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A COMPARISON OF THE INTERFACIAL INTERACTIONS OF THE APOPROTEIN FROM HIGH DENSITY LIPOPROTEIN AND β-CASEIN WITH PHOSPHOLIPIDS

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SUMMARY

The conformations adopted by β-casein and the total apoprotein from serum high density lipoprotein when spread at the air-water interface are compared; the monolayer data are consistent with the apoprotein being α-helical and the β-casein being disordered with segments distributed in loops and trains. The penetration of these hydrophobic proteins into phosphatidylcholine monolayers in different physical states was investigated. More protein can penetrate into monolayers when they are in the liquid-expanded state; for penetration at constant total surface area the lateral compressibility of the lipid is an important factor. The charge and conformation of the polar group of the phospholipid does not have a major influence on the interaction. The mixed films of lipid and protein have a mosaic structure; probably the β-casein is in a compressed state whereas the apoprotein is extended as α-helices in the plane of the interface. The chain-length dependences of the interaction of the apoprotein with phosphatidylcholine monolayers and bilayers are different; when the apoprotein binds to bilayers of shorter-chain phosphatidylcholines it alters the shape of the lipid-water interface whereas with monolayers the interface remains planar throughout.

INTRODUCTION

Recent physical studies [1, 2] have shown that in native high density lipoproteins (*d* 1.07–1.21 g/ml) the protein is situated at the surface of the particle. The lipid and protein can be separated and the isolated apoprotein will recombine with either the total lipids [3] or pure phosphatidylcholine [4]. The complex formed by the total apoprotein from porcine high density lipoprotein and dimyristoyl phosphatidylcholine has been characterised particularly thoroughly. Here again the apoprotein is in an interfacial situation; the protein forms an amphipathic α-helix which partially penetrates the lecithin bilayer and resides in the lipid-water interface [5–7]. It is clear

Abbreviation: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

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from the above that the interfacial interaction of lipid and protein is important and more needs to be known about the major factors controlling this phenomenon.

In this study we use monolayer penetration experiments [8] to compare apoprotein and β-casein, a hydrophobic protein which does not bind lipids in vivo, to see what can be learnt about the protein requirements for lipid binding. Since the lipid-lipid interactions in phospholipid monolayers and bilayers correlate [9], use of the monolayer system should give valuable information about the important lipid factors in the lipid-protein interaction. Therefore, we also use the monolayer system, where the interfacial packing of the lipid molecules can be controlled easily, to investigate the role of lipid physical state, compressibility and charge on the interfacial interaction with apoprotein.

EXPERIMENTAL

Monolayer methods

The apparatus and procedure for determining the surface pressure (π)-molecular area (A) isotherms for phospholipid monolayers spread at the air-water interface have been described before [10, 11]. The π-A isotherms of proteins were obtained by continuous compression ($\approx$ 10 min for whole curve) after the protein was spread from aqueous solution down a glass rod, as described by Trurnit [12]. The general procedure for spreading was the same as that described by Evans et al. [26]. The spreading solutions contained from 0.01–0.1 wt % protein dissolved in the phosphate buffer used as substrate. With the highly surface-active proteins under study here, incomplete spreading would be most likely to arise because of aggregation of the protein in the spreading solution. Since the apoprotein is monomeric at concentrations below 0.1 wt % [46], aggregation of the protein was not expected to be a problem in this case. β-Casein associates at concentrations approximately equal to or greater than 0.05 wt % at room temperature [47] but since the same π-A curves were obtained from 0.01 and 0.1 wt % spreading solutions aggregation does not affect the spreading from such solutions. As discussed below, the molecular areas are consistent with completely spread monomolecular films.

When determining changes in surface pressure ($\Delta\pi$) on adsorption of protein to phospholipid monolayers at constant total area, the lipid was spread from hexane/ethanol solutions and adjusted to the appropriate area/molecule or film pressure by movement of a Teflon barrier. The substrate (volume = 100 ml) was 0.01 M disodium hydrogen phosphate (pH 8.3, $I = 0.01$) contained in a Teflon trough ($20.8 \times 5.1 \times 0.8$ cm) so that its surface area to volume ratio was 1 cm^{-1}. Small volumes of a protein solution in 0.01 M Na_2HPO_4 (pH 8.3) were injected into the substrate from behind the Teflon barrier. The contents of the trough were then stirred at a constant rate with a magnetic stirrer and $\Delta\pi$ measured with a glass Wilhelmy plate as described previously [13]. Adsorption was allowed to proceed for 1 h and in most cases $\Delta\pi$ became constant after this period. Normally, $\Delta\pi$ could be reproduced to ± 1 $mN \cdot m^{-1}$. In a few experiments, the barriers were progressively moved further apart as penetration of protein occurred so that π remained constant throughout. Surface concentrations of [1-^{14}C]-acetyl-β-casein A were determined by measuring the surface radioactivity during adsorption [13, 14]. All experiments were carried out at 22±2 °C.

