Lipid monolayers: interactions with the apoprotein of high density plasma lipoprotein

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ABSTRACT Monolayer techniques were used to study the interactions of various lipids (cholesterol, lysophosphatidyl choline, phosphatidal ethanolamine, phosphatidyl choline, sphingomyelin, stearic acid, and lipids extracted from plasma high density lipoproteins and very low density lipoprotein) with the lipid-free protein subunit of rat plasma high density lipoprotein and with rat plasma albumin. The proteins were injected under the lipid monolayer at fixed area, and the increase in surface pressure (decrease in surface tension) was measured as a function of time.

With all lipids, both the rate and magnitude of this increase were greater with the apolipoprotein than with albumin. The degree of film penetration of pure lipid films (at an initial film pressure of 15 dynes/cm) by the two proteins followed the same order: cholesterol > phosphatidal ethanolamine > phosphatidyl choline > stearic acid > sphingomyelin > lysophosphatidyl choline. Other variables studied were protein concentration, initial film pressure, and pH.

Two distinctive properties of the apolipoprotein were the penetration of lipid films at pressures above the collapse pressure of the protein, and the formation of a film even at low salt concentration. High surface activity and strong interaction of HDL-protein with lipid monolayers may be associated with the flexibility of the protein molecule due to absence of disulfide bridges. The unusual surface activity of HDL-protein may be intimately related to the mechanism of formation of the lipoprotein.

KEY WORDS lipid–protein interactions · high density lipoprotein · apolipoprotein · albumin · monolayers · stearic acid · cholesterol · phosphatidyl choline · phosphatidal ethanolamine · sphingomyelin · lysophosphatidyl choline

THE IMPORTANCE OF INTERACTIONS of lipids with proteins is well recognized in several areas of biology, particularly in connection with the structure, stability, and function of cell membranes and in the structure and function of plasma lipoproteins. Most lipids and proteins will display some degree of association when they are brought together, but few methods that are capable of distinguishing between specific and nonspecific interactions are available. The methods involving specific interactions are usually based on some biological activity of the protein that is enhanced or made manifest in the presence of the lipid. Such studies have been conducted either in a bulk phase (1–4) or in films after the addition of protein or lipid (5, 6).

Most studies of interactions between lipid films and proteins have dealt with nonspecific interactions (7–9) because the number of models available for the study of specific interactions has been exceedingly limited. Very recently the protein moiety of rat plasma high density lipoprotein has been obtained as a water-soluble lipid-free molecule (10). This protein has thus become available as a model for studying specific interactions with both pure lipids and the mixture of lipids with which it is naturally associated. In this paper we report a comparison of the interaction of this HDL-protein (apolipoprotein) and of rat plasma albumin with monolayers of various lipids.

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Abbreviations: HDL, high density plasma lipoproteins; HDL-protein, lipid-free protein moiety of HDL (apolipoprotein); HDL-lipid, total lipid extracted from HDL; VLDL, very low density plasma lipoproteins; VLDL-lipid, total lipid extracted from VLDL.

Three techniques that are available for the study of lipid–protein interactions in interfacial films have recently been compared by Colacicco, Rapport, and Shapiro (9). In the procedure selected for this study, a monomolecular lipid film was spread at an air–water interface at constant area; the protein was injected into the subphase; and the decrease in surface tension (increase in surface pressure) was determined as a function of time.

One of the variables affecting the penetration of lipid films by protein is the initial film pressure. In the following studies, initial film pressures of 2 dynes/cm and 15 dynes/cm were used. At low initial film pressure, protein penetration into a lipid film is more rapid and more extensive than at higher pressure (9). Studies at both high and low initial film pressures were carried out since it is not yet known which condition is more closely related to biological systems.

A second reason for using an initial film pressure of 15 dynes/cm is that this is the collapse pressure of films of HDL-protein, and indeed of many other proteins (11). The collapse pressure of each separate component of a film is important in determining the degree of molecular interaction between substances in the film and substances which enter the film from the subphase. If a mixed film is compressed beyond the collapse pressure of an individual component, this component may be expected to leave the film unless additional stabilization occurs through noncovalent associations (interaction). Although the view has been expressed that only film penetration which takes place at an initial film pressure above the collapse pressure of the protein is significant for demonstrating lipid–protein interaction (7, 12), more recent work indicates that the important criterion is the *total* film pressure rather than the initial one (8, 9).

The third reason for studies with an initial film pressure of 15 dynes/cm stems from the observation that lecithin films are not penetrated at this high film pressure by proteins examined previously (7, 12). Since lecithin represents a very substantial portion of HDL-lipid, it was of interest to determine whether HDL-protein would behave differently with lecithin.

