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Interaction of Apolipoprotein AI from Human Serum High-Density Lipoprotein with Egg Yolk Phosphatidylcholine†

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ABSTRACT: Apolipoprotein AI from human serum high-density lipoprotein has been recombined with egg yolk lecithin from ternary complexes of detergent–lipid–protein to form homogeneous spherical particles with maximum binding of 220 mol of lipid/2 mol of AI. This complex differs from those formed when *n*-alkyl detergents or short chain saturated diacylphosphatidylcholines interact with AI in that the maximum hydrophobic volume accommodated by the protein is increased as the result of increased α-helix content. Additionally, it is shown that no interaction occurs between AI and didecanoylphosphatidylcholine or egg yolk lecithin above their thermotropic phase transitions and in the absence of single-tail amphiphiles.

Serum lipoproteins are water-soluble complexes of proteins and lipids designed as intravascular transport vehicles for phospholipids, cholesterol, cholesteryl esters, and triglycerides. The major apolipoproteins from high-density lipoprotein (HDL),[1] AI and AII, and from LDL and VLDL, apo B, are characterized by a high degree of "conformational adaptability" in keeping with their physiological function of binding variable amounts of lipid in vivo. AI, which is the subject of this paper, has a molecular weight of 28 400; the primary sequence contains no long regions of solely hydrophobic residues; the predicted secondary structure consists of regions of amphipathic helices; the protein is soluble in aqueous media in the absence of bound amphiphiles. [See Osborne & Brewer (1977) and Scanu et al. (1982) for reviews of the known properties of this protein.] There have been numerous investigations of the amphiphilic binding properties of AI [recent reviews are Reynolds (1982) and Scanu et al. (1982)], and the results can be briefly summarized. (1) *N*-Alkyl detergents bind to a set of three to four independent sites on AI, inducing a conformational change in the protein. (2) The total hydrophobic volume of *n*-alkyl detergents that can be accommodated by one molecule of AI in this conformationally altered state is approximately 4.3×10^4 A^3 and independent of the ligand head group. (3) As first demonstrated by Pownall and co-workers [e.g., see Pownall et al. (1978)] and subsequently repeated by others, association of AI with saturated, bilayer-forming diacyl phospholipids (i.e., those with a minimum alkyl chain length of 14 carbons) is a slow process and highly temperature dependent with the maximum rate of association occurring at the transition temperature of the lipid. Phosphatidylcholines containing unsaturated fatty acyl chains and uncontaminated with single-tail amphiphiles such as fatty acids and lysolecithins have transition temperatures below 0 °C, and these lipids do not complex spontaneously with AI above their transition temperatures (Assmann & Brewer, 1974; Pownall et al., 1981).

Pownall has proposed that interaction of AI with phospholipid bilayers at the thermotropic phase transition in the *absence* of contaminating single-tail amphiphiles occurs by insertion of the protein moiety into regional "defects", a similar mechanism to that proposed for the binding of phospholipase to lipid bilayers (Volwerk & De Haas, 1982). It is significant that a single homogeneous species is not formed when AI is incubated with dimyristoyl- or dipalmitoylphosphatidylcholine at the phase transition temperature, but rather multiple products are observed [e.g., see Jonas et al, (1980) and Swaney (1980)]. Further, reported levels of "saturation" of AI with these phospholipids vary among laboratories. These observations are consistent with previous suggestions that this interaction is a kinetically limited process and the products observed are not necessarily the thermodynamically stable states (Reynolds, 1982; Pownall et al., 1981).