Materials

Lipids. Chromatographically pure, saturated 1,2-diacyl phosphatidylcholines and dimyristoyl phosphatidylethanolamine were synthesized in this laboratory and have been described before [10]. Hen egg lecithin and the monosodium salt of ox brain phosphatidylserine, which were pure by thin-layer chromatography, were purchased from Lipid Products (South Nutfield, U.K.). Pure sodium dicetyl phosphate was obtained from Albright and Wilson Ltd, (Birmingham, U.K.). Docosyl trimethylammonium bromide was generously provided by Dr J. Mingins (Unilever Research Laboratory, Port Sunlight, U.K.). Cholesterol oleate (99 %+pure) was purchased from Sigma (London) Chemical Co. (London, U.K.) and cholesterol was a sample from British Drug Houses (Poole, U.K.) which was recrystallised before use. The solvents used for spreading the lipids have already been described [10].

Proteins. The preparation and purification of high density lipoprotein by conventional ultracentrifugal techniques, delipidation to yield the water-soluble high density lipoprotein apoproteins, as well as the fractionation by ion-exchange chromatography (DEAE) in 8 M urea of this apoprotein into two major components* has been described before [18, 19]. Apoproteins from both human and porcine [19] high density lipoproteins were prepared by this method. All proteins were lyophilised and stored at −25 °C. The ratio of apo A-I to apo A-II in the human apoproteins was about 3 : 1 whereas the equivalent ratio for porcine apoprotein was about 9 : 1. The apo A-I from pig and human high density lipoproteins is very similar [20, 21]. Both the human and porcine apoproteins recombined readily with lecithin [4–7] indicating that they were not irreversibly denatured during isolation. For monolayer experiments the proteins were dissolved in 0.01 M disodium hydrogen phosphate solution pH 8.3.

[1-^{14}C]Acetyl-β-casein A was prepared as described previously [13] and used in phosphate buffer pH 7 ($I = 0.1$) or in the above sodium phosphate solution pH 8.3 for penetration experiments with phospholipid monolayers. The protein β-casein A consists of a single polypeptide chain of 209 amino acids, of which eleven are lysines. Conditions of acetylation were chosen such that two lysines were acetylated; this modification had no detectable effect on the surface properties of the protein [13].

Analytical grade reagents and triple-distilled water were used in the preparation of all solutions and buffers.

RESULTS

(1) *Monolayer characteristics of lipids used*

The saturated phosphatidylcholine homologues form monolayers of all possible physical states at room temperature [9, 10]. Because the gel to liquid crystalline transition temperatures (T_c) of the egg phosphatidylcholine and ox-brain phosphatidylserine are below 0 °C their monolayers are fully expanded at 22 °C and are of similar form to that for dimyristoyl (C_{14})phosphatidylcholine. The total high density lipoprotein lipids behave similarly but the π-A curve is somewhat unreproducible and the monolayers are unstable at higher pressures because of the presence of

* The two major apoproteins from serum high density lipoprotein have been designated [15] as apo A-I and apo A-II. The amino acid sequences of these two apolipoproteins from human plasma are known [16, 17].

long chain cholesterol esters. We confirmed that these cholesterol esters do not form stable monolayers when spread alone at the air-water interface [22, 23], and that they are readily squeezed out from mixed monolayers with phospholipid. The anionic sodium dicetyl (C_{16})phosphate forms completely condensed monolayers with a limiting area of about 40 Å^2/molecule. The cationic docosyl (C_{22})trimethylammonium bromide forms liquid expanded monolayers for which $\pi \approx 2$ mN · m^{-1} at 100 Å^2/molecule and $\pi \approx 20$ mN · m^{-1} at 50 Å^2/molecule (Feinstein, M. E., unpublished result).

(2) *Surface characteristics of proteins used*

It has been shown before [24] that the total apoprotein from human high density lipoprotein, apo A-I and β-casein all have similar effects upon the surface tension of water and adsorb to maximum film pressures π of about 23 mN · m^{-1}. As a result of this high surface activity, it is easy to spread the apoproteins and β-casein quantitatively at the air-water interface to form insoluble monolayers. The π-*A* isotherms for the total apoprotein from porcine high density lipoprotein and β-casein are compared in Fig. 1. The form of the curve for spread β-casein with the inflection