MATERIALS AND METHODS

Organic solvents were redistilled reagent grade products and were stored in dark bottles. All chemicals were reagent grade.

Analytical Methods

Phosphorus was determined by a modification of the method of Beveridge and Johnson (13) after perchloric acid digestion. Protein was determined by the method of Lowry, Rosebrough, Farr, and Randall (14), with rat plasma albumin as the standard. Total cholesterol and glycerides were measured according to the methods of Abell, Levy, Brodie, and Kendall (15) and van Handel, Zilversmit, and Bowman (16), respectively.

TABLE 1 CHARACTERISTICS OF HDL, HDL-PROTEIN, AND ALBUMIN FROM RAT PLASMA

	$S_{20,w}$	Mol. Wt.	Composition		
			Protein	Phospholipid	Total Cholesterol
			%		
HDL	5.8	246,000	41.7	39.6	18.7
HDL-protein	2.1	26,800	>99	—*	—
Albumin	4.5	65,000	>99	—	—

*—, not detected at a sensitivity level corresponding to 1%.

Proteins

HDL and the lipid-free protein subunit of rat plasma HDL were prepared as described by Camejo (10). The lipid-free protein is soluble in aqueous solutions of neutral salts and is homogeneous according to the criteria of gel electrophoresis and boundary sedimentation (10). The albumin was isolated from rat plasma according to the method of Schwert (17) and was also homogeneous. This albumin was subjected to the same delipidation procedure that was used to obtain the lipid-free HDL-protein. The proteins were stored at 2°C in Pyrex glass tubes at a concentration of 5 mg/ml in 0.17 M NaCl. The properties of the two proteins are summarized in Table 1.

Lipids

Total HDL lipids were extracted from purified rat plasma HDL by the method of Folch, Lees, and Sloane Stanley (18). Neutral HDL-lipids, obtained from the total lipids by silicic acid column chromatography, were shown by thin-layer chromatography to be free of phospholipid. Total and neutral VLDL-lipid were prepared similarly. Phosphatidyl choline (egg), cholesterol, and stearic acid were commercial products. The last two were recrystallized. Phosphatidal ethanolamine, prepared by partial alkaline hydrolysis of the ethanolamine glycerophosphatide fraction obtained from bovine brain white matter by diethylaminoethyl-cellulose chromatography, was supplied by Dr. M. Frosolono. Sphingomyelin was isolated from bovine heart (19). Lysophosphatidyl choline was obtained from the choline phosphatide fraction of bovine heart by treatment with rattlesnake venom (3). Lipids used for monolayer experiments were stored for not longer than 5 days in glass vials at 2°C at a concentration of 0.5 mg/ml in chloroform–methanol 85:15.

Water and Buffers

Protein and buffer solutions were prepared with distilled water that was redistilled over alkaline permanganate in an all-glass still. The conductivity of the water was 1 micromho/cm. Glycine (0.01 M) and phosphate (0.01 M) buffers were prepared according to Miller and Golder (20). Ionic strength was adjusted to 0.1 with NaCl. When not specified, experiments were performed in phosphate buffer of pH 7.6. Buffer solutions were stored in polyethylene bottles for not longer than 7 days.

Surface Pressure Measurements

We used a modification of the circular trough of Dawson (21), which consisted of a Pyrex crystallizing dish 6 cm in diameter and 3.5 cm high, with a glass barrier 4 cm long, 2 mm wide, and 1.5 cm deep sealed to the rim. The upper half of the dish and the barrier walls were covered with a coat of purified paraffin (mp 52°C). The trough was filled completely with buffer (60 ml) and the liquid surface was cleaned in the usual way (9); the final volume was 50 ml. The two surfaces thus formed were separated by the barrier above and had a common liquid chamber below. The larger surface (18.3 cm^2) was used to spread the lipid. The smaller surface was used for injecting the protein into the subphase without disturbing the film.

We determined the surface isotherms by adding successive portions (0.5 μl) of a lipid solution (0.5 mg/ml) in chloroform–methanol 85:15 and measuring the surface tension after each addition. The isotherm of phosphatidyl choline (lecithin) determined in this way coincided with that obtained by compression in a rectangular trough (6). Surface tension was measured by means of a Wilhelmy plate (22) (2.5 cm long, sand-blasted platinum) connected by a silk thread to the arm of a torsion balance. Changes in surface tension of 0.2 dynes/cm could be measured reproducibly. All experiments were carried out at an ambient temperature of 24 ± 1°C.

