, or oleic acid charge. Together with the ultrasensitive composition dependence of substrate hydrolysis, the data suggest that phospholipid-based organization of the surface is a primary regulator of enzyme binding and catalysis.

Enzyme-catalyzed lipid hydrolysis is a classic model for heterogeneous biocatalysis (1, 2). For a water-soluble lipase to act on an insoluble lipid substrate, such catalysis must be preceded by the partitioning of the enzyme from the bulk aqueous phase to the surface phase (3). This adsorption, although it may not require substrate (4, 5), brings the enzyme and substrate into proximity, increasing the probability for their direct interaction. Because it occurs in the highly asymmetric environment of the lipid-water interface, adsorption is sensitive to the physical properties of the surface phase. Useful models for studying the surface dependence of such enzyme-surface interactions have been the carboxylester lipases (EC 3.1.1.13) (CELs) found in pancreatic juice and milk. This family of lipases shares little homology with other known lipase families but is more closely related to cholinesterases (6). Recent evidence (7) supports the suggestion (8) that pancreatic CEL functions not only after gastric lipase but also after pancreatic, colipase-dependent lipase interaction. Thus, its action may be regulated by fatty acids.

A previous study (5) shows that the human pancreatic CEL has a high affinity for siliconized glass beads, a simple hydrocarbon-water interface. Subsequent work (9, 10) elucidates an apparent specificity for adsorption to surfaces containing product fatty acid, but other data suggest that enzyme denaturation was occurring in the absence of fatty acid (10) or a bile salt cofactor (11). Indeed, it has been found that, under non-denaturating conditions, porcine diisopropylphosphoryl-CEL binds to films of substrates, products, and other lipids with comparable affinities. The exception was 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) for which both CEL affinity and number of CEL-binding sites were relatively low (12, 13). Moreover, in mixed lipid surfaces containing POPC, native and diisopropylphosphoryl-CEL binding affinities were reduced 10–20 fold, even when fatty acid or substrate was present at 0.67 mol fraction. This was qualitatively attributed to excluded area effects due to the presence of POPC.

Recently, the susceptibility to hydrolysis of CEL substrates in the presence of POPC was measured (14). The results showed an ultrasensitive dependence of hydrolysis on surface composition in the presence of high bulk (CEL). Specifically, little or no hydrolysis occurred up to a substrate-specific composition above which hydrolysis was virtually complete. Comparison of the composition dependence of this "switching" of catalytic activity with the diisopropylphosphoryl-CEL binding to POPC-1,3-dioleoylglycerol films described above was paradoxical. It revealed that CEL adsorption should have been sufficient at lower substrate mol fractions to catalyze complete substrate hydrolysis. That hydrolysis was negligible suggests either that the chemically inactivated CEL used earlier is not a good model for the native enzyme or that some other property of the surface prevents productive interaction between adsorbed enzyme and substrate. To resolve this issue, we have investigated the adsorption of native CEL to POPC-substrate and POPC-product films, with emphasis on the compositional range where there is little hydrolysis of substrate. The results show that the derivatized CEL is a good model for the native enzyme. Moreover, they show substantial differences between binding to substrate-POPC and fatty
acid-POPC films which suggest differences in the organization of the interfacial lipid molecules.

MATERIALS AND METHODS

Lipids—Trioleoylglycerol (TO), 1,3-dioleoylglycerol (1,3-D0), 1,2-dioleoylglycerol (1,2-D0), 1,3-monoleoylglycerol (1,3-MO), oleyl-methanol (OM), and oleic acid (OA) were purchased from Nu-Chek Prep, Inc. (Elysian, MN). The purity of each was checked by thin layer chromatography and from measured detection limits, after spraying with corrosive sulfuric acid and charring, purity was shown to be greater than 99%. POPC was purchased from Avanti Biochemicals (Birmingham, AL). DuPont-New England Nuclear supplied [1-14C]OA (50.7 mCi/mmol) and 1-palmitoyl-2-11[14C]oleoyl-sn-glycero-3-phosphocholine ([14C]POPC) (62.5 mCi/mmol). Purity of the phospholipid concentration in stock solutions was determined by assaying aliquots for organic phosphorus (15). Lipids were dissolved in hexane containing 5% ethanol (v/v).