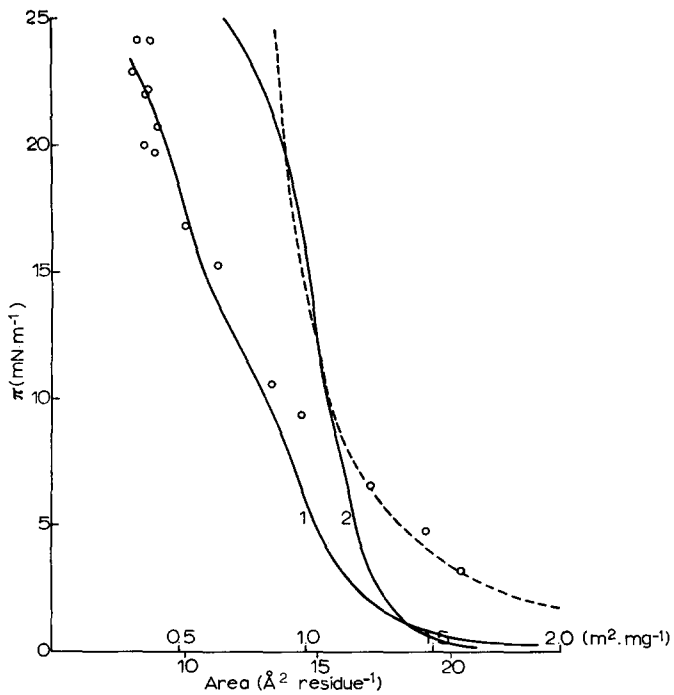

Fig. 1. Surface pressure (π)-molecular area (*A*) isotherms for β-casein spread (—, curve 1) and adsorbed (0) at the air-phosphate buffer (0.1 M, pH 7) interface at 21 °C. The equivalent isotherm for spread monolayers of the total apoprotein from porcine high density lipoprotein is also shown (—, curve 2). A random coil protein with every residue (mean residue weight = 115) in the surface and occupying a limiting area of 15 Å^2/residue would exhibit the π-*A* curve shown by the dashed line [33, 45].

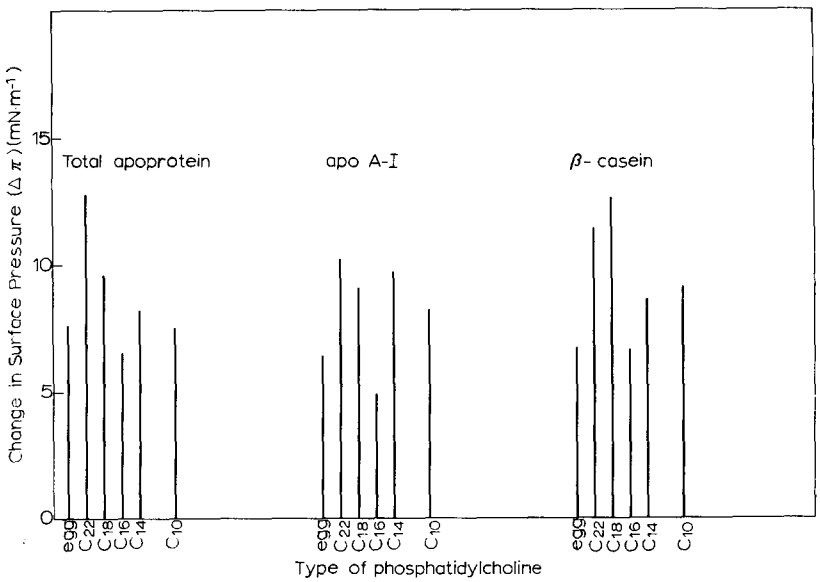

Fig. 2. Variation with phosphatidylcholine hydrocarbon chain lenght of the increase in surface pressure ($\Delta\pi$) after penetration of human apoprotein, apo A-I and β-casein into monolayers of saturated 1,2 diacyl-L-phosphatidylcholines and egg yolk phosphatidylcholine at an initial pressure (π_i) of 5 mN · m^{-1}. The initial substrate concentration of protein was $2 \cdot 10^{-5}$ wt%; the substrate was 0.01 M Na_2HPO_4 solution, pH 8.3; the temperature was 22 °C. $\Delta\pi$ was recorded after 1h. At the clean solution-air interface the proteins would generate equilibrium film pressures ranging from 0.5–9 mN · m^{-1} but, under our experimental conditions, no changes in π could be detected in 1 h in the absence of a lipid monolayer.

at $\pi \approx 10$ mN · m^{-1} is in agreement with that reported before [25, 26]. The inflection point at ≈ 0.8 m^2 · mg^{-1} corresponds to a surface packing of about 15 Å^2/residue and the limiting area is about 0.4 m^2 · mg^{-1} (7 Å^2/residue). In contrast, the more expanded apoprotein film only reaches a limiting area of ≈ 0.8 m^2 · mg^{-1} (15 Å^2/residue) when $\pi = 23$ mN · m^{-1}.

(3) *Penetration experiments*

Because the apoproteins combine most readily with phosphatidylcholine dispersions above the appropriate T_c (Henry, R., unpublished observations), our penetration experiments at constant total area have been conducted at an initial pressure (π_i) of 5 mN · m^{-1} where the egg and shorter-chain phosphatidylcholine monolayers are liquid expanded. It can be seen from Fig. 2 that $\Delta\pi$ under these conditions for the liquid-expanded monolayers of C_{10} and C_{14} phosphatidylcholine is about 8 mN · m^{-1} whereas the values obtained at 70 Å^2/molecule (the molecular area characteristic of liquid crystalline bilayers) are generally close to 4 mN · m^{-1} [24]. Generally interaction of the protein with condensed phosphatidylcholine monolayers (C_{22}, C_{18}) gives rise to larger values of $\Delta\pi$ (Fig. 2). It is also clear that for the saturated phosphatidylcholines $\Delta\pi$ passes through a minimum for the C_{16} homologue. If the initial substrate protein concentration (C_p) is increased to above $2 \cdot 10^{-5}$ wt %, then larger values of $\Delta\pi$ are observed. Plots of $\Delta\pi$ against C_p are of the same form as that for π at the air-water interface [13].

