Lipid Structural Reorganization Induced by the Pancreatic Lipase Cofactor, Procolipase†

William E. Momsen, Maureen M. Momsen, and Howard L. Brockman*

The Hormel Institute, University of Minnesota, Austin, Minnesota 55912

Received January 10, 1995; Revised Manuscript Received March 31, 1995⊗

ABSTRACT: Pancreatic colipase and its precursor, procolipase, facilitate interfacial lipid hydrolysis catalyzed by pancreatic lipase. To better understand how procolipase functions, its interactions with mixed-lipid monolayers at the argon−buffer interface have been characterized. The lipid mixtures consisted of 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine and either 1,3-dioleoylglycerol, a model lipase substrate, or 13,16-*cis,cis*-docosadienoic acid, a model lipase product. Analysis of the lipid composition dependence of procolipase-induced surface pressure increases shows thermodynamically that procolipase interacts strongly and preferentially with the lipase substrate or product. This finding was confirmed by fluorescence measurements of procolipase interaction with pyrene lipid analogs. Analysis of the quantity of procolipase adsorbed to the lipid monolayers shows that interfacial packing obeys a simple, geometric model. The partial molecular areas obtained for procolipase (708 Å^2) and the phosphatidylcholine (70 Å^2) agree with their known cross-sectional areas. However, the areas for the fatty acid (14 Å^2) and diacylglycerol (18 Å^2) are less than half the expected values, indicating the formation of substrate multilayers. Overall, the results indicate a previously unrecognized role for procolipase, recruiting substrate laterally to its vicinity and, hence, to pancreatic lipase with which procolipase forms a 1:1 interfacial complex. Accompanying this preferential interaction of procolipase with lipase substrates is their rearrangement normal to the interface. These previously unrecognized properties of this lipase cofactor should have relevance for the regulation of other lipases, like lipoprotein lipase, which are regulated by cofactor proteins.

The hydrolysis of dietary glycerides in the intestine is catalyzed by pancreatic, colipase-dependent lipase (Verger, 1984) and carboxylester lipase (Rudd & Brockman, 1984). This process occurs in the presence of biliary phosphatidylcholine which facilitates dispersion of the glycerides (Linthorst et al., 1977) and bile salts which aid in the micellar transport of reaction products to the intestinal villus membrane (Thomson & Dietschy, 1981). The two lipases function in sequence with pancreatic lipase acting on the 1(3) positions of tri- and diacylglycerols and carboxylester lipase continuing the hydrolysis of diacylglycerols and 1(3)- and 2-monoacylglycerols (Lindstrom et al., 1988; Bernbäck et al., 1990). The sequential nature of glyceride hydrolysis arises from differences in the regulatory properties of the lipases and from changes in the state of dispersion of the lipids as lipolysis proceeds (Staggers et al., 1990).

The ability of pancreatic lipase to function prior to carboxylester lipase depends on the presence of a protein cofactor, colipase. From work carried out using model systems, it is clear that the primary role of colipase and possibly its procolipase precursor is to enable the adsorption of pancreatic lipase to the substrate-containing, lipid−water interface in the presence of bile salts and diacyl phospholipids (Erlanson-Albertsson, 1992). Either of these surfactants is able to interfere with the adsorption of lipase to the interface in the absence of colipase or procolipase and, thereby, prevent or impede substrate hydrolysis. At a pH ≥ 8.0 and in the presence of Ca^{2+} at millimolar concentrations, the colipase-dependent, pancreatic lipase-catalyzed hydrolysis of glycerides is facilitated by the presence of fatty acids in the system and by prior cleavage of a pentapeptide from the N-terminus of procolipase to give colipase (Borgström et al., 1979; Wieloch et al., 1981; Borgström, 1980; Wieloch et al., 1982; Larsson & Erlanson-Albertsson, 1986). *In vivo*, the fatty acids are generated by the gastric hydrolysis of triacylglycerols (Gargouri et al., 1989). Cleavage of the pentapeptide occurs at a trypsin-sensitive site (Erlanson-Albertsson, 1992). It has been shown that both the clustering of Ca^{2+} soaps of fatty acids and the proteolytic activation of procolipase to colipase affect the pancreatic lipase adsorption step of catalysis. The former creates additional adsorption sites for the cofactor, and the latter increases its affinity for phosphatidylcholine-covered interfaces.

More recently, the model of pancreatic lipase regulation described above has come into question as a consequence of the nonphysiological milieu in which most prior studies were performed. At pH values ≤7.0, which more closely resemble those encountered by pancreatic lipase *in vivo*, proteolytic activation of procolipase is not required for efficient lipolysis in the presence of phosphatidylcholine and bile salts (Larsson & Erlanson-Albertsson, 1991). This observation, together with other evidence that the pentapeptide can function as a satiety factor (Erlanson-Albertsson, 1992), has led to the proposal that the conversion of procolipase to colipase is inconsequential for intestinal lipolysis (Larsson & Erlanson-Albertsson, 1991). With the crystallization of the pancreatic lipase−procolipase 1:1

†This work was supported by UHPHS Grant HL49180 and by the Hormel Foundation.

* Address correspondence to this author at The Hormel Institute, University of Minnesota, 801 16th Avenue NE, Austin, MN 55912. Telephone: 507-437-9620. Fax: 507-437-9696 or -9606. E-mail: hlbroc@maroon.tc.umn.edu.

⊗ Abstract published in *Advance ACS Abstracts,* May 15, 1995.

0006-2960/95/0434-7271$09.00/0

complex in the presence of bile salt and phosphatidylcholine, the role of procolipase as an anchor for pancreatic lipase has been expanded to that of an anchor-activator. In crystals prepared at pH 6.0 procolipase stabilizes the opening of the lid which covers the active site of pancreatic lipase and other lipases in the absence of an interface (van Tilbeurgh et al., 1993). That procolipase can serve this role again suggests that its proteolytic activation may be unnecessary for its intestinal function.

With respect to the role of fatty acids as activators, measurements of intestinal contents from human volunteers show that Ca^{2+} soaps of fatty acids are a minor lipid phase at physiological pH (Staggers et al., 1990). Moreover, monolayer measurements of the miscibility of fatty acids and phosphatidylcholines show that in the absence of Ca^{2+} at pH 6.6, lateral phase separation of a fatty acid-rich phase does not occur (Smaby et al., 1994). It has been observed, however, that the activity of pancreatic lipase in phosphatidylcholine-containing monolayers in the absence of colipase or procolipase depends on the presence of ester substrate or fatty acid in a composition-dependent, all-or-none manner (Muderhwa & Brockman, 1992a). This activation has been explained on the basis of percolation theory as applied to the lateral organization of the lipid molecules at the interface.

The relative lack of information about the regulation of pancreatic lipase activity by its substrates, products, and cofactors in the range of physiological pH prompted us to undertake a systematic investigation of the system at pH 6.6. The monomolecular film approach was used as a model for natural substrate emulsion surfaces because of its many experimental advantages for the study of interfacial reactions (Brockman, 1984; Verger & Pieroni, 1986). The first steps, as noted above, were to study the miscibility of a phosphatidylcholine and either a model ester substrate or fatty acid product and to measure pancreatic lipase activity in the absence of either procolipase or colipase. In this paper we report measurements of the interaction of procolipase with these mixed lipid monolayers in the absence of pancreatic lipase. The results reveal interactions between procolipase and either the ester substrate or fatty acid which are much stronger than interactions between procolipase and phosphatidylcholine. Quantitative analysis of this data suggests a novel role for procolipase at interfaces in addition to serving as an anchor-activator for pancreatic lipase, i.e., that it recruits substrate to the vicinity of its active site.

MATERIALS AND METHODS

1,3-Dioleoylglycerol (DO)[1] and 13,-16-*cis,cis*-docosadienoic acid (DA) were purchased from NuChek Prep, Inc. (Elysian, MN), 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (SOPC) was from Avanti Biochemical (Birmingham, AL), and 1-pyrenedecanoic acid (PDA) and 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphocholine (PDA-PC) were from Molecular Probes (Eugene, OR). Solvents, water, buffer, and lipid solutions were prepared as described (Smaby & Brockman, 1990).

Porcine procolipase was purified as described previously (Cunningham et al., 1989) except that for the final chromatographic step the Mono-Q HR column was replaced with a 2.5 × 14.5 cm FFQS Sepharose column (Pharmacia, Piscataway, NJ), developed with a 0−0.2 M NaCl gradient in 20 mM Tris, pH 8.0. The lack of proteolytic conversion of procolipase to colipase was confirmed by N-terminal amino acid sequence analysis data. Protein concentration was determined by absorbance at 280 nm based on an $E^{1\%}$ of 3.6 (Canioni et al., 1980) and a molecular weight of 10 300 calculated from the amino acid sequence (Sternby et al., 1984). Procolipase activity was determined by its ability to stimulate pancreatic lipase. The assay consisted of 27 units of porcine pancreatic lipase in 3.0 mL of an emulsion comprised of 1.67% tributyroylglycerol (v/v), 1 mM Tris, pH 7.0, 1 mM $CaCl_2$, 0.02% NaN_3, 0.1% Brij 35, 0.15 M NaCl, and 6.0 mM sodium taurodeoxycholate as described (Cunningham et al., 1989). The specific activity of procolipase in this assay was 33 000 units/mg.