Other Reagents—[14C]Phosphoric acid was obtained from DuPont-New England Nuclear, and Brij-35 detergent was from Pierce Chemical Co., Inc. Glycerol (99%) and p-nitrophenyl butyrate were purchased from Sigma. Ethanol was distilled from KOH and zinc (16); hexane (nonspectro grade) and acetone were from Burdick and Jackson Laboratories, Inc. (Muskegon, MI). Chloroform, diethyl ether, and methanol were redistilled before use. Petroleum ether (b.p. 65–85°C) was purified according to the procedure described earlier (17). All solvents were shocked with an insignificant level of impurities as described elsewhere (18). Hydrophobic paper for surface collection (Type 1PS), 7-cm diameter, Whatman, was prepared for use by solvent washing and water equilibration as previously reported (14). Water was purified by reverse osmosis, mixed-bed deionization, adsorption on activated charcoal, and filtration through a 0.22-μm Durapore membrane (Millipore Corp., Bedford, MA). Before use, buffers were filtered through a Diaflo hollow fiber with a molecular weight cut-off of 10,000 (Amicon Corp., Danvers, MA), degassed, and stored under argon. Lipase Type XI (pfs) from Rhizopus arrhizus was from Sigma. All other chemicals were of reagent grade and used without further purification.

Methods—The monomeric form of pancreatic carboxylester lipase (choleretic esterase, EC 3.1.1.13, M, 74,000) was purified from porcine pancreas as described previously (19). Its activity was determined from the rate of formation of p-nitrophenolate from p-nitrophenyl butyrate by a modification of the procedure described by Shirai and Jackson (20). The assay mixture consisted of 2.94 ml of 50 mM sodium phosphate buffer, pH 7.25, 500 units of R. arrhizus cholesterol esterase, EC 3.1.1.13, M, 74,000; 0.1 M NaCl and 0.1% (w/v) Brij-35, 30 μl of acetonitrile containing 1.5 μmol of p-nitrophenyl butyrate, and 30 μl of enzyme solution. Relative enzyme concentration substrates by 123I determination using liquid scintillation counting. It was found that typical subphase carryover was 0.5–2.5 μCi/mm2 of surface. Film recovery was calibrated in separate experiments using 123IPOPC (5.0 mCi/mmol) at surface pressure of 40 mN/m. It was found to be 82±2% of lipid added and all adsorption measurements reported were corrected to 100% using this value.

The surface pressure area isotherms of substrate-POPC and OA-POPC mixtures were published earlier (21). In the present study, at lipid compositions for which surface pressure area and surface potential area isotherms had not been measured directly, values of lipid molecular area and surface potential were obtained by interpolation from adjacent isotherms. Film compressibility was calculated in a similar manner from the surface pressure area data. Results