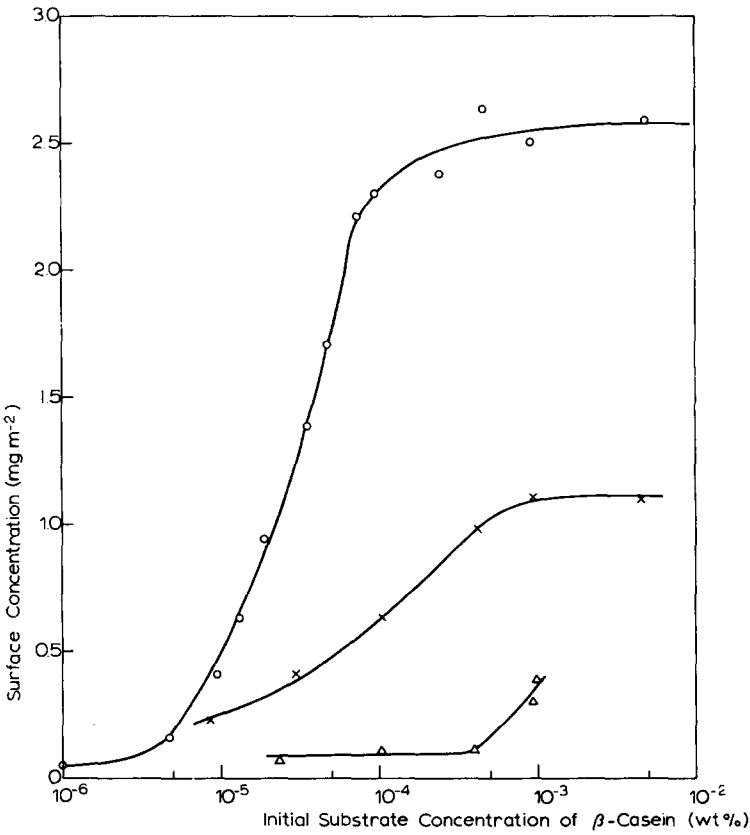

Fig. 3. Adsorption isotherms (semilog scale) for [1-C^{14}]acetyl-β-casein A at the air-phosphate buffer (pH 7, $I = 0.1$) interface (0); dimyristoyl (C_{14})phosphatidylcholine monolayer ($\pi_i = 10$ mN · m^{-1}) (X); dibehenoyl (C_{22})phosphatidylcholine monolayer ($\pi_i = 10$ mN · m^{-1}) (Δ).

The surface concentrations (Γ) of [1-^{14}C]acetyl-β-casein have been determined at air-water and phosphatidylcholine monolayer-covered interfaces. It is clear from Fig. 3 that at the air-water interface, monolayer coverage ($\Gamma \approx 2.5$ mg/m^2; 7.7 Å^2/amino-acid residue) is complete when $C_p \approx 10^{-4}$ wt % and there is no further adsorption when C_p is increased to 10^{-2} wt %. When $C_p = 10^{-5}$ wt %, approximately half of the total protein in the system ultimately reaches the interface, whereas when $C_p = 10^{-3}$ wt %, only a few percent of the total β-casein are adsorbed. The data in Fig. 3 indicate that whenever a phosphatidylcholine monolayer is present at the air-water interface and penetration is allowed to occur at constant total area, Γ for β-casein is reduced below the equivalent value for the initially clean interface. It is also apparent that the initial physical state of the phosphatidylcholine monolayer has a marked effect. For a given C_p, the Γ of β-casein is much greater with dimyristoyl than dibehenoyl phosphatidylcholine monolayers (Fig. 3). In the Γ-C_p plots for adsorption to liquid-expanded phosphatidylcholine monolayers at constant total area, Γ eventually reaches a plateau as C_p is increased. Because the variation of $\Delta\pi$ with C_p is the same for β-casein and the apoproteins [24] and the π-A curves of these proteins are