Adsorption of procolipase to lipid monolayers was measured using a two-compartment, keyhole-shaped Teflon trough filled with 10 mM phosphate, pH 6.6, 0.1 M NaCl, and 0.01% NaN_3 at 24 °C. A measured aliquot of lipid solution was spread onto the clean buffer surface to give a surface pressure <5 mN/m after which the monolayer was compressed as necessary to the desired initial surface pressure and, hence, lipid concentration at a trough area of 26−27 cm^2 (Tsujita & Brockman, 1987). The sample chamber (24.6 mL) was stirred at 100 rpm for 30 min after injection of procolipase. The monolayer was collected on solvent-cleaned, water-equilibrated hydrophobic paper discs, after which procolipase was eluted with 4.0 mL of the tributyroylglycerol emulsion and assayed as described above. Carryover of nonadsorbed procolipase was determined by inclusion of 5 μCi of [^{32}P]phosphate in the subphase as described (Tsujita et al., 1989). Values for adsorbed procolipase were also corrected for recovery of the lipid monolayer, 84 ± 3%, determined in separate experiments using [^{14}C]phospholipid at surface pressures between 1 and 35 mN/m. Preliminary experiments at selected compositions (100% PC, 45% DO/ 55% PC, 89% DO/ 11% PC, and 100% DA) and at surface pressures near film collapse showed that, with a subphase concentration of 240 nM procolipase, the surface concentration of procolipase reached a maximum within 30 min.

For fluorescence measurements, samples containing PDA or PDA-PC with DA and SOPC in organic solvent were initially dried with a stream of N_2 after which they were maintained under high vacuum for 16 h. To each was added 10 mM potassium phosphate buffer, pH 6.6, containing 0.1 mM NaCl and 1 mM EGTA to bring the lipid concentration to 0.4 mM. The suspensions were vortexed for 60 s to disperse the lipid and split into two parts. Procolipase was added to one of each pair to give a free fatty acid/protein mole ratio of 73:1 or 7.3:1 after which the samples were frozen in an acetone−dry ice bath for 60 s and thawed. Following nine additional freeze−thaw cycles to help equilibrate procolipase, if present, with the dispersions, the samples were incubated at 24 °C for 24 h during which time aliquots were taken and diluted 41-fold into the incubation

[1] Abbreviations: DO, 1,3, dioleoylglycerol; DA, 13,16-*cis,cis*-docosadienoic acid; SOPC, 1-stearoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine; PDA, 1-pyrene decanoic acid; PDA-PC, 1-hexadecanoyl-2-(1-pyrenedecanoyl)-*sn*-glycero-3-phosphocholine; Γ_P, procolipase surface excess; Γ_L, total lipid surface excess; A_P, partial molecular area of procolipase; A_L, partial molecular area of lipid; A_{DO}, partial molecular area of 1,3-dioleoylglycerol; A_{DA}, partial molecular area of 13,16-*cis,cis*-docosadienoic acid; A_S, partial molecular area of 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; I_M, pyrene monomer fluorescence emission intensity; I_E, pyrene excimer fluorescence emission intensity.

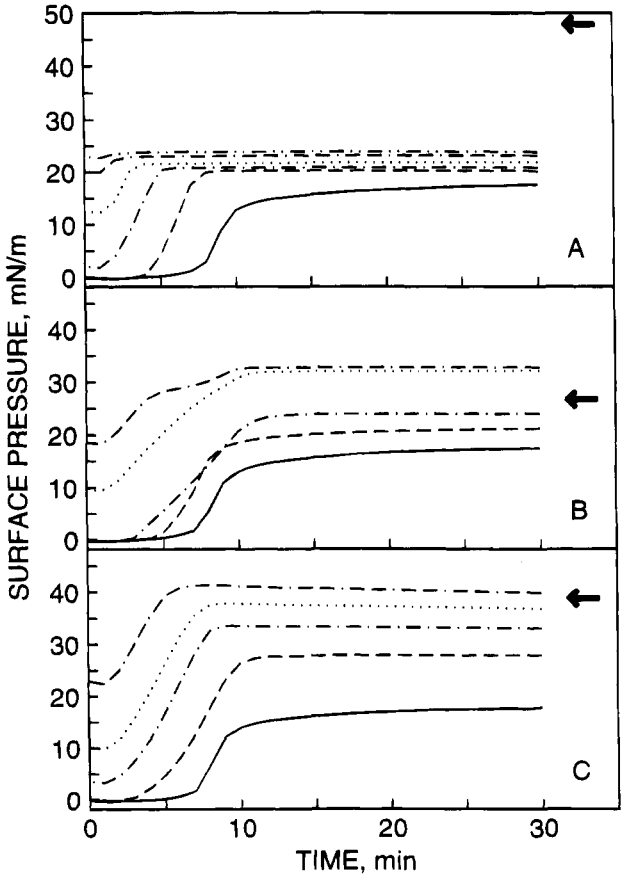

FIGURE 1: Time-dependent surface pressure changes induced by injection of colipase beneath lipid monolayers. Arrows indicate the collapse surface pressure of the lipid monolayer alone. Procolipase (240 nM) was injected under monolayers of (A) SOPC, (B) DO, or (C) DA at initial lipid concentrations of (A) 0 (—), 68 (- - -), 171 (-•-•-), 216 (•••), 241 (- - •- -), and 249 (••-••), (B) 0 (—), 60 (- - -), 135 (-•-•-), 214 (•••), and 242 (- - •- -); (C) 0 (—), 240 (- - -), 347 (-•-•-), 453 (•••), and 534 (- - •- -) pmol/cm^2.

buffer for determination of I_E/I_M and light scattering or into methanol for quantitation of PDA and PDA-PC. The latter measurements showed that the pyrene concentration in all paired samples was the same within 3−10 % after preparation and remained in that range over the 24 h incubation. Pyrene fluorescence was measured using a SPEX Fluoromax spectrofluorometer with an excitation wavelength of 340 nm at a band-pass of 1.06 nm. Fluorescence emission intensity of pyrene monomer was measured at 375 nm and that for excimer at 471 nm with a band-pass of 2.13 nm. Apparent fluorescence due to light scattering at the excitation wavelength was used to monitor vesicle aggregation state.

RESULTS

Procolipase Is Not Denatured by Adsorption at the Gas−Liquid Interface. In the absence of a lipid monolayer, adsorption of colipase to the argon−buffer interface was indicated qualitatively by the increase in surface pressure from zero to near 18 mN/m (Figure 1A−C, solid lines). From sixteen such determinations, the average increase in surface pressure was 17.7 ± 0.4 mN/m. Collection and measurement of the adsorbed procolipase in each case confirmed its association with the interface and yielded a procolipase surface concentration of 23 ± 3 pmol/cm^2. Essentially identical results were obtained at a bulk procolipase concentration of 120 nM, indicating that saturation of the lipid−water interface had been achieved with 240 nM procolipase. The value of the surface concentration of colipase at saturation is comparable to the value of 23.8 pmol/cm^2 which can be estimated for a hexagonally close-packed, two-dimensional array of spherical protein molecules having the molecular weight of procolipase. This saturation density and the retention of cofactor activity following collection and assay of the adsorbed procolipase indicate that it forms a monolayer of undenatured procolipase at the argon−buffer interface. This interfacial stability confers an advantage in studying the interaction of procolipase with lipids, namely, that lipid concentrations from near zero to the collapse packing of the lipid monolayer can be used without interference from protein denaturation.