We have previously discussed the kinetic problems inherent in an investigation of the interactions of components one or more of which have limited solubility in the aqueous milieu (Mimms et al., 1981; Watt & Reynolds, 1981; Dhawan & Reynolds, 1983). One experimental approach is to disperse the hydrocarbon-soluble components in detergent-mixed micelles. We have demonstrated that removal of detergent from such multicomponent system of protein–lipid–detergent micelles leads to the formation of the morphological structure

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[1] Abbreviations: EYL, egg yolk phosphatidylcholine; SDS, sodium dodecyl sulfate; GdmCl, guanidinium chloride; HPLC, high-performance liquid chromatography; PPOPC, L-α-palmitoyl-2-palmitoleoylphosphatidylcholine; diC_{10}PC, didecanoylphosphatidylcholine; HDL, high-density lipoprotein(s); LDL, low-density lipoprotein(s); VLDL, very low density lipoprotein(s); EDTA, ethylenediaminetetraacetic acid.

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found in vivo. That is, membrane-bound proteins and phospholipids spontaneously form vesicular structures with the protein incorporated in a phospholipid bilayer, but apolipoproteins form water-soluble complexes in which the protein rather than the lipid has directed the final structure.

In this study, we have investigated the interaction of AI with the naturally occurring, long-chain phospholipid EYL by reconstituting a water-soluble protein–lipid complex from detergent systems in which the protein and lipid are completely dispersed. Under these conditions, the phospholipid retains no "memory" of its natural bilayer state, and the structural information directing the final complex is present in the amino acid sequence of the protein.

Experimental Procedures

Materials

The AI polypeptide of human serum HDL was prepared as described previously (Reynolds, 1976). Egg yolk phosphatidylcholine (EYL) was purchased from Lipid Products, Nutley, England, and showed less than 0.5% contamination by fatty acid or lysolecithin when chromatographed on Ultrasphere Si in hexane–2-propanol–water (6:8:1.4) on a Beckman HPLC. Sodium dodecyl sulfate was British Drug House Chemical Corp. "specially pure" grade purchased from Gallard Schlesinger. Sodium dodecyl [^{35}S]sulfate was obtained from Amersham. Octyl glucoside was from Calbiochem, and [^{14}C]octyl glucoside was from Research Products International, Elk Grove Village, IL. Sodium tauro[^{14}C]cholate was purchased from Amersham. Chromatographic supports, Sephacryl S200 superfine and S-1000, were obtained from Pharmacia, Inc. All other chemicals were standard reagent grade.

Methods

Gel exclusion chromatography was carried out by using Bio-Rad Econocolumns (0.7 × 50 cm) containing polyethylene support films. All lipid-containing samples were chromatographed on columns presaturated with EYL as described previously (Reynolds et al., 1983). Recovery of protein and lipid from the column was in all cases greater than 95%.

Analytical Procedures. Protein concentration was determined either from the previously published extinction coefficient of 1.09 (0.1% in a 1-cm cell) (Friedberg & Reynolds, 1976) or by the method of Lowry et al. (1951). A microcell of volume 50–100 μL was used in a Cary 17D spectrophotometer to measure the optical density of small-volume samples eluted from the gel filtration column. Lipid phosphorus was assayed according to the micro method of Bartlett (1959). Radioactive ligands were measured in a Beckman LS 100C liquid scintillation counter using ACS (Amersham) scintillation fluid.

Circular dichroic spectra were recorded on a Jobin-Yvon Dichrographe III calibrated with *d*-10-camphorsulfonic acid. Cells were 1-mm path length, and a mean residue weight of 116.7 was used to calculate molar ellipticity.

Fluorescence spectra were obtained by using a Perkin-Elmer MPF 44A spectrofluorometer and micro cells kindly provided for us by Dr. Robert Gennis, University of Illinois, Urbana, IL. Concentrations of protein and protein–amphiphile complexes used were in all cases less than 0.05 mg/mL.

Sedimentation equilibrium and velocity were determined in a Beckman Model E ultracentrifuge equipped with a photoelectric scanner. The data for multicomponent complexes were analyzed according to Reynolds & Tanford (1976). $M_p(1 - \phi'\rho)$ is obtained directly from the slope of a plot of ln OD vs. r^2, where M_p is the molecular weight of the protein component and $1 - \phi'\rho$ is the buoyant density factor. This latter term can be expanded as $(1 - \bar{v}_p\rho) + \sum_i \delta_i(1 - \bar{v}_i\rho)$ where $\bar{v}_p$ is the partial specific volume of the protein, $\bar{v}_i$ is the partial specific volume of each bound ligand, and δ_i is the grams of component i per gram of protein.