We formed the monolayer by touching the aqueous surface with the tip of the needle of a 10 μl Hamilton syringe and adding portions of lipid solution not larger than 0.5 μl. After each addition, the solvent was allowed to evaporate (10 sec). Lipid was added until the desired initial film pressure was reached. Then continuous magnetic stirring was started (Teflon-coated magnetized bar in the bottom of the dish). This caused no change in surface tension. Protein solution was injected into the subphase, just above the rotating bar, by means of a microsyringe. When not specified, the protein concentration in the subphase was 2 μg/ml (0.0002%).

RESULTS

Several definitions are necessary for the presentation of the results of protein interaction with lipid films. The initial film pressure, π_i, represents the decrease in surface tension of the aqueous phase caused by the presence of the film. Injection of materials into the subphase produces a further decrease in surface tension which is called the *increase in surface pressure* ($\Delta\pi$). When no further change is observed with time, the increase in surface pressure is referred to as the $\Delta\pi$ at equilibrium ($\Delta\pi_{eq}$). When the change in surface pressure must be considered in relation to the collapse pressure of the film, a quantity of interest is the final (or total) surface pressure π_f ($\pi_f = \pi_i + \Delta\pi$).

Interactions with Individual Lipids

Phospholipids. Films prepared from phosphatidal ethanolamine, sphingomyelin, and lysolecithin showed the characteristics of liquid expanded films, as did phosphatidyl choline (Fig. 1). Films of these lipids were studied at initial film pressures of 2 dynes/cm (Fig. 2) and 15 dynes/cm (Fig. 3). The equilibrium pressure ($\Delta\pi_{eq}$)

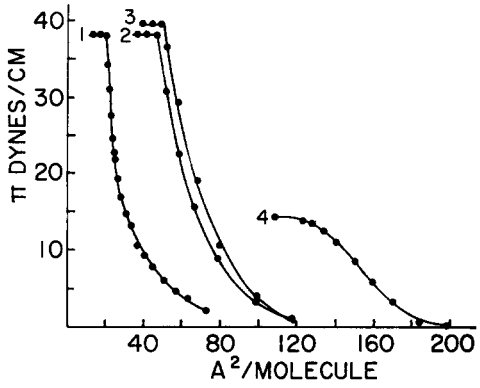

FIG. 1. Force–area curves of lipid film on phosphate buffer, pH 7.6, ionic strength 0.1. *1*, total HDL-lipids; *2*, phospholipid fraction from HDL; *3*, phosphatidyl choline; *4*, neutral lipid fraction from VLDL.

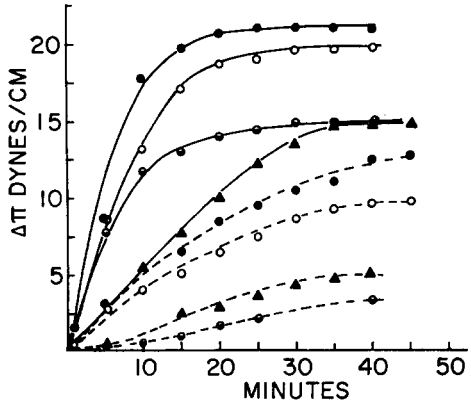

FIG. 2. Increase in surface pressure of phospholipid monolayers at a π_i of 2 dynes/cm on interaction with HDL-protein (solid lines) and albumin (broken lines). Lipid monolayers: ●, phosphatidal ethanolamine; ○, phosphatidyl choline; ◒, lysophosphatidyl choline; ▲, sphingomyelin. Subphase: phosphate buffer pH 7.6, ionic strength 0.1, protein concentration 2 μg/ml.

and rate of increase of π were greater at 2 than at 15 dynes/cm for both HDL-protein and albumin with all phospholipids. Also, HDL-protein showed a more rapid and a higher degree of interaction than albumin. With HDL-protein and a π_i of 2 dynes/cm, the $\Delta\pi_{eq}$ values followed the order phosphatidal ethanolamine > phosphatidyl choline > lysophosphatidyl choline > sphingomyelin, whereas with albumin the interaction with sphingomyelin was greater than that with lysolecithin. At 15 dynes/cm, the order of interaction with HDL-protein was phosphatidal ethanolamine > phosphatidyl choline > sphingomyelin > lysolecithin. With albumin, at this higher initial film pressure, $\Delta\pi_{eq}$ was larger with phosphatidal ethanolamine than with phosphatidyl choline, and no interaction was seen with either sphingomyelin or lysolecithin.