Table 1: Surface Pressure (π, mN/m) Parameters for Procolipase Adsorption to Lipid Monolayers

lipids	mole fraction	collapse π	max final π	lower π limit	$\pi_{i\|\Delta\pi=0}$	$\pi_{i\|\Gamma P=0}$	$\Delta\pi\|\pi_{i=0}$
DA/SOPC	0.000	46.6	23.9	2	23.9	23.9	20.6
	0.195	46.6	25.7	1	26.5	26.6	21.8
	0.401	45.9	32.0	1	31.8	29.8	22.9
	0.601	45.1	40.3	1	41.1		24.5
	0.798	42.1	42.7	2	49.4		27.6
	1.000	38.4	40.6	8	44.4		35.5
DO/SOPC	0.200	47.5	27.3	5	29.2	33.4	21.0
	0.252	46.9	26.8	0	30.2	34.0	21.7
	0.398	37.3	38.8	3	36.8	38.8	22.4
	0.497	33.9	36.9	4	40.3	39.8	23.3
	0.602	31.6	33.9	1	48.9		24.2
	0.702	30.1	34.6	3	44.3		25.5
	0.797	29.0	34.0	7	40.1		27.8
	1.000	27.7	32.9	9	33.9		31.1

Procolipase Alters the Surface Pressure of Lipid Monolayers. For modeling the lipid−water interface at which procolipase functions, SOPC was chosen as a representative matrix lipid, DO as a pancreatic lipase substrate, and DA as a fatty acid product. As shown earlier, DA exhibits interfacial behavior similar to that of oleic acid but forms much more stable monolayers at physiological ionic strength with respect to dissolution into the aqueous subphase (Tsujita & Brockman, 1987). DA is also a good "substrate" for pancreatic lipase-catalyzed exchange of its carboxyl oxygens with water (Muderhwa & Brockman, 1992a) and, therefore, will also be referred to as a substrate below. Each of the lipids alone exhibits liquid-expanded monolayer behavior from near zero to its collapse surface pressure (Smaby & Brockman, 1990). Monolayers of each lipid were spread to the desired concentration or, if necessary, spread and compressed to an initial surface pressure corresponding to the desired lipid concentration. The value of the collapse surface pressure for each lipid is tabulated in Table 1 and is indicated in Figure 1A−C by an arrow. In most cases subsequent addition of procolipase to the stirred subphase to a concentration of 240 nM produced a time-dependent increase in the surface pressure. As shown by the representative data in Figure 1, achievement of equilibrium in the procolipase−monolayer interaction was indicated by the stability of the surface pressure between 10 and 30 min following procolipase addition. Saturation of the interaction was confirmed by the identical values of the final surface pressure obtained with 120, 240, and 360 nM procolipase at selected initial surface pressures (data not shown).

With monolayers of SOPC alone the highest initial surface pressure at which addition of procolipase produced an increase in surface pressure was 24 mN/m (Figure 1a). This is similiar to the recently reported value of ~23 mN/m obtained with egg lecithin at an initial surface pressure of 5 mN/m and a subphase pH of 7.4 (de La Fournière et al., 1994). These results suggest that procolipase adsorbs to and is miscible with SOPC up to, but not beyond, 24 mN/m. In contrast, with monolayers of either DO or DA, surface pressure and procolipase surface concentration increased following procolipase addition for all initial surface pressures. This occurred even if the DO or DA monolayer was initially compressed to near its collapse pressure of 28 or 39 mN/m, respectively (Figure 1B,C), or even if it was compressed to half of its molecular area at collapse prior to procolipase addition (not shown). An increase in surface pressure indicates a lowering of interfacial free energy. Because interaction of procolipase with monolayers rich in lipase substrates results in final surface pressures which are (a) higher than those obtained in the absence of lipid, (b) greater than the value of 24 mN/m obtained with SOPC, and (c) higher than the collapse surface pressures of the substrate monolayers themselves, the data indicate a stronger interaction of procolipase with the lipase substrates compared to SOPC.

The data in Figure 1 indicate that the adsorption of procolipase to the lipid–water interface depends more on the lipid species comprising the monolayer than the value of the surface pressure. At pH 6.6 and approximately physiological ionic strength, SOPC is miscible with either DO (Cunningham et al., 1989) or DA (Smaby et al., 1994) in monomolecular films and those mixtures exhibit liquid-expanded surface pressure–area characteristics at all surface pressures from near zero to the collapse surface pressure of the film. To determine how surface pressure increases and procolipase adsorption depend on lipid species composition of the monolayers, measurements of the type shown in Figure 1 were made with SOPC–DO and SOPC–DA mixtures. For each data set obtained at a particular lipid composition, including those obtained with DO, DA, and SOPC alone, a plot of the change in surface pressure versus the initial surface pressure showed a negative linear correlation above a lower limit of initial surface pressure given in Table 1. Such behavior is typical for protein adsorption to lipid monolayers [e.g., Quinn and Dawson, (1970)]. The values of initial surface pressure extrapolated to the point at which the change in surface pressure was zero, abbreviated $\pi_i|_{\Delta\pi=0}$, are summarized in Table 1. For comparison, the collapse surface pressure of each lipid mixture is also given. The data show that $\pi_i|_{\Delta\pi=0}$ increases as the mole fraction of DO or DA is increased. Importantly, above 0.5 mole fraction of DO or DA, it exceeds both the collapse surface pressure of the lipid film in the absence of procolipase and the surface pressure of 17.7 ± 0.4 mN/m achieved with procolipase addition in the absence of lipid. Extending the results obtained with monolayers of a single lipid species, these results show that procolipase interacts strongly and preferentially with lipase substrates even in the presence of SOPC.

Procolipase-Induced Surface Pressure Increases Reflect Procolipase Adsorption. Implicit in the interpretation of the data given above is the assumption that it is procolipase and not a surface-active impurity in the procolipase preparation which is responsible for the observed surface pressure changes following procolipase addition. At the conclusion of each experiment described above, the lipid monolayer was collected, and the two-dimensional concentration of procolipase in the monolayer, Γ_P, was determined. Up to a lipid mole fraction of 0.5 DO or DA with SOPC, values of Γ_P for monolayers having initial surface pressures above zero showed a negative linear correlation with initial surface pressure (not shown). Like the changes in surface pressure, linearity of protein surface concentration with initial surface pressure is often observed for protein adsorption to lipid monolayers [e.g., Ibdah and Phillips, (1988)]. The values of initial surface pressure extrapolated to the initial surface pressure at which procolipase no longer adsorbs to the interface, designated $\pi_i|_{\Gamma_P=0}$, are given in Table 1. Figure 2 shows that for each lipid composition up to 0.5 DO or DA these values exhibit a 1:1 correlation with the values of $\pi_i|_{\Delta\pi=0}$ obtained with the same lipid mixtures. Unless an impurity is present which has surface properties identical to those of procolipase, this 1:1 proportionality in quantities that reflect the strength of the lipid–protein interaction indicates that the surface pressure changes are a direct consequence of procolipase adsorption over this range of lipid compositions. Above 0.5 mole fraction of DO or DA, positive values of procolipase adsorption were obtained at all initial surface pressures, but these were not linear with initial surface pressure. This lack of the expected linearity appears to reflect again the significant nonidealities of mixing between procolipase and DO or DA in substrate-rich interfaces shown above for surface pressure changes.

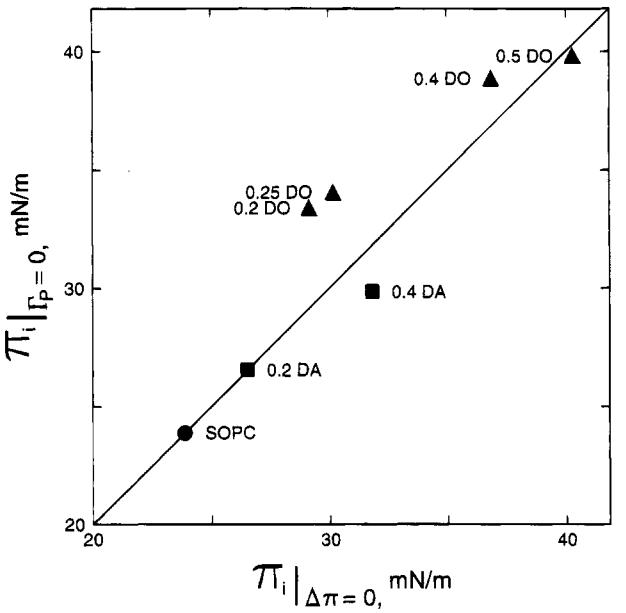

FIGURE 2: Relation of extrapolated initial monolayer surface pressure at which procolipase adsorption is blocked to that at which the surface pressure does not change following procolipase injection beneath mixed-lipid monolayers. The numbers and abbreviations indicate the mole fraction of one of the two lipid components comprising the film. The solid theoretical line has a slope of 1.0.