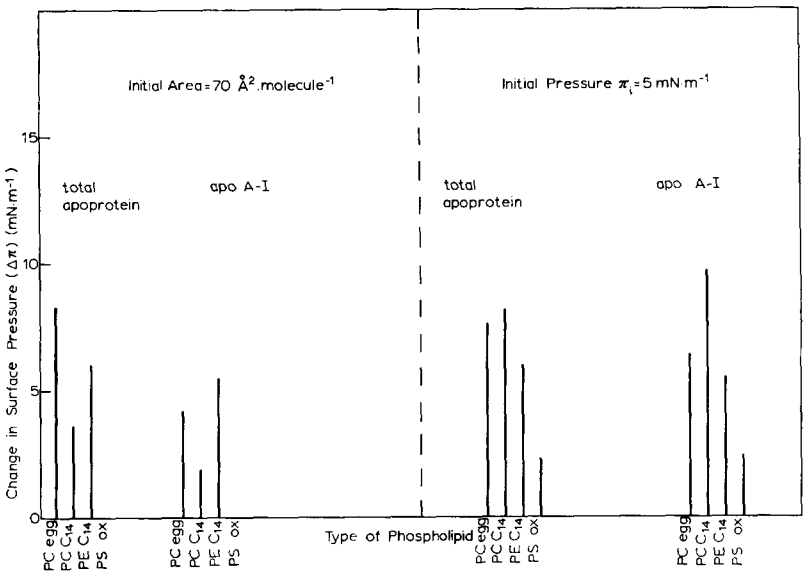

Fig. 4. Variations in $\Delta\pi$ after penetration of human apoprotein and apo A-I into monolayers of egg yolk phosphatidylcholine, dimyristoyl phosphatidylcholine, dimyristoyl phosphatidylethanolamine and ox brain phosphatidylserine at initial areas of 70 Å²/molecule and $\pi_i = 5$ mN · m^{-1}. The other conditions were as described in the legend to Fig. 2.

not too dissimilar (Fig. 1), the variation of Γ with C_p for apoprotein will be qualitatively similar to the curves shown in Fig. 3. When the proteins were allowed to adsorb to phosphatidylcholine monolayers at constant π, Γ continued to increase until either the substrate became too depleted in protein or until the barriers could not be withdrawn any further. Under our conditions, Γ was the same for both liquid-expanded and condensed monolayers when adsorption occurred at the same constant π.

The effects of the lipid polar group structure and charge on $\Delta\pi$ for apoprotein and apo A-I at $C_p = 2 \cdot 10^{-5}$ wt % have been investigated and the results are summarised in Fig. 4. It is clear that the different orientations for the zwitterionic polar groups of dimyristoyl phosphatidylcholine and phosphatidylethanolamine [27] are of relatively minor importance when compared to the effect of the net negative charge of phosphatidylserine. $\Delta\pi$ is much reduced in the latter case because of the repulsion between the similarly charged phospholipid and protein molecules. The inhibiting effect of a net negative charge on the lipid monolayer was confirmed with dicetyl phosphate. With a condensed monolayer of this lipid at $\pi_i = 5$ mN · m^{-1}, $\Delta\pi$ for apo A-I was approx. 2 mN · m^{-1} whereas with condensed phosphatidylcholine monolayers $\Delta\pi$ was about 10 mN · m^{-1} (Fig. 2). $\Delta\pi$ remained at 2 mN · m^{-1} for dicetyl phosphate when, in addition, EDTA was present in the subphase at a concentration of 10^{-4} M or calcium chloride was added to give a Ca^{2+} concentration of 10^{-5} M. When the net charge on the protein was reversed by reducing the substrate pH from 8.3 to 3.5 with HCl, $\Delta\pi$ with dicetyl phosphate increased to about 11 mN · m^{-1} whereas there was no significant change in $\Delta\pi$ for phosphatidylserine monolayers. It is interesting that when the monolayer was formed ($\pi_i = 5$ mN · m^{-1}) from the cationic amphiphile docosyl

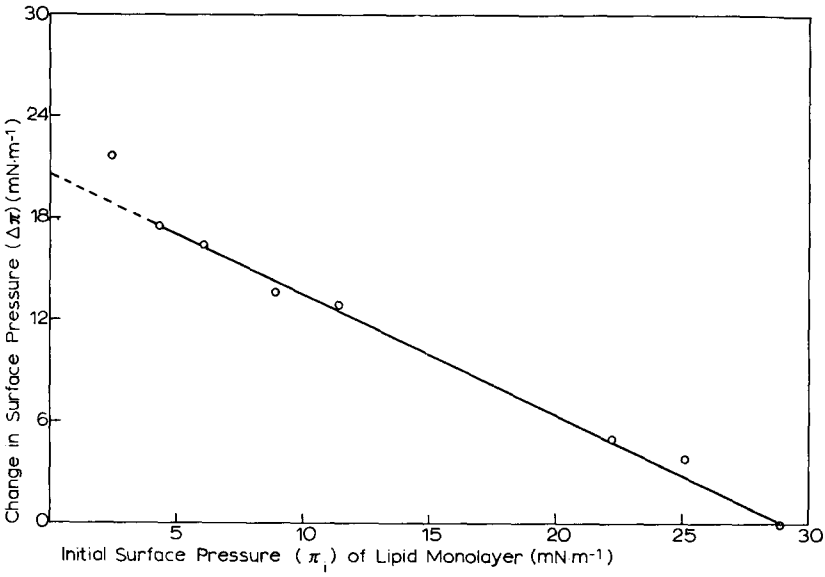

Fig. 5. Dependence of $\Delta\pi$ on π_i for the penetration of human apoprotein into monolayers of total high density lipoprotein lipids. The other conditions were as described in the legend to Fig. 2.

(C_{22})trimethylammonium bromide, no change in π was observed with $C_p = 2 \cdot 10^{-5}$ wt % for apo A-I at pH 8.3.