Cholesterol and Stearic Acid. Films of these substances are much less compressible than phospholipid films. The $\Delta\pi$–time curves show that both cholesterol and stearic acid films are readily penetrated by HDL-protein, and more rapidly and to a greater extent than by albumin (Figs. 4 and 5). The interaction of albumin with the stearic acid film was relatively weak: at an initial film pressure of 2 dynes/cm, the initial rate of interaction with albumin was only 1/10 that with HDL protein and, at 15 dynes/cm, no penetration by albumin was detected (Fig. 5). The interaction of albumin with cholesterol was decidedly greater than with any of the phospholipids; such a marked difference was not seen with HDL-protein.

Interactions with Mixed Lipid Films

The total lipid extracted from rat HDL had the following composition: phosphatidyl choline, 54.7%; sphingomyelin, 6.7%; lysophosphatidyl choline, 6.7%; cholesteryl esters, 28.7%; and free cholesterol, 3.1%. The π–A isotherm, determined on the basis of an average molecular weight of 727, shows that the film of this lipid has the characteristics of an expanded film *at low pressure*, with a collapse pressure of 38 dynes/cm at 20 A^2/molecule (Fig. 1, curve *1*). The film of total lipid shows a marked contraction compared to the film formed by its phospholipid components (curve *2*). The small area/molecule is due to special properties of films of cholesteryl ester–lecithin mixtures. At low film pressure, the area/molecule is the average of the areas of lecithin and cholesteryl ester. At high film pressure, the extraordinarily small area/molecule (20 A^2 at 38 dynes/cm) is due to the fact that, upon compression, cholesteryl esters are ejected from the monolayer, which at the end consists solely of lecithin (Colacicco, unpublished data). The isotherm of the phospholipid fraction (80% lecithin, 10% sphingomyelin, 10% lysolecithin) is very similar to that of purified lecithin (Fig. 1, curves *2* and *3*). The neutral HDL-lipid fraction (90% cholesteryl esters, 10% cholesterol) did not produce a stable film at

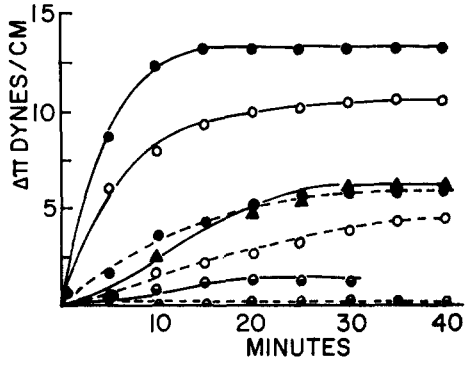

FIG. 3. Increase in surface pressure of phospholipid monolayers at a π_i of 15 dynes/cm on interaction with HDL-protein (solid lines) and albumin (broken lines). Lipid monolayers: ●, phosphatidal ethanolamine; ○, phosphatidyl choline; ◒, lysophosphatidyl choline; ▲, sphingomyelin. Subphase: as in Fig. 2.

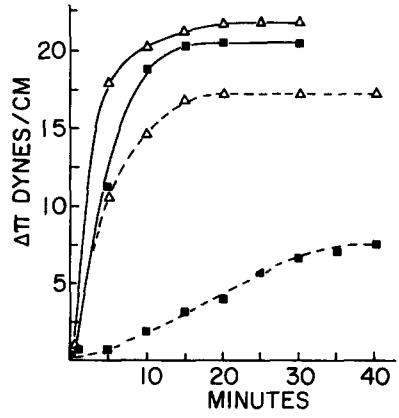

FIG. 4. Increase in surface pressure of monolayers of cholesterol and stearic acid at a π_i of 2 dynes/cm on interaction with HDL-protein (solid lines) and albumin (broken lines). △, cholesterol; ■, stearic acid. Subphase: as in Fig. 2.

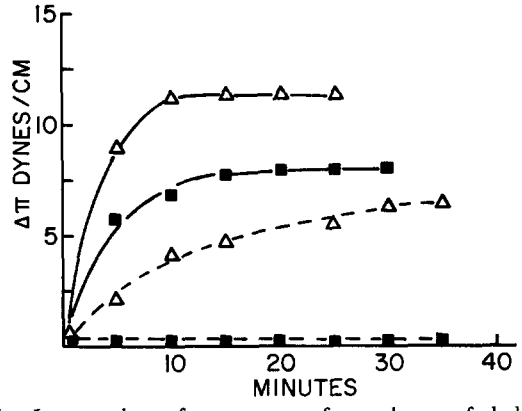

FIG. 5. Increase in surface pressure of monolayers of cholesterol and stearic acid at a π_i of 15 dynes/cm on interaction with HDL-protein (solid lines) and albumin (broken lines). △, cholesterol; ■, stearic acid. Subphase: as in Fig. 2.