Procolipase Preferentially Perturbs Substrate Fluorescence. To further characterize the indicated preferential interaction of procolipase with lipase substrates as compared with phosphatidylcholine, the fluorescent pyrene-containing fatty acid and phosphatidylcholine analogs PDA and PDA-PC were used in aqueous lipid dispersions. Two lipid mixtures were prepared. The first was composed of 0.15

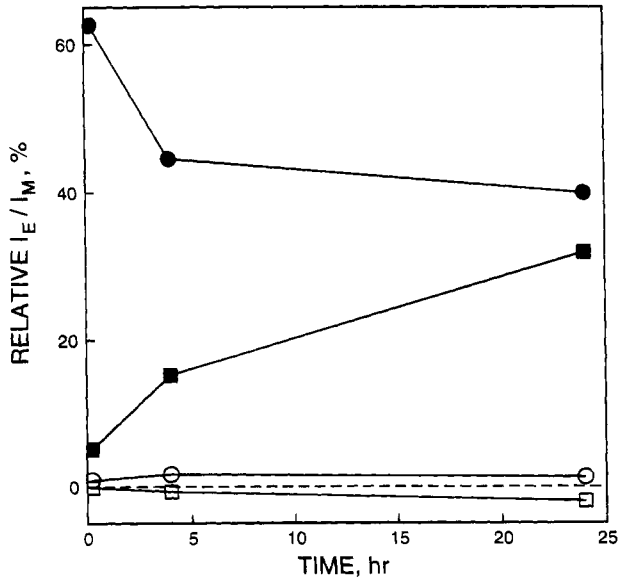

FIGURE 3: Time dependence of procolipase-induced changes in pyrene excimer to monomer ratios, I_E/I_M, relative to protein-free controls. Pyrenedecanoic acid probe was present as the free fatty acid (●, ■) or incorporated into phosphatidylcholine (○, □). Lipid concentration was 400 μM, and procolipase concentration was 2.74 μM (■, □) or 27.4 μM (●, ○).

mole fraction of PDA, 0.35 DA, and 0.50 of SOPC and the second of 0.50 mole fraction of DA, 0.15 of PDA-PC, and 0.35 of SOPC. Thus, in both mixtures the pyrene probe was present in 15% of the molecules which is equivalent to 10% of fatty acyl groups. An aliquot of each lipid mixture in organic solvent was dried and dispersed in aqueous buffer solution as described in Materials and Methods. This was split into two samples, concentrated procolipase solution was added to one of each pair of dispersions to a final concentration of 2.74 or 27.4 μM, and the pairs of tubes were frozen and thawed 10 times. After preparation the tubes were incubated at 24 °C. The fluorescence emission of a fresh dilution of each sample was measured at 0.25, 4, and 24 h after preparation. From these data values of I_E/I_M were calculated for each sample at each time point, and the percentage change in I_E/I_M for each procolipase-containing sample relative to its protein-free control at that time point was calculated. Measurements of apparent fluorescence at the excitation wavelength, i.e., light scattering, showed that the lipid dispersions underwent minimal changes in aggregation state during the 24 h incubation period.

As shown in Figure 3, if the fluorescent label was present in the phosophatidylcholine, the relative change in I_E/I_M was less than 2% over the 24 h incubation period. If unesterified pyrenedecanoic acid was present in the sample, increases in I_E/I_M of 15−45% relative to protein-free controls were observed between 4 and 24 h (Figure 3). At the 10-fold higher procolipase concentration the relative increase in I_E/I_M was larger at all time points. These results show that the presence of procolipase increases I_E/I_M of pyrenyl groups only if the probe is present as the free fatty acid.

Additional experiments were conducted to further test the apparent preference of procolipase for interaction with lipase substrates relative to phosphatidylcholine. In one set, PDA and PDA-PC were again used at 0.15 mole fraction with the lower procolipase concentration following the protocol described above, but the total mole fraction of free fatty acid was 0.15 in all mixtures. The observed changes in I_E/I_M were comparable to those obtained at the same level of procolipase when the total mole fraction of unesterified fatty acid was 0.50. Comparable results were also obtained when the freeze−thaw cycles were replaced by sonication for 1 h. In addition, monolayer experiments of the type described in the preceding section were also performed using lipid mixtures with 0.15 mole fraction PDA and either 0.15 or 0.50 total mole fraction of fatty acid with SOPC. The increases in surface pressure following procolipase addition to the aqueous subphase, values of $\pi_i|_{\Delta\pi=0}$, and the levels of adsorbed procolipase were also comparable to those observed at similar mole fractions of fatty acid in the absence of PDA. To measure the ability of procolipase to solubilize fatty acid, the quantity of PDA in the aqueous phase following 30 min exposure of a monolayer containing 0.15 mole fraction of PDA to a stirred subphase containing 240 nM procolipase was determined. This amounted to <3% of the total PDA initially present in the film. Overall, these results suggest that the presence of PDA in the monolayers and bilayers does not markedly perturb the interaction of procolipase with the lipids and confirm that procolipase does not catalyze dissolution of fatty acid (see Discussion).

The Procolipase−Substrate Interaction Is Not Stoichiometric. Over the range of low lipid concentrations, for which the initial surface pressure is approximately zero, the measured increase in surface pressure following procolipase addition depends on the lipid composition as well as the lipid concentration in the monolayer. In this range for monolayers of SOPC alone, the surface pressure increase is essentially constant near the value observed in the absence of lipid. As the initial surface pressure exceeds zero, the change in surface pressure then decreases. With DO or DA, however, the increase in surface pressure due to procolipase addition goes through a maximum as surface lipid concentration increases. These differences in behavior are exemplified in Figure 4, which shows the change in surface pressure as a function of surface lipid concentration, Γ_L, at selected lipid compositions. Γ_L is the sum of the two-dimensional concentrations of SOPC, DO, and DA, i.e., $\Gamma_S + \Gamma_{DO} + \Gamma_{DA}$, and is used here in place of initial surface pressure simply because the initial surface pressure is effectively zero for most of the low lipid concentrations. The higher maxima in the plots at higher substrate mole fractions again reflect the stronger interaction of procolipase with the substrates as opposed to SOPC. Because the surface concentration of procolipase decreases linearly with increasing lipid concentration (Figure 5A,B) over the lipid concentration range which includes the maxima in surface pressure changes (Figure 4), it was possible that the maxima shown in Figure 4 might reflect a stoichiometric interaction between procolipase and the non-phospholipids. However, no normalization of the abscissa of Figure 4 with respect to species, acyl group concentration, or lipid/procolipase ratio supported this hypothesis.

In testing for the presence of stoichiometric interactions, it was noted that the lipid concentration at which each maximum shown in Figure 4 occurred, estimated by differentiating a cubic spline approximation, correlates with the lipid concentration which defines the end of the gaseous to liquid-expanded monolayer phase transition for the lipid film in the absence of procolipase. Effectively, this is the concentration at which the initial surface pressure of the lipid monolayer becomes nonzero. To accurately estimate this

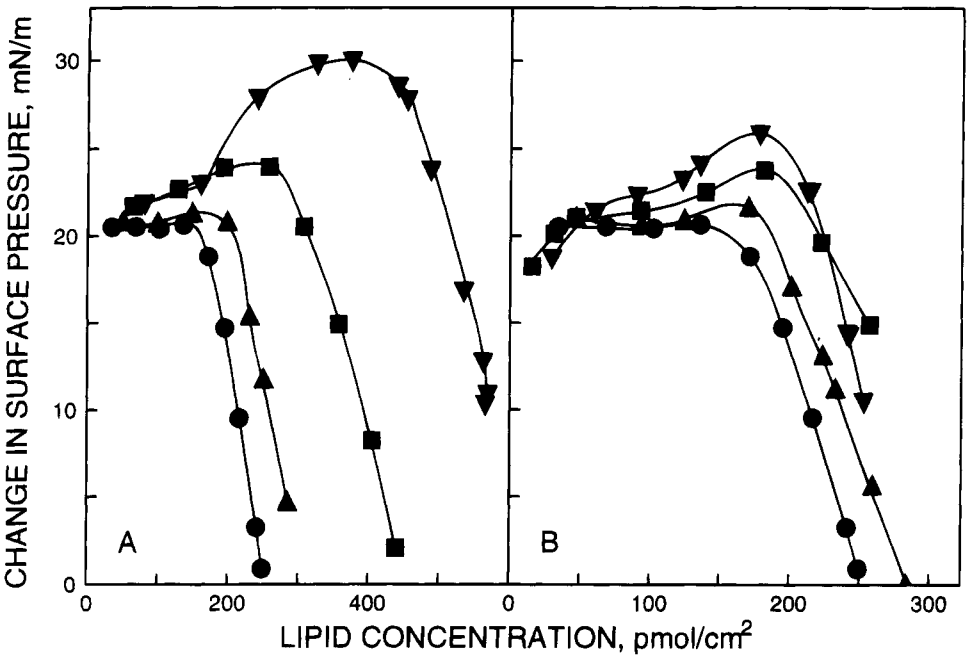

FIGURE 4: Lipid concentration dependence of equilibrium surface pressure changes at selected compositions following procolipase injection beneath mixed-lipid monolayers . The mole fraction of DA (panel A) or DO (panel B) in mixtures with SOPC was 0.0 (●), 0.20 (▲), 0.60 (■), or 1.00 (▼).