The Stokes radius of the complex was obtained by measurement of the sedimentation coefficient and $M_p(1 - \phi'\rho)$ using the relationship

$$R_s = \frac{M_p(1 - \phi'\rho)}{6\pi\eta sN}$$

where η is the solvent viscosity, s is the sedimentation coefficient, and N is Avogadro's number.

All AI complexes were formed in a solution containing 0.15 M NaCl, 0.02 M $NaHCO_3$, pH 8.0, and 0.001 M EDTA. Lipid-containing solutions were protected from light and blanketed with argon to avoid oxidative or photoinduced degradative reactions.

Binding of octyl glucoside to AI and EYL and of SDS to EYL was measured by equilibrium dialysis using radioactive detergent (Steinhardt & Reynolds, 1969).

Large multilamellar liposomes were prepared by bath sonication of EYL for 15 min at 4 °C with the solution protected from light and blanketed with argon.

Single-wall vesicles of EYL were prepared as described previously (Mimms et al., 1981; Reynolds et al., 1983) by removal of detergent from a solution of mixed micelles of octyl glucoside and EYL. The average diameter of the vesicles was determined by using gel exclusion chromatography on S-1000 (Reynolds et al., 1983).

Results

Conditions Leading to No Complex Formation between AI and EYL. (A) Condition 1. Incubation (24 h, 25 °C) of AI at concentrations between 1.7×10^{-5} and 7×10^{-5} M with EYL mutlilamellar liposomes and EYL single-walled vesicles of diameter 1700 Å did not result in the formation of a "tight" complex. Centrifugation of the protein–lipid mixture containing multilamellar liposomes at 12000*g* for 30 min produced a pellet of phospholipid free of protein and a supernatant consisting of protein only. Gel filtration chromatography of both of these mixtures (AI–multilamellar liposomes and AI–single-wall vesicles) on S200 superfine gave an elution pattern in which the total lipid emerged in the void volume and the total protein was included at an elution position corresponding to that of AI in the absence of lipid.

(B) Condition 2. Incubation of AI and EYL liposomes for 24 h in the presence of 6 M GdmCl followed by dialysis to remove the GdmCl also resulted in no complex formation by the same criteria described under condition 1. Centrifugation or gel filtration chromatography of the final mixtures led to total separation of the lipid and protein components.

Formation of a Heterogeneous, Partially Saturated Complex. When detergents are added to EYL liposomes at low detergent/EYL ratios, the detergent partitions between the aqueous phase and the lipid bilayer but does not totally disrupt the bilayer organization. When EYL is so treated and mixed with AI, complex formation ocurs, but the complexes are heterogeneous and contain far less EYL than is present in complexes formed after complete disruption of the bilayer organization. This is illustrated by the following two experiments.

Sodium dodecyl sulfate was added to EYL liposomes at a molar ratio of 1.6 (4.05×10^{-3} M SDS and 2.53×10^{-3} M EYL). The solution was turbid and did not clear over a period

of 2 h. Independent binding measurements showed a maximum of 0.3 mol of SDS associated with 1 mol of EYL under these conditions, the remainder being present as free SDS. AI was added at a concentration of 2.6×10^{-5} M and the mixture incubated overnight at room temperature. The solution which was still turbid was dialyzed to remove the detergent and chromatographed as described under Methods. Part of the lipid emerged free of AI in the void volume of the S200 column. All of the protein was well included in the column matrix and was found to be associated with a small portion of the added lipid. Lipid binding was heterogeneous across the protein peak, ranging from 27 mol/mol in the leading edge to 2 mol/mol in the trailing edge. The peak tube with an average molar binding ratio ($\bar{\nu}$) of 9 mol/mol was subjected to analytical ultracentrifugation, and the complex was found to contain AI in dimeric form (analytical result, 1.9 AI's per particle).