25°C, nor did synthetic cholesteryl stearate. In contrast, the neutral lipid fraction from rat VLDL (80% glycerides, 6% cholesteryl esters, 6% cholesterol) gave a stable film with a collapse pressure of 15 dynes/cm, and

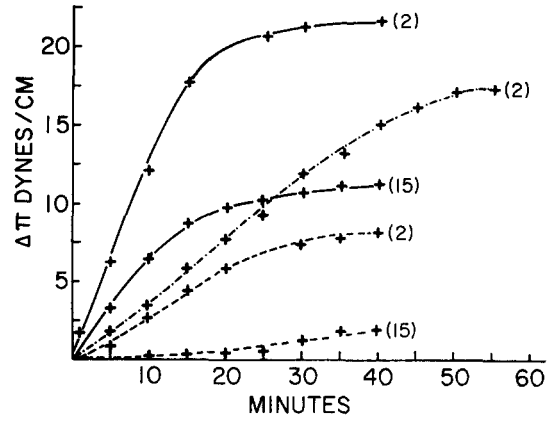

FIG. 6. Increase in surface pressure of monolayers of HDL-lipid on interaction with various proteins at π_i of 2 or 15 dynes (in parens)/cm. —, HDL-protein; -·-, HDL; ---, albumin. Subphase: as in Fig. 2.

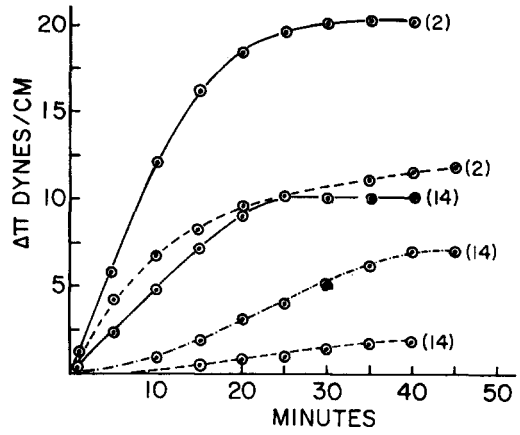

FIG. 7. Increase in surface pressure of monolayers of neutral VLDL-lipid on interaction with various proteins at a π_i of 2 or 14 dynes/cm. —, HDL-protein; -·-, HDl; ---, albumin. Subphase: as in Fig. 2.

was useful for studying protein penetration into lipid films of low collapse pressure.

The $\Delta\pi$–time curves for the interaction of HDL-protein, albumin, and HDL with total HDL-lipid (Fig. 6) show that the increase in surface pressure ($\Delta\pi_{eq}$) produced by HDL-protein was about 3 times as large as that found with plasma albumin. The rate of protein penetration into the film was about 4 times as great for HDL-protein as for albumin.

The interaction of albumin with a film of neutral VLDL-lipid (Fig. 7) at π_i values of either 2 or 14 dynes/cm did not produce increases in π above the collapse pressure of the lipid film (15 dynes/cm). In contrast, HDL-protein did cause an increase in pressure of 6–10 dynes above the collapse pressure of the film of mixed lipid, while intact HDL showed a similar, though smaller, increase above the collapse pressure.

Effect of pH

The extent of penetration of proteins into lipid films is affected by the pH of the aqueous subphase. Human and bovine serum albumin, γ-globulin, and hemoglobin show maximum penetration when the pH of the subphase is below the isoelectric point of the protein, irrespective of the kind of lipid film (7, 8, 12). This was also found for the interaction between rat plasma albumin and HDL-lipid: the $\Delta\pi$–pH curve (Fig. 8) shows higher values below pH 4 and a minimum between pH 7 and 8. In marked contrast, the interaction between HDL-protein and HDL-lipid was not greatly affected by pH between the values of 4 and 10 (Fig. 8).

Effect of Initial Film Pressure

A linear reciprocal relationship was found between $\Delta\pi_{eq}$ and π_i for HDL-protein injected under a monolayer of phosphatidyl choline (Fig. 9). With albumin, penetration

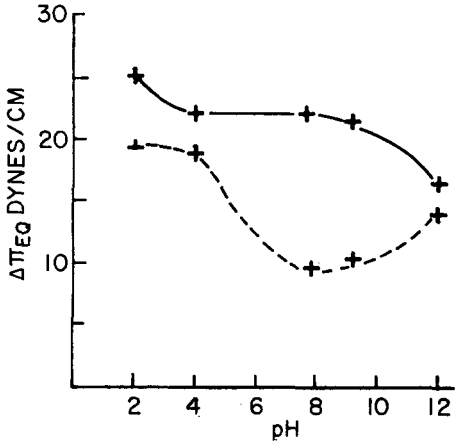

FIG. 8. Effect of pH on the penetration of monolayers of HDL-lipid by HDL-protein (solid line) and albumin (broken line). $\Delta\pi_{eq}$ values were obtained from the plateau region of surface pressure–time curves with films at a π_i of 2 dynes/cm and a protein concentration of 2 μg/ml.