The interaction of total apoprotein with monolayers of the total high density lipoprotein lipids has been studied and the data are summarised in terms of the variation of $\Delta\pi$ with π_i (Fig. 5). $\Delta\pi$ decreases linearly with increasing π_i and is zero when $\pi_i \approx 29$ mN · m^{-1}. A very similar relationship has been observed for the penetration of egg lecithin monolayers by apoprotein [28]. Clearly, the apoprotein has the capability of penetrating lipid monolayers for which π_i is greater that the collapse pressure of the protein. The presence of cholesterol oleate in phosphatidylcholine monolayers (mol ratio 1 : 1.3) reduces the magnitude of the $\Delta\pi$ observed with apoprotein. For example, with dimyristoyl phosphatidylcholine monolayers at $\pi_i =$ 5 mN · m^{-1}, $\Delta\pi$ is reduced from 8 to 2 mN · m^{-1} by the above levels of cholesterol oleate; this probable arises because the mixed monolayer is much more compressible at low π [22].

DISCUSSION

Protein conformation

Because the apoproteins and β-casein are hydrophobic [20, 29, 30] and very surface-active they penetrate readily into phospholipid monolayers spread at the air-water interface. However, β-casein does not interact with phosphatidylcholine dispersions whereas the apoprotein complexes with phosphatidylcholine in solution [24]. Therefore, something more specific than simple hydrophobicity is involved in the lipid-binding capabilities of the apoproteins in vivo. It is important to note that, in

contrast to highly water-soluble globular proteins which bind primarily by electrostatic attraction and tend to remain outside the monolayer or bilayer, hydrophobic proteins penetrate into the apolar regions of monolayers [13, 31] and bilayers [5–7, 32]. The most likely reason for the inability of β-casein to bind phosphatidylcholine in solution is that it cannot adopt a suitable conformation at the lipid-water interface; the apoprotein molecules which have been designed to reside at the lipid-water interface in vivo [1, 2] must be able to do this readily. Consistent with this, the π-A data in Fig. 1 demonstrate that the interfacial conformations of apoprotein and β-casein are different.

The more-or-less random coil β-casein molecule adsorbs flat at low surface coverage so that essentially all the residues are in the surface [13]. As a result when π is less than about $7\,mN \cdot m^{-1}$ the adsorbed film (which is at equilibrium) can be described by theories based on polymer statistics (see dashed line in Fig. 1) [33]. At higher pressures, residues leave the interface and form loops in one or other bulk phase. As a result the limiting area is smaller than the minimum area of 15 $Å^2$/residue into which amino acids can be compressed. The polypeptide chain of β-casein cannot fold to form an α-helix because of the large number of proline residues distributed along the chain [34]. In contrast to this the apoprotein molecule contains some 50 % α-helix and this increases on interaction with lipid [35]. The π-A curve for apoprotein in Fig. 1 is consistent with a film containing largely α-helical molecules because the limiting area is $\approx 15\,Å^2$/residue. This figure is in excellent agreement with a theoretical estimate of the area per residue for α-helix derived from measurements of space-filling molecular models [36]. The sequence of human apo A-I [16] indicates that the regions of α-helix are likely to be amphipathic (i.e. have polar and apolar sides). Therefore the protein is probably situated at the air-water interface with the helix in the plane of the interface and apolar side-chains protruding into the air and polar side-chains protruding into the water. In complexes of apoprotein and phosphatidylcholine, the protein is orientated in this fashion at the surface of a phospholipid bilayer [5–7]. Clearly this orientation would also be preferred in mixed monolayers of apoprotein and phospholipid. Because the apoprotein interacts with both phosphatidylcholine monolayers and bilayers, details of the lipid packing learnt from the monolayer data should also apply to the bilayer situation.

Structure of mixed monolayers and lipid packing

The parallel changes in π observed when β-casein and the apoproteins adsorb to the phosphatidylcholine monolayers (Fig. 2) indicate that, qualitatively at least, the penetration is similar. A given $\Delta\pi$ will not necessarily correspond to the same Γ for the apoprotein and β-casein because the π-A curves of the two proteins are different (Fig. 1). It is clear from Fig. 3 that the presence of a phosphatidylcholine monolayer inhibits the adsorption of β-casein to the air-water interface [13, 31]. Furthermore, a condensed phosphatidylcholine monolayer (e.g. distearoyl), where the phosphatidylcholine molecules are in a quasi-crystalline packing, reduces Γ more than a liquid-expanded monolayer. These facts strongly suggest that protein molecules cannot occupy the same areas of the interface as the lipid molecules and that the interfacial area available to the protein is thereby decreased. As has been discussed before [13, 31], the adsorption of the hydrophobic β-casein molecules to phosphatidylcholine monolayers can be described by a geometric model in which the area per lipid mole-

cule decreases from its value at π_i to a value at $(\pi_i + \Delta\pi)$ consistent with the π-A isotherm of the lipid, if some additional condensation of lipid and/or protein is included. The extent of interaction is consistent with the concept of a boundary layer of restricted lipid molecules around each protein molecule [31, 37]; presumably the hydrocarbon chains interact with loops of hydrophobic β-casein residues which extend into the air. Such an interaction readily explains the greater stability against collapse of proteins in this situation, as compared to pure protein films at the air-water interface.