A similar experiment was performed with sodium taurocholate in which the detergent was added to EYL liposomes at a molar ratio of 4.25 (7.27×10^{-3} M detergent and 1.71×10^{-3} M EYL). The solution remained turbid over a 2-h period. AI was added to a final molar concentration of 2.4×10^{-5} M and the solution dialyzed to remove the taurocholate. The resultant turbid solution was centrifuged, and the supernatant which contained 100% of the protein was chromatographed on an S200 column. The included protein peak displayed heterogeneous binding, the leading edge corresponding to 48 mol of EYL/mol of AI and the trailing edge 17 mol of EYL/mol of AI.

Investigation of Saturation Binding of EYL to AI. In order to assure total dispersion of EYL in detergent–lipid mixed micelles, a minimum ratio of 10 mol of detergent/mol of EYL was used in excess of the critical micelle concentration. In the experiments reported, the AI concentration was (2–7) $\times 10^{-5}$ M and EYL concentration was varied from 1.4×10^{-4} to 0.03 M. The mixture of protein, lipid, and detergent was incubated at room temperature for 2–3 h and the detergent removed by dialysis. Radioactive detergent was used to monitor the rate of removal, which was rapid for sodium taurocholate and octyl glucoside but extremely slow for SDS. Typically, 1 mL of solution dialyzed against three 2-L changes of dialysate over a 24-h period lowered the concentration of the former two detergents (in the sample) to less than 10^{-6} M. Removal of SDS to the same levels required at least 72 h.

The resultant complexes of AI and EYL were chromatographed on S200 superfine presaturated with EYL, and the effluent was monitored for protein and EYL as described under Methods. The average EYL/AI molar ratio in the complexes ($\bar{\nu}$) is plotted in Figure 1A. The results demonstrate an apparent saturation of the protein at 110 ± 10 mol/mol (corresponding to 220 mol of EYL per mol of particle containing 2 mol of AI). Also shown in Figure 1 are the increase in Stokes radius with binding (B), the change in molar ellipticity, $[\theta_{208}]$ (C), and the number of copies of AI per particle as determined by analytical ultracentrifugation (D). The Stokes radii were determined by measurements of sedimentation velocity and sedimentation equilibrium on the same sample (see Methods under Experimental Procedures). In all cases, a single-sedimenting boundary was observed in sedimentation velocity experiments, and a plot of ln OD vs. the square of the radial distance, r^2, obtained from sedimentation equilibrium was linear. (It should be noted that binding heterogeneity is not readily detectable in ultracentrifugation procedures since the contribution of bound EYL to the buoyant density factor is less than 15% in all experiments.)