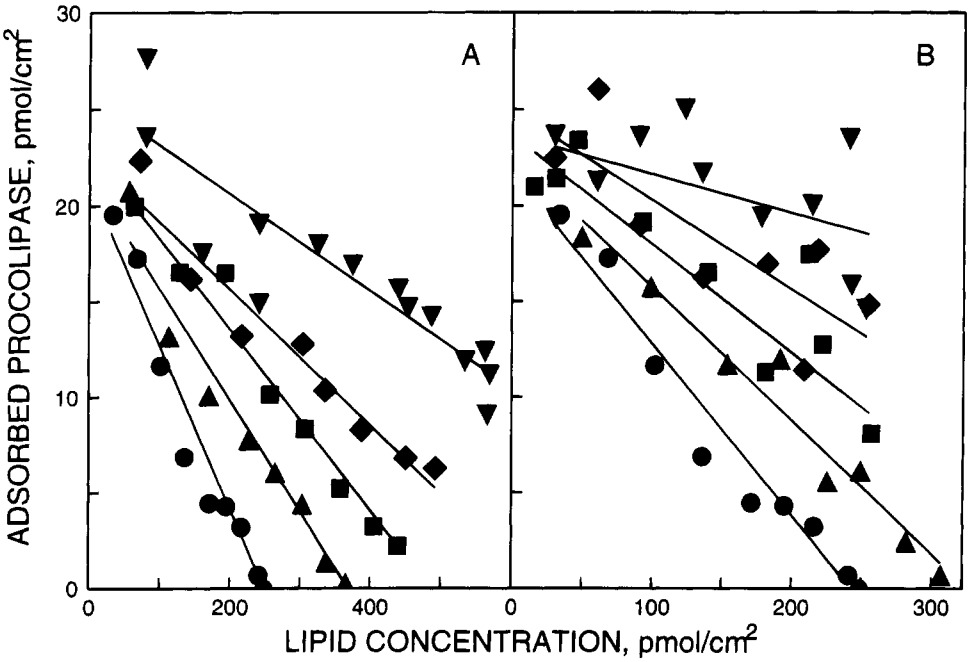

FIGURE 5: Lipid concentration dependence of equilibrium procolipase adsorption at selected compositions following its injection beneath mixed-lipid monolayers. The mole fraction of DA (panel A) or DO (panel B) in mixtures with SOPC was 0.0 (●), 0.40 (▲), 0.60 (■), 0.80 (◆), or 1.00 (▼). Solid lines are least-squares fits.

"lift-off" concentration, the liquid-expanded region of the surface pressure−area isotherm for each lipid mixture obtained in the absence of procolipase was fitted to an equation for that state described earlier (Smaby & Brockman, 1992). The fitting parameters obtained were then used to calculate the lipid concentration in the film extrapolated to a surface pressure of zero. Figure 6 shows that with the exception of SOPC alone (filled circle), which did not show a clearly defined maximum in Figure 4, the lipid concentration at which the increase in surface pressure was maximal following procolipase addition shows a good correlation (r = 0.985) with calculated lift-off concentrations. The solid, theoretical line in the figure has a slope of 1.0. Thus, the data in Figures 4 and 6 suggest that the differences between the interaction of procolipase with SOPC and the lipase substrates do not arise from stoichiometric interactions of procolipase with DO and DA. Rather, they reflect the more pronounced deviations from mixing ideality for procolipase interactions with DO and DA.

Procolipase−Lipid Packing Obeys a Simple, Geometric Model. In testing for the existence of stoichiometric interactions between procolipase and lipids, as described above, a negative, linear correlation was noted between the surface concentration of lipid and the surface concentration of

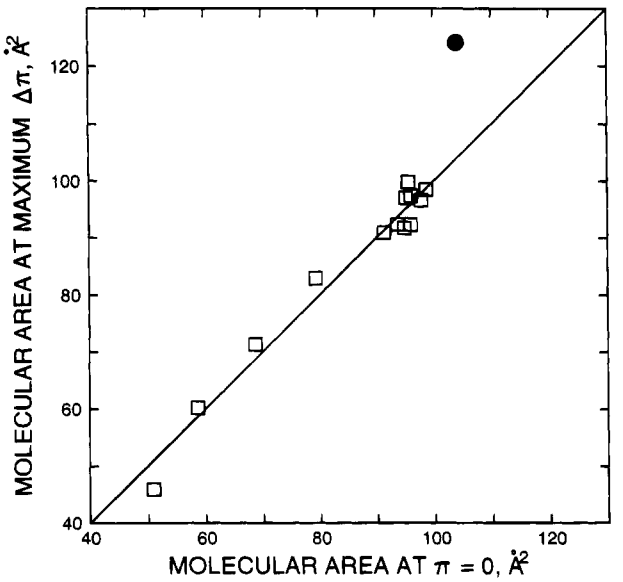

FIGURE 6: Relation of the molecular area at which the maximal change in surface pressure is induced by procolipase to the calculated average lipid molecular area at zero surface pressure. Squares (□) represent data obtained at lipid compositions other than that for SOPC alone (●). The solid theoretical line has a slope of 1.0.

procolipase. This is shown by the representative data sets plotted in Figure 5. Good linearity was observed at most lipid compositions and over a wide range of lipid/procolipase ratios. Not shown, for clarity, are additional data sets obtained with 0.2 mole fraction of DA and with 0.20, 0.25, 0.5, and 0.7 mole fractions of DO. The data sets showing the most scatter of points about their respective regression lines are those obtained at mole fractions of DO > 0.6. At each lipid composition used, the negative linear correlation between the surface concentration of procolipase and the surface concentration of lipid implies that the surface phase can be considered as being occupied by procolipase and, collectively, lipid molecules with procolipase and lipid each having a partial molecular area which is independent of the lipid/procolipase ratio in the monolayer and the surface pressure of the monolayer (Muderhwa & Brockman, 1990). If the partial molecular areas of procolipase and lipid in all monolayers having the *i*th lipid composition are A_{iP} and A_{iL} and their measured surface concentrations are Γ_P and Γ_L, then from simple geometric considerations it is readily shown that

$$\Gamma_P = 1/A_{iP} - \Gamma_L A_{iL}/A_{iP}$$

For each data set of the type exemplified in Figure 5, A_{iL} and A_{iP} were determined from its regression line. The values obtained and the regression coefficients are summarized in Table 2. The values of A_{iP} are reasonably constant and average 717 ± 34 Å²/molecule of procolipase. This supports the notion that the conformation of procolipase in the presence of different lipid mixtures is constant at a surface pressure- and lipid composition-independent value similar to the value of 725 ± 14 Å² determined in the absence of lipid.

As might be anticipated from the use of different lipid mixtures, the values of A_{iL} were not constant. Instead, they decreased monotonically with increasing mole fraction of DO or DA (Table 2). Plotting A_{iL} versus the mole fraction

Table 2: Partial Molecular Areas of Lipid and Procolipase in Mixed Films

lipids	mole fraction	lipid area, (Å²)	procolipase area, (Å²)	regression coefficient
DA/SOPC	0.000	69.0	766	0.979
	0.195	54.9	718	0.993
	0.401	46.0	764	0.980
	0.601	34.9	702	0.987
	0.798	26.0	733	0.969
	1.000	16.2	656	0.895
DO/SOPC	0.200	58.4	673	0.982
	0.252	56.6	749	0.962
	0.398	51.2	738	0.980
	0.497	49.7	747	0.976
	0.602	40.2	698	0.956
	0.702	33.1	722	0.907
	0.797	30.1	672	0.801
	1.000	15.0	699	0.544

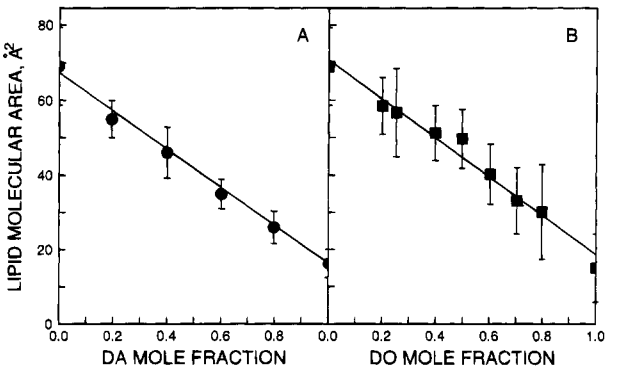

FIGURE 7: Lipid composition dependence of lipid partial molecular area. Partial molecular areas were calculated from the *X*-intercepts of fitted lines exemplified in Figure 6.

of DO or DA yields a straight line for each set of mixtures (Figure 7). This linearity indicates that in the mixed lipid–protein monolayer the partial molecular area of each lipid species, like that of procolipase, is essentially constant at all surface pressures and lipid compositions as well as at all procolipase/lipid ratios. Thus, for all lipid–procolipase mixed monolayers,

$$\Gamma_P = 1/A_P - \Gamma_S A_S/A_P - \Gamma_{DO} A_{DO}/A_P - \Gamma_{DA} A_{DA}/A_P \quad (1)$$

where the absence of the subscript *i* indicates that these values are effectively independent of lipid composition, surface pressure, and procolipase/lipid ratio.