Since the interfacial conformations of the apoproteins are different from that of β-casein in that they tend to lie as α-helical rods in the surface, the details of the interaction with phosphatidylcholine are presumably different. In this case the protein is mainly in direct contact with the glycerol backbone region of the phosphatidylcholine molecules [5–7]. Unlike β-casein, which is embedded in a compressed state in the monolayer, the apoprotein is extended in the plane of the interface. In both cases the mixed film has a mosaic structure.

The interaction of both β-casein and the apoproteins involves penetration by the protein and insertion of hydrophobic groups among the lipid molecules and the lateral compressibility $[C = -1/A\ (\delta A/\delta\pi)_T]$ of the lipid monolayer has an important effect [38, 39]. It can be shown [31] that

$$\Gamma = \frac{\Delta\pi \cdot C}{A_p} \qquad [1]$$

where A_p is the average area per protein molecule. It is clear from Eqn 1 that the Γ values for a protein penetrating into two different lipid monolayers should be in the ratio of their compressibilities as long as $\Delta\pi$ and A_p are the same in both cases. Since the compressibilities of phosphatidylcholine monolayers in a given physical state are very similar (see π-A curves in refs 10 and 11), the $\Delta\pi$ data in Fig. 2 suggest that protein penetration is independent of phosphatidylcholine chain length for monolayers in the same physical state.

The effects of changing the initial physical state of the phosphatidylcholine monolayer can be deduced from the data for protein penetration into monolayers of the C_{10}–C_{22} phosphatidylcholine homologues at $\pi_i = 5\ mN \cdot m^{-1}$ (Fig. 2). $\Delta\pi$ is inversely proportional to the compressibility of the lipid monolayer (Eqn 1) and therefore for a given Γ, larger $\Delta\pi$ values are recorded with condensed lipid monolayers. Whereas liquid-expanded monolayers with fluid chains are highly compressible, fully condensed monolayers are extremely incompressible. The larger $\Delta\pi$ in the latter situation occurs despite the fact that Γ is reduced at a given C_p. It is to be expected that the stoichiometry and packing would be different when the lipid molecules are initially in a quasi-crystalline array (e.g. dibehenoyl phosphatidylcholine monolayers). In this case the protein is more likely to penetrate only partially the monolayer and to remain largely situated below the film [31].

The penetration of protein into dipalmitoyl phosphatidylcholine monolayers is of particular interest because the monolayer at room temperature exhibits the transition from the condensed to expanded state [9–11]. When two-dimensional crystallisation occurs, the compressibility increases dramatically [39] and from Eqn 1 it is clear that $\Delta\pi$ will decrease if Γ and A_p remain constant. This explains why $\Delta\pi$ for

dipalmitoyl phosphatidylcholine in Fig. 2 is at a minimum and neatly confirms the concept of lipid compression by penetrating β-casein or apoprotein molecules.

It is well established [8, 40] that proteins tend not to penetrate into lipid monolayers when π_i is approximately equal to or greater than 30 $mN \cdot m^{-1}$. Furthermore, it has been shown [28, 31, 40, 41] with several proteins that $\Delta\pi$ decreases linearly with increasing π_i. In this sense, the apoprotein/total high density lipoprotein lipid system is behaving normally (Fig. 5) and as the initial packing density in the lipid monolayer is increased so the amount of protein which penetrates is caused to decrease. It is apparent from Fig. 5 that $\Delta\pi = 0$ when $\pi_i \approx 29\ mN \cdot m^{-1}$. This pressure is about 6 $mN \cdot m^{-1}$ greater than the maximum pressure the proteins exert when alone at the interface so it is clear that the apoprotein is also stabilised at the interface by the total high density lipoprotein lipids.

As might be expected, the interaction of apoprotein, which is negatively charged under the experimental conditions used, is less with phosphatidylserine than with phosphatidylcholine monolayers (Fig. 4). Similarly, reversal of the charge on both lipid and protein, e.g. by interacting apoprotein with positively charged lipids at pH 4.0, also inhibits penetration and Ca^{2+} does not seem to be able to promote interaction of negatively charged lipid and protein by a bridging mechanism. In conclusion, it seems that electrostatic interactions play only a secondary role in the process of monolayer penetration by total apoprotein, apo A-I and β-casein. With these proteins the interaction is dominated by the intrinsic surface-activity (amphipathicity) of the macromolecule.