Substrate-POPC Films—Previously, the hydrolysis of four substrates by 123 nm (CEL) was measured in mixed monolayer films with POPC. They were OM, TO, 1,3-D0, and 1,2-D0. All showed hydrolytic ultrasonicity (14, 23) with respect to their abundance in the film. For TO, 1,3-D0, and possibly 1,2-D0 a product of their partial hydrolysis is 1,3-MO, which itself is a substrate for CEL. (14). 1,3-MO is also better able to lower surface tension, i.e. is more amphiphilic, exhibiting an equilibrium spreading and collapse surface pressures of 43 mN/m (21) compared with ≤50 mN/m for the other substrates. This collapse pressure is comparable to that of POPC, suggesting that 1,3-MO might be more available to CEL in the presence of POPC than the other substrates. To test this, the composition dependence of 123I-POPC hydrolysis by CEL in mixed monolayers with POPC was determined. The bulk enzyme concentration (123 nm) and reaction time (10 min) was identical to those used for the other substrates not above and studied elsewhere. As shown in Fig. 1, the susceptibility of 123I-1,3-MO to CEL-catalyzed hydrolysis is initially low but rises abruptly near 100%. This behavior is qualitatively similar to that for the less amphiphilic substrates (14). The composition at which the increase occurs (Fig. 1) is ~0.75 mol fraction of 1,3-MO. This is a higher mol fraction than observed with other substrates which showed such behavior in the mol fraction range of 0.2–0.6 (14). The 12% maximum extent of hydrolysis below
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FIG. 1. Composition dependence of 1(3)-MO hydrolysis by monomeric, porcine pancreatic carboxylester lipase in mixed-lipid films with POPC. Bulk phase enzyme concentration was 123 nM. Hydrolysis was measured at an initial surface pressure just below film collapse (36-41 mN/m).

FIG. 2. Composition dependence of carboxylester lipase adsorption to substrate or OA in mixed lipid films with POPC. POPC with TO (A), 1,3-DO (B), 1,2-DO (C), OM (D), 1(3)-MO (E), and OA (F). Filled symbols in F correspond to films in which OA was substituted for each substrate in panels A-E at levels expected following complete substrate hydrolysis.

0.75 mol fraction of 1(3)-MO (Fig. 1) is somewhat greater than for the other substrates (14), possibly because 1(3)-MO, more than the other substrates, tends to desorb into the aqueous phase adjacent to the lipid film.

Because substrate hydrolysis must be preceded by CEL adsorption to the surface (14), an obvious reason for the lack of reaction at low substrate abundance could be a deficiency of enzyme at the interface. To examine this possibility, the adsorption of native CEL to mixed POPC-substrate monolayers was measured under the same conditions as for hydrolysis studies (e.g. Fig. 1 and Ref. 14). It should be noted that measurements of adsorbed CEL are corrected for the carryover of enzyme which accompanies monolayer collection and adsorbed CEL is determined from recovered enzymatic activity, not protein. Thus, we are measuring the surface excess of catalytically active enzyme. Fig. 2, A-E shows CEL adsorption data for each of five substrates over the compositional region where hydrolysis is relatively low. In all panels of Fig. 2, the value of 0.20 ± 0.006 (S.D.) pmol/cm² at zero substrate mol fraction is the average of four determinations. For each substrate, adsorption increases monotonically with its abundance. The highest levels observed are ~0.6 pmol/cm² at a 1,3-DO mol fraction of ~0.5. This corresponds to about one-sixth of the total surface area being covered by CEL (12, 13). Moreover, for each set of data in Fig. 2, A-E, at the highest value of the surface concentration of CEL, there are ~550 substrate molecules in the monolayer for each bound CEL molecule. Because the enzyme is a catalyst which inhibits turnover numbers of 1500-3000 min⁻¹ (8), the small extents of substrate hydrolysis observed under these conditions (14 and in Fig. 1) cannot be due simply to a paucity of CEL in the surface phase.

For the measurements shown in Fig. 2, A-E, each lipid film was initially at a surface pressure close to its collapse pressure. Fig. 3A shows the change in surface pressure of the films following addition of enzyme to the subphase, plotted as a function of the initial film pressure. The figure shows that the adsorption of CEL is accompanied by only small changes in surface pressure. The decrease of ~4-6 mN/m for 1(3)-MO-containing films reflects the instability of such films at high surface pressures. Because adsorption levels (Fig. 2, A-E) were as high as one-sixth of an enzyme monolayer, the minimal changes in surface pressure suggest the absence of any significant perturbation of lipid packing or hydration by CEL.