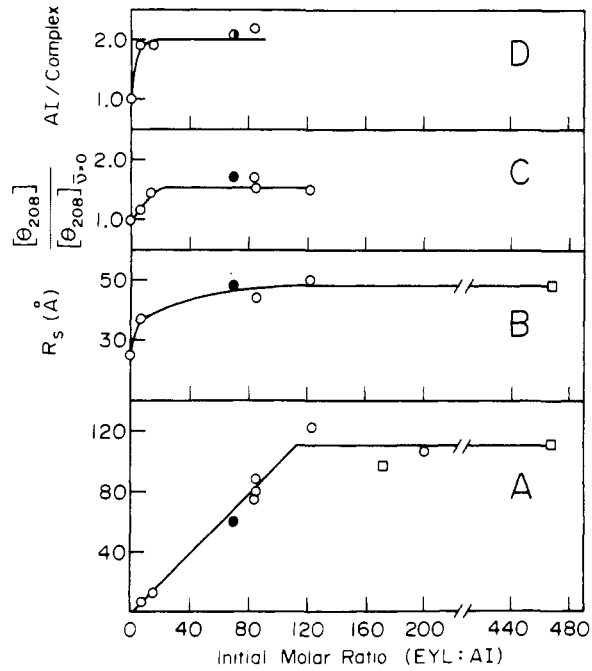


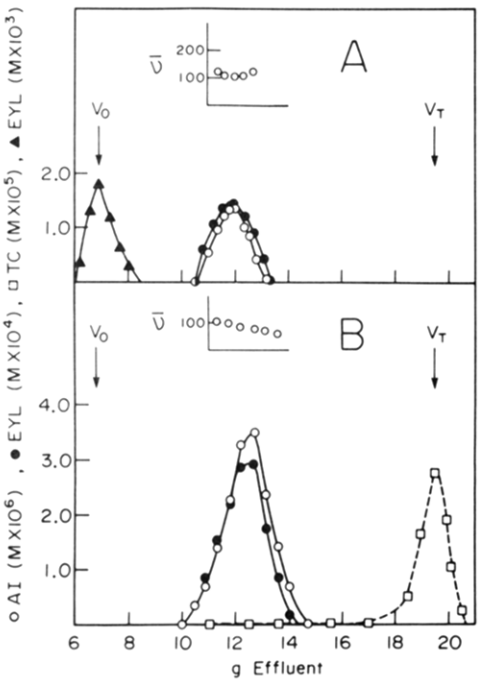
FIGURE 1: Complexes of AI–EYL. (A) $\bar{\nu}$ as a function of initial molar ratios of EYL to AI. (B) Stokes radii of EYL–AI complexes. (C) Increase in $[\theta_{208}]$ as a function of EYL binding. (D) Number of copies of AI per particle. (○) Complex formed from SDS; (●) complex formed from taurocholate; (□) complex formed from octyl glucoside.

When the initial ratio of EYL to AI is 100 or greater, homogeneous complexes are formed as shown in Figure 2A. This is representative of a large number of gel exclusion experiments with the three different detergents and initial EYL/AI molar ratios ≥100. On the other hand, at lower initial lipid to protein ratios, binding heterogeneity is observed as shown in Figure 2B. A Stokes radius of 48 Å for the $(AI)_2(EYL)_{220}$ complex corresponds to an $f/f_{min} = 1.07$ (dehydrated particle) or $f/f_0 = 1.04$ (allowing 9 mol of H_2O/mol of EYL bound), indicating the particle is very close to spherical.

Negative-stain electron microscopy with uranyl acetate as well as phosphotungstate (1%, pH 7.4) revealed small, apparently spherical particles (Figure 2C). The maximal diameter observed at apparent saturation (2 mol of AI/220 mol of EYL) was 90 ± 20 Å, in good agreement with the Stokes radius obtained by analytical ultracentrifugation. Complexes that were not fully saturated with EYL such as are shown in Figure 2B were heterogeneous in size, as would be predicted from the heterogeneity in binding, but also appeared nearly spherical. In no case were stacked disks (rouleaux) observed such as have been previously reported when complexes are formed between saturated diacylphosphatidylcholine and AI. Pownall et al. (1982) also observed spherical particles when AI–PPOPC complexes were formed by detergent removal from the ternary system AI–PPOPC–cholate.

The increase in α-helical content suggested by the increase in $[\theta_{208}]$ is much larger than that observed when the ligand is a single-tail amphiphile, i.e., a factor of 1.5–1.6 as compared to 1.0–1.2 (see Table II). Two spectra for EYL–AI complexes of $\bar{\nu}$ 6 and 100 are shown in Figure 3.