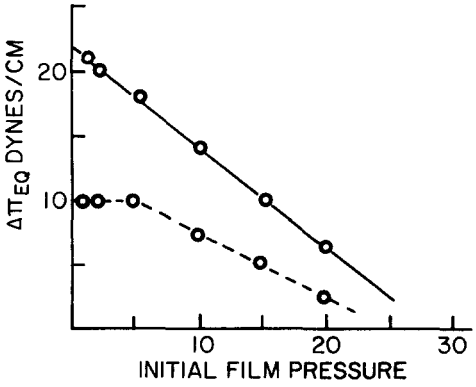

FIG. 9. Effect of initial film pressure on protein penetration into films of phosphatidyl choline. Solid line, HDL-protein; broken line, albumin. Subphase: as in Fig. 2.

of the film was constant for values of π_i between 1 and 5 dynes/cm.

Relation between Film Penetration and Protein Concentration in the Bulk Phase

The effect of the concentration of HDL-protein and albumin was studied with films of phosphatidyl choline and cholesterol. Changes in surface pressure were measured until equilibrium was reached. For protein concentrations below 0.5 μg/ml this required 150–180 min. Linearity of the plot of $1/\Delta\pi_{eq}$ vs $1/c$ (where c is the protein concentration in the bulk phase) showed that film penetration follows a Langmuir relationship:

$$1/\Delta\pi_{eq} = 1/\Delta\pi_m + K/\Delta\pi_m \cdot 1/c$$

where $\Delta\pi_m$ is the maximum increase in surface pressure and K is a constant (Fig. 10). Such a linear function permits the use of the reciprocal of the slope ($\Delta\pi_m/K$) as a measure of the capacity for film penetration (23). The values recorded (Table 2) show that this quantity was 5.8 and 2.8 times as large for HDL-protein as for albumin with films of lecithin at π_i values of 2 dynes/cm and 15 dynes/cm, respectively. With cholesterol at 2 dynes/cm, the value of $\Delta\pi_m/K$ for HDL-protein was 6.1 times as large as for albumin. If surface concentrations of protein for penetration of lecithin monolayers are calculated from the Gibbs equation in the form suggested by Eley and Hedge (24), higher values are obtained for HDL-protein than for albumin (Table 2). The free energy changes for these surface concentrations were calculated according to Mankowich (25).

Surface Activity of HDL-Protein

HDL-protein showed a remarkable capacity to collect at the air–water interface from its aqueous solutions ("surface denaturation"). This was not seen with either albumin or ribonuclease (Fig. 11 A). Ribonuclease was included because it had previously been found to have a high degree of interaction with lipid films (9).

HDL-protein could also be spread as a film if small portions (0.5 μl) of a protein solution (200 μg/ml) were placed at the air–water interface (0.01 M phosphate buffer, pH 7.6, ionic strength 0.10). The force–area curve of this film had a collapse pressure of 15 dynes/cm and a limiting molecular area (at zero pressure) of 3400 A² (Fig. 12). With albumin, ribonuclease, and trypsin the surface tension changes observed in this way were small, short-lived, and not reproducible.

Studies were then made of the interaction of HDL-protein and albumin with a monolayer of HDL-protein at a π_i of 2 dynes/cm. HDL-protein caused a rapid increase in surface pressure with $\Delta\pi_{eq}$ of 16 dynes/cm. With albumin, the increase was much slower and was only 6 dynes/cm. The $\Delta\pi_m/K$ values obtained from Fig. 10 are 730 ergs-cm/μg for HDL-protein and 22 ergs-cm/μg for albumin (Table 2).