Comparison of the interaction of apoprotein with phospholipid monolayers and bilayers

It has been established that lipid-lipid interactions in phospholipid monolayers correlate with those in bilayers [9, 10]. Now, comparison of the present information on the apoprotein/phospholipid monolayer interaction with that for the apoprotein/bilayer system [5–7] indicates that the lipid-protein interaction is qualitatively the same in both cases. Firstly, it is clear that the hydrophobic interaction of the apoprotein with the monolayer occurs more readily (i.e. Γ higher) when the film is in the liquid state (i.e. $T > T_c$). Complex formation in bulk also involves maintaining the bilayer in the liquid-crystalline state [4]. Secondly, the monolayer data indicate that the net charge of the phospholipid polar group or conformation of the zwitterion are not critical in the interaction; this is consistent with the bulk situation where the apoprotein can complex with phosphatidylcholine, phosphatidylserine or phosphatidylethanolamine. Unfortunately, the stoichiometries of the monolayer and bilayer interaction cannot be compared simply, because in the monolayer experiment it is a function of C_p.

Differences in the monolayer and bilayer interaction probably arise from four effects: (1) whether penetration occurs at constant π or constant area, (2) in monolayer experiments the interface remains planar whereas in the bilayer case the curvature of the lipid-water interface can be altered by the apoprotein, (3) differences in the adsorption energies of the apoprotein to the monolayer and bilayer [42], (4) differences in the temperatures of monolayer and bilayer experiments. Penetration into the monolayer occurs at either constant area or constant π whereas the conditions for the bilayer interaction are not known; it has been shown [43] that the interaction of a related apoprotein with egg phosphatidylcholine bilayers causes a lateral expansion

more-or-less equal to that associated with the transition from gel to liquid crystal. However, it is not known whether this expansion is simply due to insertion of the protein or whether the lipid-packing density is also altered during this interaction. The interaction of apoprotein with phosphatidylcholine bilayers in bulk is dependent upon the hydrocarbon chain-length because the nature of the complex is a function of bilayer stability [4, 7, 44]. Shortening the chain-length makes the bilayer less stable and this allows the apoprotein to alter the geometry of the lipid-water interface. Thus, with phosphatidylcholine bilayers, which are less stable than those formed by dimyristoyl phosphatidylcholine, it seems to be the protein which determines the morphology of the resulting complex, whereas with more stable lipid bilayers (e.g. dipalmitoyl phosphatidylcholine and higher homologues) the bilayer appears to become the structure-determining part [44] (Hauser and Phillips, unpublished observation). Of course, monolayers of phosphatidylcholine spread at the air-water interface remain planar whatever the chain-length of the molecules. This explains why, in contrast to the bilayer situation, for monolayers in a given physical state the interaction is independent of chain-length.

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REFERENCES

1 Atkinson, D., Davis, M. A. F. and Leslie, R. B. (1974) Proc. R. Soc. Lond. Ser. B. 186, 165–180
2 Finer, E. G., Henry, R., Leslie, R. B. and Robertson, R. N. (1975) Biochim. Biophys. Acta 380, 320–337
3 Scanu, A. (1967) J. Biol. Chem. 242, 711–719
4 Kruski, A. W. and Scanu, A. M. (1974) Chem. Phys. Lipids 13, 27–48
5 Hauser, H., Henry, R., Leslie, R. B. and Stubbs, J. M. (1974) Eur. J. Biochem. 48, 583–594
6 Barratt, M. D., Badley, R. A., Leslie, R. B., Morgan, C. G. and Radda, G. K. (1974) Eur. J. Biochem. 48, 595–601
7 Andrews, A., Atkinson, D., Barratt, M. D., Finer, E. G., Hauser, H., Henry, R., Leslie, R. B., Owens, N. L., Phillips, M. C. and Robertson, R. N. (1975), to be published
8 Colacicco, G. (1970) Lipids 5, 636–649
9 Phillips, M. C. (1972) Prog. Surface Membrane Sci. 5, 139–221
10 Phillips, M. C. and Chapman, D. (1968) Biochim. Biophys. Acta 163, 301–313
11 Paltauf, F., Hauser, H. and Phillips, M. C. (1971) Biochim. Biophys. Acta 249, 539–547
12 Trurnit, H. J. (1960) J. Colloid Sci. 15, 1–13
13 Phillips, M. C., Evans, M. T. A. and Hauser, H. (1973) Proceedings of the 6th International Congress on Surface Activity, Vol. 2, pp. 381–391, Carl Hanser Verlag, Munich
14 Adams, D. J., Evans, M. T. A., Mitchell, J. R., Phillips, M. C. and Rees, P. M. (1971) J. Polymer Sci. Part C 34, 167–179
15 Kostner, G. and Alaupovic, P. (1971) FEBS Lett. 15, 320–324
16 Baker, H. N., Delahunty, T., Gotto, Jr, A. M. and Jackson, R. L. (1974) Proc. Natl. Acad. Sci. U.S. 71, 3631–3634
17 Brewer, Jr, H. B., Lux, S. E., Ronan, R. and John, K. M. (1972) Proc. Natl. Acad. Sci. U.S. 69 1304–1308
18 Edelstein, C., Lim, C. T. and Scanu, A. M. (1972) J. Biol. Chem. 247, 5842–5849
19 Scanu, A. M., Lim, C. T. and Edelstein, C. (1972) J. Biol. Chem. 247, 5850–5855
20 Davis, M. A. F., Henry, R. and Leslie, R. B. (1974) Comp. Biochem. Physiol. 47B, 831–849