The interaction of AI with EYL also results in an alteration of the intrinsic fluorescence of the polypeptide as shown in Figure 4. The emission spectrum of uncomplexed AI (excited at 278 nm) has a maximum at 336 nm, characteristic of tryptophan emission in undenatured proteins. No pronounced shoulder is present at 305–310 nm, indicating quenching of



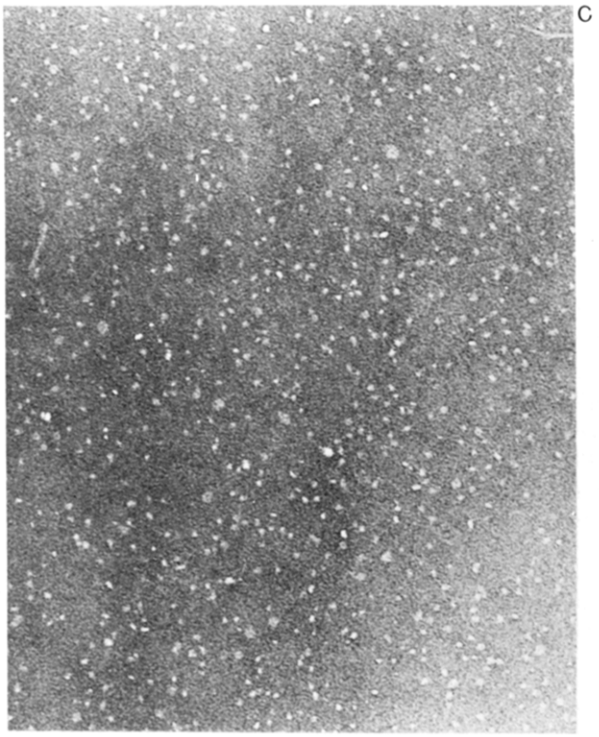


FIGURE 2: Elution profile of AI–EYL complexes from Sephacryl S200. (A) Complex formed from a 200:1 molar ratio of EYL:AI. (B) Complex formed from a 72:1 molar ratio of EYL:AI. (O) Protein concentration; (●, ▲) EYL concentration; (□) taurocholate concentration. (C) Negative-stain electron micrograph of the complex discribed in (B). Microscopy performed with uranyl acetate and phosphotungstate (1%, pH 7.4). Magnification 120000×.

the tyrosine residues in this form of AI. The low fluorescence yield of tyrosine can be attributed to a number of commonly observed factors such as energy transfer to nearby tryptophans, peptide bonds, and hydrogen bond accepting carbonyl groups. When AI–SDS or AI–EYL complexes are excited at 295 nm, the emission maximum is blue shifted by 4 nm and the intensity reduced relative to unliganded AI (data not shown). Excitation of these complexes at 278 nm results in a blue shift in the emission spectra and enhanced tyrosine fluorescence as shown in Figure 4. Clearly, combination with either ligand

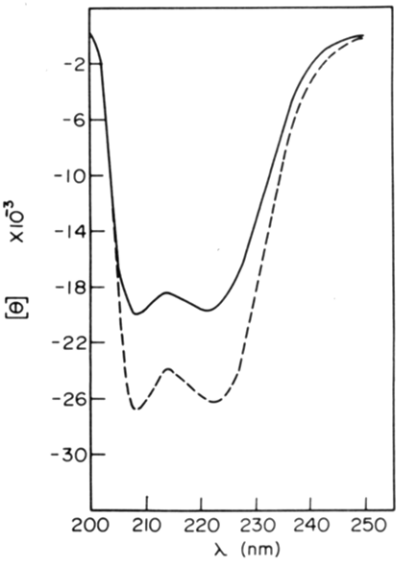


FIGURE 3: Circular dichroic spectra of AI–EYL complexes. (—) $\bar{\nu}$ = 6; (---) $\bar{\nu}$ = 100.

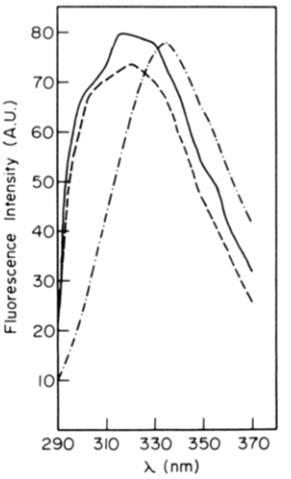


FIGURE 4: Fluorescence emission of AI–amphiphile complexes (excitation at 278 nm). (–·–) AI (no ligand); (---) AI–SDS at saturation; (—) AI–EYL at saturation.

results in disruption of internal energy transfer. Since AI is monomeric in the presence of SDS and dimeric when liganded with EYL, the altered spectral properties observed in Figure 4 are clearly ligand induced and not related to the state of association of the protein in the particle.