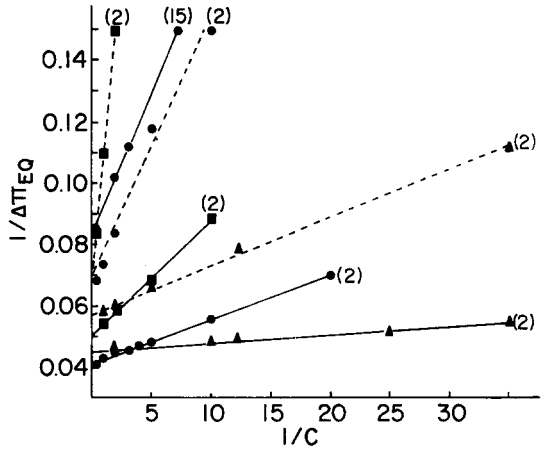

FIG. 10. Relationship between protein concentration in the subphase and increase in surface pressure at equilibrium ($\Delta\pi_{eq}$). Solid lines, HDL-protein; broken lines, albumin. ▲, cholesterol; ●, phosphatidyl choline; ■, HDL-protein, at initial film pressure given in parentheses. Subphase: as in Fig. 2.

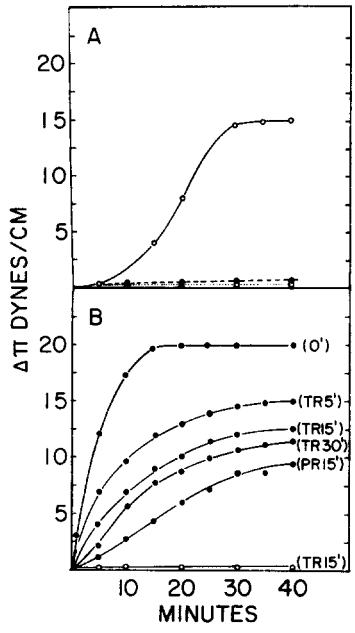

FIG. 11. Surface activity of HDL-protein and of the products of its enzymatic hydrolysis. A: Surface adsorption of protein from its solution. ○, HDL-protein; □ (dotted line), albumin; ● (dashed line), ribonuclease (see also B: ○, HDL-protein after 15 min of tryptic hydrolysis). B: Penetration of phosphatidyl choline films at π_i of 2 dynes/cm by HDL-protein (●) before and after enzymic hydrolysis. TR5′, TR15′, and TR30′ represent 5, 15, and 30 min tryptic hydrolysates; PR15′ is the hydrolysate after 15 min with pronase. ○, HDL-protein after 15 min tryptic hydrolysis. Subphase: as in Fig. 2.

Effect of Proteolytic Enzymes on the Surface Activity of HDL-Protein

HDL-protein is rapidly attacked by trypsin and pronase. Incubation in 0.5 ml of 200 μg of HDL-protein with 4 μg

TABLE 2 VALUES OF $\Delta\pi_m/K$, SURFACE CONCENTRATIONS OF PROTEIN PENETRATING THE FILM (Γ) AND FREE ENERGY CHANGES (ΔG) FOR INTERACTION OF HDL-PROTEIN AND ALBUMIN WITH FILMS OF PHOSPHATIDYL CHOLINE (LECITHIN) AND CHOLESTEROL

Type of Film	π_i	Injected Protein	$\Delta\pi_m/K$	Γ	ΔG
	dynes/cm		*ergs-cm/μg*	*pmoles/cm²*	*cal/mole*
Lipid					
Lecithin	2	HDL-protein	690	110	−7345
Lecithin	2	albumin	120	43	−6524
Lecithin	15	HDL-protein	118	13	−6472
Lecithin	15	albumin	62	3	−4571
Cholesterol	2	HDL-protein	3900	—	—
Cholesterol	2	albumin	640	—	—
Protein					
HDL-protein	2	HDL-protein	730	—	—
HDL-protein	2	albumin	22	—	—

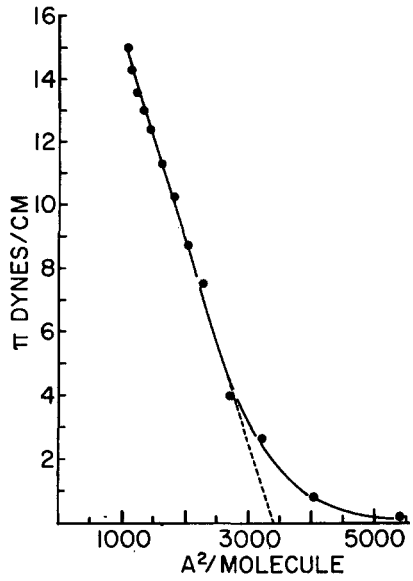

FIG. 12. Force–area curve of HDL-protein spread as a film at the air–water interface. Subphase buffer pH 7.6, ionic strength 0.1.

of trypsin at pH 7.4 for 5 min converted 84% of the protein to trichloroacetic acid-soluble peptides. After either 15 min or 30 min, 93% was soluble in trichloroacetic acid. With pronase under the same conditions, 99% of the HDL-protein was altered in 15 min.