The similarities of the adsorption data and surface pressure changes for the different substrates suggest a common mode of regulation of CEL adsorption. A shared feature of POPC-substrate monolayers is that the total fraction of surface area occupied by POPC decreases and, hence, that of substrate increases with increasing substrate mol fraction (21). This suggests that surface area not occupied by POPC may be required for adsorption. Secondly, the small, but finite, CEL adsorption measured in the absence of substrate suggests that a fraction of the area of POPC itself is available. From earlier studies (12, 13, 24), it can reasonably be assumed that at the high level of enzyme used, adsorption is equilibrated during the 10 min of incubation. If CEL adsorption is proportional to the fraction of surface not occupied by POPC and A₀,∞ is the area of each POPC to which CEL cannot bind, then

\[ A_{CEL} = C \left( 1 - \frac{A_0,\infty}{A_{\infty}} \right) \]

FIG. 3. Change in surface pressure versus initial surface pressure for adsorption of carboxylester lipase to (A) substrates and (B) OA in mixed lipid films with POPC. Conditions and symbols are as in Fig. 2.
where $A'_{pc}$ is the apparent area of a POPC molecule and $C$ is a proportionality constant. The variable $A'_{pc}$ is simply the total surface area divided by the number of POPC molecules and is readily calculated at the final surface pressure, i.e., after the adsorption period, using surface pressure area data reported earlier (21). Using these and the measured $\Gamma_{CEL}$ values given in Fig. 2, $A'_{pc}$ plots of $\Gamma_{CEL}$ versus $1/A'_{pc}$ were constructed. As exemplified by one set of data obtained with POPC-1,3-DO films (Fig. 4A), such plots were reasonably linear. For each of the five substrates, the coefficient of correlation of each plot is given in Table I together with the value of $A_{pc}$ calculated from the slope and intercept of the line. The values of $A_{pc}$ are quite similar, averaging 43.5 ± 1.0 Å²/POPC. If the initial, rather than final, surface pressure of each film was used for determining $A'_{pc}$, a nearly identical average, 43.6 ± 1.1, is obtained. Thus, the data are consistent with there being a common area of 43–44 Å² for POPC to which enzyme cannot bind.

If $A_{pc}$ solely regulates adsorption under these conditions, Equation 1 should be obeyed when $A'_{pc}$ is varied at a fixed lipid composition. Unfortunately, for any given composition only a small range of areas can be attained because the surface pressure must be kept above 18–20 mN/m to prevent CEL adsorption. For each of the five substrates, the coefficient of correlation of each plot is given in Table I together with the value of $A_{pc}$ calculated from the slope and intercept of the line. The values of $A_{pc}$ are quite similar, averaging 43.5 ± 1.0 Å²/POPC. If the initial, rather than final, surface pressure of each film was used for determining $A'_{pc}$, a nearly identical average, 43.6 ± 1.1, is obtained. Thus, the data are consistent with there being a common area of 43–44 Å² for POPC to which enzyme cannot bind.

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as shown in Fig. 1 does not disproportionately inhibit CEL adsorption.

As noted above, for the experiments involving OA-POPC films, initial conditions were chosen to mimic corresponding substrate-POPC films. In all cases, hydrolysis of the substrate by CEL had been shown to be low. In addition to those experiments, CEL binding to 1,3-DO-POPC films was measured in the mol fraction range where 1,3-DO hydrolysis was complete. The results (not shown) for 20A-POPC films were essentially identical to those reported earlier for binding of native CEL to 13,16-docosadienoic acid-POPC films (13). Specifically, in the present study surface concentrations of CEL increased monotonically from ~0.75 to ~4.0 pmol/cm² with an increase in OA mol fraction from 0.56 to 1.0. These values for bound CEL were also identical to those obtained with 1,3-DO-POPC films, presumably because the 1,3-DO was rapidly hydrolyzed to 20A. In the latter experiment, the mol fraction range of 1,3-DO present before addition of enzyme was 0.6–1.0.