From the intercept of the regression line for each set of mixtures, i.e., at a DO or DA mole fraction extrapolated to zero (Figure 7), values of 70.7 and 66.9 Å² were obtained for the partial molecular area of SOPC, A_S. From the slopes and intercepts the partial molecular area of DO, A_{DO}, was 18.4 Å² and that of DA, A_{DA}, was 15.3 Å². Because of the linearity of the data sets shown in Figures 5 and 7 and because the values of A_P and A_S determined from them are, within error, identical, the entire array of Γ_P versus lipid concentration data points was fitted to eq 1 using multiple linear regression. For this analysis the residuals, determined as the measured less the calculated values of Γ_P, are plotted in Figure 8A as a function of Γ_P. They show a fairly uniform distribution about zero, except at the highest values of Γ_P. The more deviant points were from the mixtures rich in DO noted above. The values of the partial molecular areas for procolipase, SOPC, DO, and DA, calculated from the

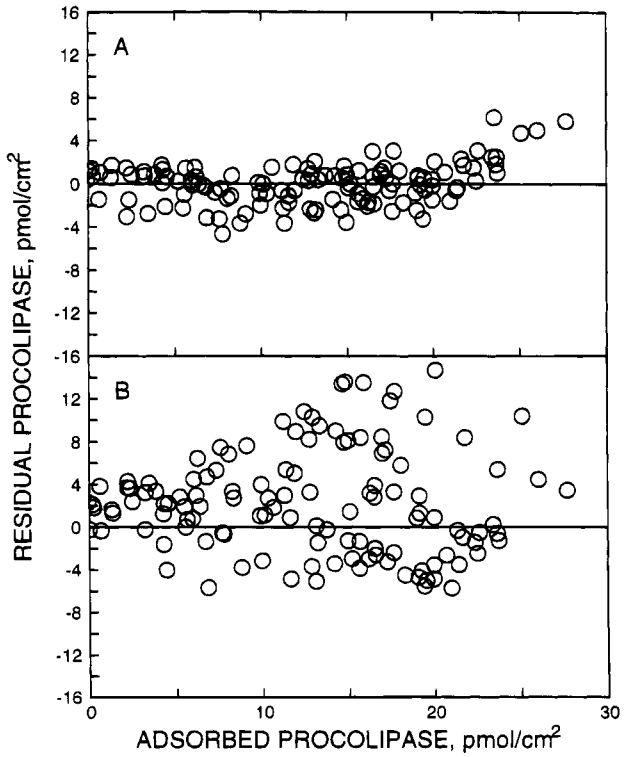

FIGURE 8: Residuals for procolipase adsorption to mixed lipid monolayers determined by multiple linear regression fitting of all procolipase adsorption data to interfacial models. Data were fitted to eq 1 with optimization of partial molecular areas of procolipase and each lipid species (panel A) or procolipase only with lipid areas for each point determined from independent measurements as described in the text (panel B).

regression parameters, are 708, 70.1, 17.9, and 14.4 Å^2/molecule, respectively. They are, within error, identical to those obtained by the stepwise analysis, consistent with the notion that with a saturating level of procolipase in the subphase, each lipid species and procolipase occupies an interfacial area which is independent of surface pressure, lipid composition, and procolipase/lipid ratio. For comparison, the complete data set was also fitted to a compressibility-based model (see Discussion). Specifically, using an equation of the form of eq 1, at each data point all lipid molecular areas were set to those measured in the absence of procolipase, at the final surface pressure attained in the presence of procolipase and only the molecular area of procolipase was optimized. The regression coefficient for this compressibility model is only 0.58, compared with 0.96 for the building block model, and the partial molecular area of procolipase obtained is 580 Å^2.

DISCUSSION

Most globular proteins will adsorb to an air−water or oil−water interface, causing a decrease in the interfacial tension, i.e., an increase in the surface pressure. A typical characteristic of nonspecific, i.e., charge-independent, protein adsorption to phospholipid-containing interfaces is the linear relationship between protein- induced change in the surface pressure and the initial surface pressure of the monolayer at a fixed protein concentration in the bulk aqueous phase (Camejo et al., 1968; Quinn & Dawson, 1970; Bougis et al., 1981; Weinberg et al., 1992). Similarly, the concentration of protein adsorbed to a phospholipid-covered interface generally decreases as the initial surface pressure of the monolayer is increased [e.g., Ibdah and Phillips, (1988)]. For the adsorption of many globular proteins, the experimental range over which the initial surface pressure of the lipid monolayer can be varied is limited because of protein denaturation at initial surface pressure values <10−20 mN/m. This means that the change of lipid molecular area over which protein adsorption can be studied is typically less than 30%, even if the monolayers are liquid expanded. Exceptions to the general rule are the serum apolipoproteins A which, by virtue of being able to form amphipathic helices, adsorb reversibly to air− or lipid−water interfaces [e.g., Weinberg et al., (1992)]. This property has allowed comparison of the adsorption of, for example, apolipoproteins A in the presence and absence of lipid and forms the basis of current thinking about nonspecific, i.e., charge-independent, adsorption of undenatured proteins to biological interfaces (Ibdah & Phillips, 1988; Ibdah et al., 1989; Weinberg et al., 1992). It has been concluded that apolipoproteins compress the lipid molecules in the monolayer to the molecular area that they would occupy in the absence of adsorbed protein but at the value of the surface pressure reached in the presence of protein. As defined, this compressibility model is limited to cases of lipid−protein immiscibility or nearly ideal miscibility in the interface. It successfully describes the adsorption of apolipoproteins A when the fraction of surface area available for protein adsorption is between zero and 0.25 of total monolayer surface (Ibdah & Phillips, 1988; Ibdah et al., 1989; Weinberg et al., 1992) and, in most cases, shows that the area occupied by the adsorbed protein is similar to that obtained in the absence of a lipid monolayer. This identity and the observation that apolipoproteins A and C can adsorb to monolayers having initial surface pressure values 9−11 mN/m higher than the value of the surface pressure generated by the same bulk concentration of protein in the absence of lipid (Krebs et al., 1983, 1988; Ibdah & Phillips, 1988; Weinberg et al., 1992) indicate miscibility of the lipid and protein. Recently, a more nonideal example of apolipoprotein A-I adsorption was observed with 1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine (Parks & Thuren, 1993). In this case the apparent area per protein molecule was 35% larger than at the air−water interface. Within the context of the compressibility model, it was necessary to postulate that the polyunsaturated acyl moiety of the phosphatidylcholine was forced into a more compact conformation than it would have occupied in the absence of protein.

Procolipase is also stable to denaturation in the presence of a high energy oil−water interface. In this case, however, stability stems from disulfide cross links which prevent protein unfolding at the interface. The crystal structures of the human lipase−procolipase complexes (van Tilbeurgh et al., 1992, 1993) show that the surface activity of procolipase arises not from amphipathic helices but rather from three hydrophobic "fingers" which constitute one face of the molecule. Thus, aside from its functional significance in the interfacial activation of pancreatic lipase, procolipase is an apparently unique model protein with which to study lipid−protein interactions. To provide a complete set of data from which to evaluate these interactions, 123 measurements of procolipase-induced surface pressure changes and surface concentrations of procolipase were obtained over a range of lipid concentrations which would provide variation of the fraction of surface area available for procolipase adsorption

from zero to one. Moreover, because procolipase functions in the presence of fatty acids, glycerides, and phospholipids *in vivo*, its adsorption was characterized using SOPC−DO and SOPC−DA mixed monolayers covering the range of DO and DA compositions from zero to one. Because pancreatic lipase was absent, no changes in lipid composition occurred during the adsorption measurements.

One unexpected finding, observed as a consequence of the wide range over which the surface concentration of lipid was varied, was the existence of maxima in plots of the change in surface pressure versus lipid concentration (Figure 4). These correlated not with a particular lipid−procolipase ratio but rather with the end of the gaseous-to-liquid expanded monolayer phase transition (Figure 6). As a consequence of these maxima, at any lipid composition the *X*-intercept of a plot of the change in surface pressure versus the initial surface pressure, $\pi_i|_{\Delta\pi=0}$, does not coincide with the corresponding *Y*-intercept of the line, $\Delta\pi|_{\pi i=0}$ (Table 1). This observation suggests that, for protein adsorption to different lipid mixtures, the difference $\Delta\pi|_{\pi i=0} - \pi_i|_{\Delta\pi=0}$ can be used as a relative measure of mixing nonideality in the lipid−protein monolayer. Using this measure or the increasing values of the maximum surface pressure attained following procolipase addition (Figure 1 and Table 1), a clear conclusion from measurements of procolipase-induced surface pressure changes is that procolipase interacts more strongly with DO and DA, both substrates of lipases, than with SOPC, a prototypical surface component of substrate emulsions.