In order to determine whether the peptides from HDL-protein exhibited surface activity similar to that of the intact protein, we injected portions of the enzymatic digest equivalent to 100 μg of protein under films of lecithin at a π_i of 2 dynes/cm. Although a substantial reduction of $\Delta\pi$ was observed in comparison with that observed with intact HDL-protein (Fig. 11 B), the digestion products of both trypsin and pronase still displayed a large capacity for film penetration. However, the film-forming ability (which was typical of HDL-protein) was lost (Fig. 11 B).

DISCUSSION

In general, the effects of lipid structure and initial film pressure on film penetration ($\Delta\pi$) by both HDL-protein and serum albumin are in accordance with previous findings (8, 9). Values of $\Delta\pi$ at a given π_i were in the order cholesterol > phosphatidal ethanolamine > phosphatidyl choline > sphingomyelin. At small values of initial film pressure, the rate and extent of film penetration were greater than at large values.

A most remarkable property of HDL-protein was its capacity to form a film at the air–water interface of very dilute salt solutions. Other proteins do this only at high salt concentrations (26). Furthermore, it was found that HDL-protein was transferred almost completely from the bulk phase to the interface (this result is obtained by comparison of the quantity, 4.5 μg, calculated from a film at half-maximal pressure from the Langmuir equation, with that found experimentally, 4.0 μg, when HDL-protein was added from above the interface). The area per amino acid residue (15.5 A²) and the film thickness (9.5 A) were calculated by using the value of 0.724 ml/g for the partial specific volume (v) of HDL-protein (10, 26). This may indicate that HDL-protein is present in the film as an extended polypeptide chain as suggested by Yamashita and Bull (26), who found similar values for lysozyme spread on 3M $(NH_4)_2SO_4$ or 3.5M KCl (14.8 A² for the mean area per amino acid residue and 9.0 A for the film thickness).

Two structural characteristics that enhance the surface activity of proteins are the presence of hydrophobic side chains and the absence of barriers to unfolding of the polypeptide chain. The absence of disulfide bridges from HDL-protein (10) is probably the major reason for its behaving differently from albumin. When such bridges are present, high salt concentration or the addition of a denaturing agent is required to enable the peptide chain to become coplanar with the interface (26). The absence of disulfide bridges (as indicated by the very low half-cystine content) appears to be a feature of proteins associated with lipids either in soluble lipoproteins or in cell membranes (27–30).

Several characteristics distinguish the penetration of HDL-protein into monolayers of lipid from those observed with albumin at physiological pH. First, the reaction is more rapid. Second, it is much less dependent on pH (possibly again because of the greater flexibility of the polypeptide chain resulting from the absence of disulfide bridges in HDL-protein). Third, the extent of penetration as judged from the magnitude of the increase in surface pressure is much greater. Particularly important is

the capacity of HDL-protein to react with lipid films at an initial film pressure that permits little interaction with other proteins. For example, albumin and other proteins hardly penetrate films of lecithin or mixed lipids at 15 dynes/cm, but HDL-protein penetrates readily.

Since the differences in penetration of different lipid films by HDL-protein are similar to those found with other proteins (9), HDL-protein apparently has no special affinity for a particular lipid. However, the interaction between HDL-protein and the neutral lipids extracted from VLDL exposes an important feature. This lipid mixture formed a film of low collapse pressure (15 dynes/cm). The final pressure reached after penetration by HDL-protein was about 25 dynes/cm or *10 dynes/cm above the collapse pressure of both the lipid and the protein components of the film*. The initial stabilization of monolayers that this implies is presumably due to extensive noncovalent bonding between the HDL-protein and the neutral lipid film (7, 8, 24). In the absence of such bonding either the protein or the lipid would leave the film when the collapse pressure of the components was reached (15–16 dynes/cm).

A direct approach to the identification of the chemical structures in proteins that are responsible for interaction with lipid films is suggested by the experiments using enzymic hydrolysates of HDL-protein. Although the peptides resulting from tryptic hydrolysis have lost their film-forming ability, they retain considerable capacity to penetrate lipid monolayers. If specific peptides with film-penetrating properties can be isolated, the structures responsible for reaction with lipid can perhaps be identified.

Finally, the tendency of HDL-protein to collect at interfaces may provide the driving force for the first step in the mechanism of lipoprotein formation proposed by Trams and Brown (31). The capacity of the protein to form complexes with lipids at interfaces could lead to the formation of lipoproteins at either the plasma membrane or the subcellular membranes. Ejection of the lipoprotein from the membranes may be related to our observation that HDL-protein, when charged with lipids, has a lesser tendency to associate with lipid films (Figs. 6 and 7).

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