Interaction between Didecanoylphosphatidylcholine and AI. Diacylphosphatidylcholines with short acyl chains differ from EYL in that their rate of spontaneous hydrolysis to generate free fatty acid anions is relatively high. Phospholipids in this category, unless freshly purified, are therefore invariably contaminated by single-chain amphiphiles and will interact with AI without a need for added detergent. We have previously reported a study of the interaction of $diC_{10}PC$ with AI (Reynolds et al., 1977). Analysis of the data revealed highly cooperative binding of the lipid at a concentration 2 orders of magnitude above the critical micelle concentration (CMC). It was demonstrated that this was a thermodynamically impossible result unless one could postulate that a contaminant present at a much lower concentration than the diacyl lipid had to be bound to the protein before the diacyl lipid itself could interact. We assumed that this contaminant was either fatty acid or lysolecithin, although we were not aware at the time of the rapid rate of spontaneous hydrolysis of this lipid. The experiments reported here confirm this

Table I: Interaction of Didecanoylphosphatidylcholine with AI

h	concentration: AI ($\times 10^5$ M)	concentration: $diC_{10}PC$ ($\times 10^3$ M)	concentration: decanoate ($\times 10^4$ M)	M_p	$diC_{10}PC$/AI (mol/mol)	unbound $diC_{10}PC$ concn ($\times 10^3$ M)
20	1.31	1.145	0.882	28 400	0	1.145
48	1.31	1.093	1.91	56 800	5	1.036
72	1.31	1.056	2.64	56 800	41	0.592

Table II: Properties of AI–Amphiphile Complexes

ligand[a]	ligand: hydrophobic volume (Å^3)	ligand: surface area[b] (Å^2)	protein complex: molecules of ligand/molecule of AI	protein complex: bound hydrophobic volume/molecule of AI (Å$^3 \times 10^{-4}$)	protein complex: surface area occupied/molecule of AI (Å$^2 \times 10^{-4}$)	% helix
none						49
OG	206	55	210	4.3	1.16	nd[c]
SDS	314	55	138	4.3	0.76	47
$C_{14}TMAC$	369	55	117	4.3	0.64	43
$C_{16}PC$	407	63	97	3.9	0.61	53
$diC_{10}PC$	493	63	95	4.7	0.60	59
PPOPC	784	63	100	7.8	0.63	70
EYL	926	63	110	10.2	0.69	73
PPOPC			55[d]	4.3[d]	0.35[d]	
EYL			45[d]	4.3[d]	0.28[d]	

[a] Abbreviations: OG, octyl glucoside; SDS, sodium dodecyl sulfate; $C_{14}TMAC$, tetradecyltrimethylammonium chloride; $C_{16}PC$, lysopalmitoylphosphatidylcholine; $diC_{10}PC$, didecanoylphosphatidylcholine; PPOPC, L-α-palmitoyl-2-palmitoleoylphosphatidylcholine (Pownall et al., 1982); EYL, egg yolk phosphatidylcholine. [b] Estimate of surface area occupied by one ligand head group when the ligands are as close to each other as they are in micelles or bilayers (±10%). [c] nd, not determined. [d] Predicted on the basis of a constant hydrophobic volume.

interpretation by direct measurement of the fatty acid concentration as a function of time and comparison with the resultant change in diacyl lipid binding.