The surface pressure measurements indicate that procolipase has the ability to laterally concentrate DO and DA in its vicinity relative to SOPC. This is a novel and unexpected observation. To study this selectivity more directly, rather than by thermodynamic inference, aqueous dispersions of pyrene-containing lipids were used. A much exploited feature of the fluorescence of pyrene-containing lipids is the formation of excimers, the concentration of which varies directly with their two-dimensional concentration in a lipid−water interface [e.g., Kinnunen et al., (1993)]. Because of this characteristic of pyrene-containing lipids, any tendency of procolipase to concentrate PDA laterally in its vicinity should increase I_E/I_M. Experimentally, it was observed that I_E/I_M increased in a procolipase-dependent manner if the pyrene probe was in the free fatty acid but did not when it was in the phosphatidylcholine. Within the usual uncertainties and limitations accompanying the interpretation of data obtained with an extrinsic fluorescent probe, these data indicate the specificity of procolipase interaction with fatty acid as opposed to SOPC.

When lipid monolayers were comprised of SOPC only, the adsorption behavior of procolipase was similar to that reported for the adsorption of the apolipoproteins A and C to phospholipids. Plots of the change in surface pressure versus initial surface pressure are linear, and the value of $\pi_i|_{\Delta\pi=0}$ is 6 mN/m higher than the surface pressure generated by procolipase in the absence of a lipid monolayer. The latter indicates the miscibility of procolipase and SOPC. Also for adsorption of procolipase to SOPC alone, the surface concentration of procolipase is proportional to the fraction of available area calculated using the compressibility-based model ($r = 0.98$, data not shown) and yields a reasonable value of 770 Å^2 for the partial molecular area of procolipase. This is somewhat higher than the value of 725 Å^2 determined in the absence of lipid and that of 707 Å^2 determined in the presence of lipid determined using eq 1. However, the compressibility model fails to accurately describe the results when applied to data obtained with DO- and DA-rich monolayers (Figure 8B).

The assumption of constant partial molecular areas of lipid species in monolayers over a subphase containing a surface-active solute was first introduced and thermodynamically justified by Pethica (1955). In the present study it was possible to measure directly the surface concentration of the adsorbing species, procolipase, and the applicability of this "building block" model, eq 1, was shown by analysis of the data obtained (Figures 5, 7, and 8A). At one level, better fitting of the data with the building block model as compared to the compressibility model is assured because it has one adjustable parameter for each monolayer component. Its strength, however, is that it does not necessitate *a priori* assignment of mixing nonidealities to one or the other of the species present but reflects them from the data. Do nonidealities arise from changes in lipid area, protein area, or both when they interact? As recently pointed out, attributing all nonideality to the lipid can create uncertainties in the interpretation of the parameters obtained when the compressibility model is used (Parks & Thuren, 1993). In the present study of procolipase−lipid interactions, the parameters obtained from the multiple linear regression analysis using the building block model clearly indicate the origin of the mixing nonidealities. The value of A_P of 707 Å^2/molecule agrees, within error, with that of 725 ± 14 Å^2 determined in the absence of lipid, suggesting no major reorientation or conformational change in the procolipase molecule when it adsorbs to an interface in which lipid is present. The value of A_S of 70 Å^2/molecule is slightly higher than that of 66 Å^2/molecule for SOPC in the absence of protein at 24 mN/m, the value of $\pi_i|_{\Delta\pi=0}$ for procolipase adsorption to SOPC alone. This indicates a positive nonideality of mixing between SOPC and procolipase in the interface. In contrast, the values of A_{DA} and A_{DO} are only 14.4 and 17.9 Å^2 per molecule, respectively, compared with their molecular areas at monolayer collapse of 28.2 and 63.3 Å^2. These unexpectedly small values for A_{DA} and A_{DO} clearly indicate that the interaction of procolipase with DA and DO is highly nonideal.

A structural basis for the molecular areas of DA and DO obtained from fitting the data to the building block model is suggested by the values being approximately one-half to one-third of the collapse areas of the lipids in the absence of procolipase. These unexpectedly low values suggest that procolipase induces a transformation of the *lipid monolayer* into a *lipid multilayer*. On the basis of the ratios of A_{DA} and A_{DO} to their respective collapse areas, DA and DO occupy a bilayer or trilayer arrangement as illustrated in Figure 9. That SOPC does not undergo this same transformation in the presence of procolipase is likely a consequence of the large free energy which would be required to dehydrate the zwitterionic phosphorylcholine headgroup and move it into a low-dielectric medium. Particularly for DO, however, the presence of SOPC seems to contribute to the stability of the multilayer structure. This is suggested by the lesser scatter in values of Γ_P observed in SOPC-rich monolayers (Figure 5).

Alternatives to the model illustrated in Figure 9 were considered, but none gave results consistent with the data.

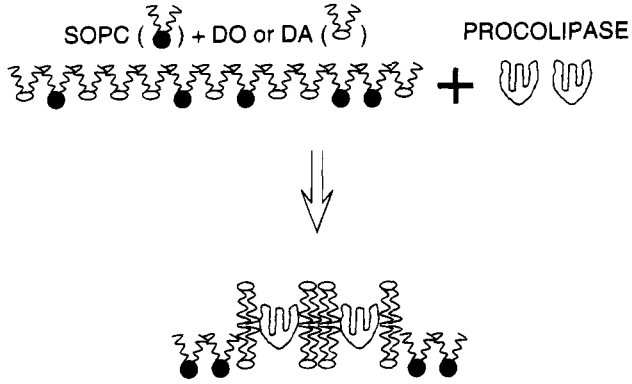

FIGURE 9: Hypothetical model for interfacial structure of procolipase-containing mixed-lipid monolayers derived from multiple linear regression analysis of procolipase adsorption data. Drawing is approximately to scale.

For example, procolipase could simply displace DA and DO from the interface to form a bulk phase of DO or DA. However, this cannot explain procolipase-induced increases in surface pressure to values higher than the collapse pressure of DO or DA and should give molecular areas of DA and DO of zero. Also, by themselves, DO and DA are more surface active than procolipase. It could be postulated that a fraction of the DO and DA molecules are displaced from the interface by procolipase while others remain as a monomolecular film. However, over the wide range of procolipase/lipid ratios studied, this type of model is inconsistent with the linearity observed in Figures 5 and 7, unless it is also postulated that the displaced and nondisplaced pools of DA and DO are filled in constant proportion. The same argument also applys to the possibility that DO and DA are solubilized into the aqueous bulk phase by procolipase. Moreover, this explanation is inconsistent with studies of the pancreatic lipase-catalyzed hydrolysis of [^{14}C]-DO in mixed monolayers with SOPC. Films exposed to 120 nM procolipase for 20 min were recovered with the same efficiency as those not exposed to procolipase (Cunningham et al., 1989; W. E. Momsen and H. L. Brockman, unpublished results). The mole fraction of DO was varied from 0.1 to 1 in this earlier study, and its hydrolysis by the 10 nM pancreatic lipase present ranged from 4 to 100%. This indicates that procolipase did not catalyze the dissolution of the [^{14}C]oleic acid formed. Thus, the building block model described by eq 1 and illustrated in Figure 9 is the simplest that is consistent with the data.

The formation of multilayer structures by uncharged lipids in the absence of protein has considerable precedent for non-phospholipid films in which the aliphatic moieties are saturated. Multilayer formation characterized by phase transitions at one-half or one-third the collapse area of the monolayer have been observed with triacylglycerols (Bursh et al., 1968; Larsson, 1973), diacylglycerols (Tajima & Gershfeld, 1978; Larsson, 1973), protonated fatty acids (Larsson, 1973; McFate et al., 1993; Weinbach et al., 1994), wax esters (Larsson, 1973), and cerebrosides (Johnson & Chapman, 1989). Although trilayer formation is well documented for protonated, saturated fatty acids, it was not observed for oleic and other fatty acids with *cis* unsaturation. Likewise, DA or DO monolayers compressed to one-third their collapse area do not exhibit a measurable phase transition (data not shown). This suggests that procolipase, by virtue of its apolar "fingers" firmly anchored at the lipid–water interface (van Tilbeurgh et al. 1993), is able to limit the motions of the acyl chains sufficiently to stabilize multilayer structures comprised of DO and DA. Also, the presence of phosphatidylcholine in the layer adjacent to the bulk water phase seems to contribute to multilayer stability. The formation of such a hybrid phospholipid–cholesteryl ester structure in the absence of protein was previously observed in studies of mixed-lipid films at the argon–buffer interface (Smaby & Brockman, 1981a,b).