**DISCUSSION**

The “switching” on of the catalytic activity with small increases in substrate abundance (e.g. Fig. 1) has been observed with CELs and other lipolytic enzymes (14, 25). It is puzzling because it is not associated with any recognized physical discontinuity in the surface phase. For all POPC-substrate mixtures previously examined, switching occurs at a composition well above that for complex, i.e. pseudo-compound, formation between the lipids (14). Also, in the switching region, these insoluble lipids constitute a single surface phase of the liquid-expanded type (21) and hydrolysis was shown to occur in the monolayer phase (14). As noted in “Results,” however, 1(3)-MO hydrolysis may also occur to a small extent in the aqueous phase because of the tendency of 1(3)-MO to desorb in the time course of our experiments. In multilamellar, liquid-crystalline bilayers comparable switching is also observed using 1,3-DO (25). For all these systems, a possible explanation for the lack of catalytic activity at low substrate compositions is the absence of surface-bound CEL. Although an earlier binding study had indicated adequate CEL should be present (13), it was limited in scope. Because it was performed before catalytic switching had been observed, few data points were obtained in the relevant compositional region. Secondly, in presumption of substrate hydrolysis, a chemically modified, catalytically inactive form of CEL was used. These limitations, in light of the subsequent demonstration of a lack of catalytic activity toward POPC-substrate films (14), prompted the present study.

The binding of native CEL to POPC-1,3-DO films was measured under the same conditions used in the earlier, more limited study of diisopropylphosphoryl-CEL binding (13). Comparison of the present data (Fig. 2B) with those obtained earlier shows almost perfect agreement (not shown, but see Fig. 7). This is fortuitous, but shows that the derivatized enzyme is a good model for native CEL. Therefore, a deficiency of adsorbed catalyst cannot explain the lack of hydrolysis at low substrate compositions (e.g. Fig. 1 and Ref. 14). Nor, as confirmed by Fig. 6, does the generation of small quantities of product fatty acid markedly decrease CEL binding. The binding data in Fig. 2, A E, are monotonic with respect to mol fraction and linear with respect to 1/A' (e.g. Fig. 4). They show no obvious discontinuities in slope at the complex composition for each POPC-substrate mixture. The latter are 0.25 for 1,3-DO, 0.26 for 1,2-DO, 0.2 for OM, 0.04 for TO, and 0.58 for 1(3)-MO (21). Thus, complex formation between POPC and substrate per se is not an important
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In the present study, the consistencies in the \( A'_\text{rec} \) dependence of CEL adsorption to POPC-substrate mixtures (Fig. 4 and Table I) as opposed to the lack thereof with respect to surface potential (Fig. 6A) and compressibility (Fig. 6B) indicate that the fraction of free area, or a parameter proportional to it, is the dominant regulator of adsorption. This is also shown indirectly by Fig. 3 in that binding occurs over a wide range of surface pressure and does not increase it, as would proteins like apolipoprotein A-I (29). Also, when plotted versus surface pressure, adsorbed CEL values for the various substrate-POPC mixtures do not define a common curve (not shown). What the POPC-substrate binding data do not address directly is the part of POPC responsible for the inhibition of binding. Comparison of POPC and substrate structures suggests that the phosphocholine moiety could sterically interfere with access of CEL to the more apolar regions of the surface phase. Data from crystal structures show an area of 48 \( \text{Å}^2 \) per phosphocholine group (32). Thus, if the head group, when fully hydrated, inhibits adsorption, it must be tilted slightly from its usual orientation in bilayers, i.e. parallel to the interface (see refs. in 28). We cannot rule out other parts of the molecule, but if the enzyme “penetrated” into the apolar region of the film, the binding would be expected to increase surface pressure more than it did (Fig 3A) CRL binding studies of the type presented here, but with different phospholipids, should better define what groups determine the excluded area.

For a simple binding mechanism, the saturation level of binding should be given by the parameter \( C \) in Equation 1. However, the values of near 1 pmol/cm\(^2\) are only one-fourth of the level achieved in the absence of the phospholipid. This discrepancy arises qualitatively from the role of POPC or its complexes with substrate in regulating the apparent affinity of CEL binding (13, 24). This regulation is also a POPC-related, excluded area effect but involves the rate constant for CEL adsorption, not area available for occupancy by CEL. In light of such complexity, the agreement of the binding data with Equation 1 at low binding levels arises presumably because affinity is relatively low and approximately constant. It is for these reasons that the parameter \( C \) should only be considered as a proportionality constant in the present study.

OA-POPC Films—Earlier, we presented limited data obtained with diisopropylphosphopheryl-CEL which suggest a common dependency of CEL binding to POPC-fatty acid and POPC-substrate films. This is clearly not the case over most of the range of substrate and corresponding OA mol fractions where substrate hydrolysis is low (compare Fig. 2, A-E, to 2F). Over much of this compositional range, where binding to POPC-substrate films increases monotonically, binding to POPC-OA films is essentially invariant. As reported in “Results,” these differences cannot be ascribed to variations in surface pressure, surface potential, monolayer compressibility, or POPC molecular area. One possible explanation is the net charge of OA. However, CEL binds with high affinity to films consisting only of OA or docosadienoic acid (12). Thus, the data suggest that charge alone is not the regulatory factor. Whatever it is, its effect is mass-dependent, as shown by the continuous inhibition with increasing substrate replacement shown in Fig. 6.

In the range of OA compositions corresponding to high 1,3-DO compositions, i.e. where 1,3-DO hydrolysis was complete, \( \Gamma_{CEL} \) increased rapidly to about 4 pmol/cm\(^2\) at an OA mol fraction of 1.0. These data matched earlier values obtained using docosadienoic acid (13). In that previous study, the binding of diisopropylphosphopheryl-CEL to 1,3-DO-POPC films showed similarities to that for the docosadienoic acid-POPC films when plotted on the basis of the fraction of surface area not occupied by the complex formed between fatty acid or 1,3-DO and POPC. In the present study, data indicate that fraction of free area should instead be calculated as \( (A'_\text{rec}-A'_\text{rec,OA})/A'_\text{rec} \) (Figs. 4, A-B, and Table I), at least for substrate-containing films. Using that definition of free area for all films, a direct comparison of CEL and diisopropylphosphoryl-CEL binding can be made at all compositions. To normalize for the large changes in CEL binding which occur with increasing 1,3-DO or OA mol fraction, the binding data can be expressed as percentage differences. Specifically, in the region of low 1,3-DO hydrolysis, data for CEL binding to OA-POPC films (Fig 2F) were fitted to an empirical function, \( y = ae^{-x} + c \), where \( y \) is the CEL surface excess, \( x \) is the area fraction, and \( a, b, \) and \( c \) are fitting parameters. The values of