The activities of both pancreatic lipase, in the absence of colipase or procolipase, and carboxylester lipase toward either ester or fatty acid substrates in liquid-expanded monomolecular films containing 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine exhibit a critical dependence on the substrate mole fraction (Muderhwa & Brockman, 1992a,b. A major contributor to this phenomenon is the steric inhibition of lipase adsorption to the monolayer by phosphorylcholine headgroups (Muderhwa & Brockman, 1990). Such inhibition of pancreatic lipase adsorption is also observed with phospholipid-stabilized triglyceride emulsions but is overcome by procolipase and/or colipase depending on conditions (Verger, 1984). The ability to overcome this inhibition of lipase adsorption has been attributed to the ability of procolipase and colipase to form a 1:1 complex with pancreatic lipase and to adsorb (a) to phospholipid-rich interfaces at higher lipid packing densities than lipase alone and (b) to clusters of the calcium soaps of fatty acids at pH 8.0. Under more physiological conditions fatty acids are largely protonated and are miscible with phosphatidylcholines in liquid-expanded monolayers (Smaby et al., 1994). This suggests that the enhancement of lipolysis by calcium-induced clustering of fatty acids will not occur. However, as shown in this study procolipase interacts with protonated fatty acid, as well as with diacylglycerol, much more strongly than with phosphatidylcholine. This suggests that procolipase may facilitate pancreatic lipase–substrate and pancreatic lipase–fatty acid interactions as a consequence of its ability to increase the two-dimensional concentration of these lipids in the vicinity of the lipase–(pro)colipase complex. Moreover, the apparent ability of procolipase to stabilize multilayer lipid structures suggests that procolipase might also regulate substrate delivery from the bulk lipid phase of an emulsion droplet. Both of these are novel functions for procolipase, to be added to its recognized role as an anchor-activator for pancreatic lipase in phospholipid-rich interfaces.

ACKNOWLEDGMENT

We thank Dr. Paavo Kinnunen for his advice concerning the interpretation of data from experiments utilizing pyrene fluorescent probes. We also thank Dr. Mark Lowe for his helpful suggestions.

REFERENCES

Bernbäck, S., Bläckberg, L., & Hernell, O. (1990) *J. Clin. Invest. 85*, 1221.

Borgström, B. (1980) *Gastroenterology 78*, 954.

Borgström, B., Wieloch, T., & Erlanson-Albertsson, C. (1979) *FEBS Lett. 108*, 407.

Borgström, B., & Erlanson-Albertsson, C. (1984) in *Lipases* (Borgstrom, B., & Brockman, H. L., Eds.) pp 151–183, Elsevier Science Publishers, Amsterdam.

Bougis, P., Rochat, H., Pieroni, G., & Verger, R. (1981) *Biochemistry 20*, 4915.

Brockman, H. L. (1984) in *Lipases* (Borgström, B., & Brockman, H. L., Eds.) pp 1−46, Elsevier Science Publishers, Amsterdam.
Bursh, T., Larsson, K., & Lundquist, M. (1968) *Chem. Phys. Lipids 2*, 102.
Camejo, G., Colacicco, G., & Rapport, M. M. (1968) *J. Lipid Res. 9*, 562.
Canioni, P., Cozzone, P. J., & Sarda, L. (1980) *Biochim. Biophys. Acta 621*, 29.
Cevc, G., Seddon, J. M., Hartung, R., & Eggert, W. (1988) *Biochim. Biophys. Acta 940*, 219.
Cunningham, B. A., Tsujita, T., & Brockman, H. L. (1989) *Biochemistry 28*, 32.
de La Fournière, L., Ivanova, M. G., Blond, J. P., Carrière, F., & Verger, R. (1994) *Colloids Surf. B: Biointerfaces 2*, 585.
Erlanson-Albertsson, C. (1992) *Biochim. Biophys. Acta 1125*, 1.
Gargouri, Y., Moreau, H., & Verger, R. (1989) *Biochim. Biophys. Acta 1006*, 255.
Graham, D. E., & Phillips, M. C. (1979) *J. Colloid Interface Sci. 70*, 403.
Ibdah, J. A., & Phillips, M. C. (1988) *Biochemistry 27*, 7155.
Ibdah, J. A., Krebs, K. E., & Phillips, M. C. (1989) *Biochim. Biophys. Acta 1004*, 300.
Johnson, D. S., & Chapman, D. (1989) *Biochim. Biophys. Acta 937*, 10.
Kinnunen, P. K. J., Koiv, A., & Mustonen, P. (1993) in *Fluorescence Spectroscopy: New Methods and Applications* (Wolfbeis, O. S., Ed.) pp 160−171, Springer-Verlag, Berlin, Heidelberg.
Krebs, K. E., Phillips, M. C., & Sparks, C. E. (1983) *Biochim. Biophys. Acta 751*, 470.
Krebs, K. E., Ibdah, J. A., & Phillips, M. C. (1988) *Biochim. Biophys. Acta 959*, 229.
Larsson, K. (1973) *Surf. Colloid Sci. 6*, 261.
Larsson, A., & Erlanson-Albertsson, C. (1986) *Biochim. Biophys. Acta 876*, 543.
Larsson, A., & Erlanson-Albertsson, C. (1991) *Biochim. Biophys. Acta 1083*, 283.
Lindstrom, M., Sternby, B., & Borgström, B. (1988) *Biochim. Biophys. Acta 959*, 178.
Linthorst, J. M., Clark, B., & Holt, P. R. (1977) *J. Colloid Interface Sci. 60*, 1.
MacRitchie, F. (1978) *Adv. Protein Chem. 32*, 283.
McFate, C., Ward, D., & Olmsted, J., III (1993) *Langmuir 9*, 1036.
Muderhwa, J. M., & Brockman, H. L. (1990) *J. Biol. Chem. 265*, 19644.
Muderhwa, J. M., & Brockman, H. L. (1992a) *J. Biol. Chem. 267*, 24184.
Muderhwa, J. M., & Brockman, H. L. (1992b) *Biochemistry 31*, 149.
Parks, J. S., & Thuren, T. Y. (1993) *J. Lipid Res. 34*, 779.
Pethica, B. A. (1955) *Faraday Soc. Trans. 51*, 1402.
Phillips, M. C., Evans, M. T. A., & Hauser, H. (1975a) *Adv. Chem. Ser. 144*, 217.
Phillips, M. C., Graham, D. E., & Hauser, H. (1975b) *Nature 254*, 154.
Quinn, P., & Dawson, R. C. M. (1970) *Biochem. J. 119*, 21.
Rudd, E. A., & Brockman, H. L. (1984) in *Lipases* (Borgström, B., & Brockman, H. L., Eds.) pp 185−204, Elsevier Science Publishers, Amsterdam.
Smaby, J. M., & Brockman, H. L. (1981a) *Biochemistry 20*, 718.
Smaby, J. M., & Brockman, H. L. (1981b) *Biochemistry 20*, 724.
Smaby, J. M., & Brockman, H. L. (1990) *Biophys. J. 58*, 195.
Smaby, J. M., & Brockman, H. L. (1992) *Langmuir 8*, 563.
Smaby, J. M., Muderhwa, J. M., & Brockman, H. L. (1994) *Biochemistry 33*, 1915.
Staggers, J. E., Hernell, O., Stafford, R. J., & Carey, M. C. (1990) *Biochemistry 29*, 2028.
Sternby, B., Engstrom, A., Hellman, U., Vihert, A. M., Sternby, N.-H., & Borgström, B. (1984) *Biochim. Biophys. Acta 784*, 75.
Tajima, K., & Gershfeld, N. L. (1978) *Biophys. J. 22*, 489.
Thomson, A. B. R., & Dietschy, J. M. (1981) in *Physiology of the Gastrointestinal Tract* (Johnson, L. R., Ed.) pp 1147−1220, Raven Press, New York.
Tsujita, T., & Brockman, H. L. (1987) *Biochemistry 26*, 8423.
Tsujita, T., Muderhwa, J. M., & Brockman, H. L. (1989) *J. Biol. Chem. 264*, 8612.
van Tilbeurgh, H., Sarda, L., Verger, R., & Cambillau, C. (1992) *Nature 359*, 159.
van Tilbeurgh, H., Egloff, M.-P., Martinez, C., Rugani, N., Verger, R., & Cambillau, C. (1993) *Nature 362*, 814.
Verger, R. (1984) in *Lipases* (Borgström, B., & Brockman, H. L., Eds.) pp 83−150, Elsevier Science Publishers, Amsterdam.
Verger, R., & Pieroni, G. (1986) in *Lipids and Membranes: Past, Present and Future* (Op den Kamp, J. A. F., Roelofsen, B., & Wirtz, K. W. A., Eds.) pp 153−170, Elsevier Science Publishers B. V., Amsterdam.
Verger, R., Rietsch, J., & Desnuelle, P. (1977) *J. Biol. Chem. 252*, 4319.
Weinbach, S. P., Kjaer, K., Bouwman, W. G., Grübel, G., Legrand, J.-F., Als-Nielsen, J., Lahav, M., & Leiserowitz, L. (1994) *Science 264*, 1566.
Weinberg, R. B., Ibdah, J. A., & Phillips, M. C. (1992) *J. Biol. Chem. 267*, 8977.
Wieloch, T., Borgström, B., Pieroni, G., Pattus, F., & Verger, R. (1981) *FEBS Lett. 128*, 217.
Wieloch, T., Borgström, B., Pieroni, G., Pattus, F., & Verger, R. (1982) *J. Biol. Chem. 257*, 11523.

BI9500537