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**Fig. 7. Area dependence of normalized carboxylester lipase adsorption to POPC-1,3-DO films.** Percentage differences and area fractions were calculate as described in the text. Binding data for native CEL (\( C, \square \)) were from experiments listed in Table I and those for diisopropylphosphopheryl-CEL (\( \square \)) from Ref. 13. The shaded area indicates the region over which 1,3-DO hydrolysis increases from 0 to \( \sim 100\% \) (see text) and the solid line is drawn through the points to guide the eye.
x were calculated using $A_{o,\infty} = 43.5 \text{ Å}^2$/POPC from the data of Table I. With the parameters obtained, $y$ was calculated for each area fraction at which CEL (Fig. 2B) or diisopropylphosphoryl-CEL (13) binding had been measured. The fractional difference was calculated as $(\Gamma_{\text{CEL}} - y)/y$. The same fitting procedure was applied to the compositional region where 1,3-DO hydrolysis was complete using the corresponding values of CEL bound to OA-POPC to obtain $a$, $b$, and $c$. The fractional differences calculated are shown in Fig. 7 as percentages. The shaded area indicates the range over which hydrolysis of 1,3-DO by CEL increases from 5 to 98% (0.50–0.60 mol fraction of 1,3-DO). Two significant characteristics of the binding behavior are evident. The first is that the adsorption of CEL (open and crossed circles) and diisopropylphosphoryl-CEL (filled squares) are identical in the low hydrolysis range over which they can be compared. Thus, the derivatized CEL is a good model for the native enzyme. Secondly, although the difference in adsorption to OA- and 1,3-DO-containing films is 150% to the left of the shaded area (low 1,3-DO hydrolysis), it falls to near zero on the right (high 1,3-DO-hydrolysis). This suggests that the factor(s) responsible for the differences in CEL binding in the low hydrolysis region is absent or negligible at higher 1,3-DO mol fractions. In the high hydrolysis range, only data obtained with the derivatized enzyme could be used but, as judged by the left hand region, it is a good model enzyme.

Implications—Because catalysis is inhibited in the presence of adsorbed CEL (left region of Fig. 7), the mode of binding of CEL could preclude effective enzyme-substrate interaction. However, such a mechanism explains neither the differences in binding behavior are evident. The first is that the surface organization is the key factor in regulating both CEL adsorption and catalysis. As the substrate mol fraction exceeds its complex stoichiometry, domains of uncomplexed substrate may appear, interrupting the organization locally and allowing more CEL binding. Part or all of this substrate in the vicinity of bound CEL may even be hydrolyzed, accounting for the finite hydrolysis at low substrate compositions even with insoluble substrates (e.g. Fig. 1 and Ref. 14). However, if the overall organization of the POPC-rich surface remains intact, products may not be freely exchanged for new substrate in the surface phase. Thus, hydrolysis would stop or slow to negligible rates. The dramatic increase in hydrolysis which occurs with an additional increase in substrate mol fraction (e.g. Fig. 1) may signal the loss of phospholipid-dominated organization of the surface, i.e. the onset of connectivity of domains of uncomplexed substrate. Thus, the switching of catalysis may represent the change in the surface from one of domains of substrate in an organized network of POPC; substrate complex to domains of the complex in a sea of substrate. This possible coexistence of liquid-like domains is analogous to that observed for dmyristoylphosphatidylcholine and cholesterol in monolayers (33) but would not involve macroscopic phase separation.

For all substrates in the compositional range where hydrolysis is low, the complete replacement of substrate by OA, i.e. simulated hydrolysis, results in OA mol fraction below its complex composition. Thus, partial hydrolysis of substrate would not be expected to disrupt the POPC-organized surface. As with substrates, at a sufficiently high mol fraction of OA $\geq 0.67$, the organizational effect of POPC will be lost. Thus, at high OA composition binding is comparable to that for substrate-rich films (Fig. 7).

Overall, the regulation of the CEL, especially the catalytic switching, is what would be expected for a percolation process (34). The implication of these findings for lipolysis is to emphasize the importance of the local organization of lipid molecules in the surface as a regulatory factor. It is not necessary to postulate the formation of a specific structural defect (e.g. 35) for binding and catalysis to proceed, merely the generation of sufficiently large area domains for the enzyme to bind and sufficient domain connectivity to permit mobility of substrate and products. This can occur by addition of molecules like diacylglycerols or through generation of reaction products (e.g. 36). Also, with respect to binding, this type of regulation is probably more important for weakly surface-active enzymes, like CEL, and pancreatic phospholipase A$_2$, which are unable to displace phospholipids to create their own binding site.

Acknowledgments—We wish to thank N. K. Mizuno for preparation of carboxylester lipase and Core G of Program Project Grant HL05214 for assistance in purification of labeled substrates.

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