Table I shows the results; $diC_{10}PC$ was purified by HPLC, and interaction with AI was measured as previously described (Reynolds et al., 1977). After 20 h of incubation (AI + lipid), 8.8×10^{-5} M fatty acid was present. This was insufficient to produce interaction with diacyl lipid. No binding was observed, and AI was found to be monomeric by ultracentrifugation. At longer times of standing, the decanoate concentration increases, and the binding of diacyl lipid increases. It should be noted that these data cannot distinguish between an interaction model in which AI must bind three or four single-tail amphiphiles prior to diacyl lipid binding and one in which AI interacts directly with a lipid bilayer containing defects generated by partitioning of the decanoate into the diacyl lipid. Regardless of the mechanism of association, AI does not bind diacyl phospholipids above the phase transition in the absence of detergent-like molecules.

Discussion

Naturally occurring phospholids such as EYL have extremely low monomer concentrations and thus do not bind to the high-affinity sites on AI [e.g., see Reynolds (1982)]. AI also does not interact with the self-associated form of either $diC_{10}PC$ or EYL at 25 °C in the absence of single-tail amphiphiles. Therefore, complex formation between this protein and phospholipids was investigated by "reconstitution" from ternary systems consisting of detergent–lipid–AI. The complexes formed by these procedures are homogeneous and spherical. They are quite different from the heterogeneous and disk-shaped complexes observed when fully saturated long-chain diacyl lipids combine with AI at the thermotropic phase transition in the absence of single-tail amphiphiles [see Scanu et al. (1982) for a review of these complexes]. Since bilayers composed of fully saturated diacyl lipids do not normally occur in nature (and would not be near the phase transition temperature if they did occur), it is arguable that the complexes observed here may be more relevant to the *in vivo* assembly of HDL than the disk-shaped complexes.

We have demonstrated an apparent maximal association of 220 ± 20 mol of EYL with 2 mol of AI. This corresponds to a hydrophobic volume much larger than has been observed previously. This is in excellent agreement, however, with the AI–PPOPC spherical complex reported by Pownall et al. (1982) that was formed from a cholate-containing solution of lipid and protein. Neither of these latter two results, however, agrees with the conclusion based on our previous data for single-chain amphiphiles and for $diC_{10}PC$ that the amphiphile binding region of AI is designed to accommodate a fixed hydrophobic volume of about 4×10^4 Å^3 per AI molecule, regardless of the amphiphile head group. Predicted binding on that basis would be much less than observed, about 50 mol of EYL or PPOPC per mol of AI. At the same time, there is a marked difference in the circular dichroic spectrum when the latter ligands are bound, showing that the increase in binding is the result of a major change in the folding of the polypeptide chain of the protein. Table II summarizes the relevant data.

A plausible thermodynamic basis for the instability of the fixed-volume complex for EYL binding is suggested by calculating the *maximal* area that the polar groups of the amphiphiles can be expected to occupy at the interface between the binding volume and the adjacent aqueous solution. As Table II shows, this area would be much smaller for EYL (and PPOPC) than for the ligands we have previously studied if the same conformation of the protein with the same fixed binding volume were used. The conformational change results in an increase in the total area of ligand head groups per AI to a level comparable with that of previously studied ligands. The thermodynamic driving force for such a conformational change can be understood in terms of the well-established

principle (Tanford, 1980; Israelachvili et al., 1976) that excess space between aggregated amphiphiles and water leads to instability because it must lead to unfavorable contacts between water and hydrocarbon. To fulfill the foregoing thermodynamic requirements, additional binding regions are created on AI by the formation of additional (presumably amphipathic) helices.

The increase in tyrosine emission observed when AI binds amphiphilic ligands is not dependent on the state of association of the protein nor is it a function of increased helical content (see Table II for a comparison of SDS and EYL helix content). The simplest explanation for this phenomenon is that binding to amphipathic helical "pockets" in the protein results in a spatial separation of tyrosine and tryptophan such that efficient energy transfer cannot occur. All the tryptophan residues are present in the first half of the polypeptide chain (positions 8, 50, 72, and 108). There are four tyrosines at positions 115, 166, 192, and 236, so it is possible that these residues have been removed from close contact with the tryptophan-containing region of AI by interaction with the hydrophobic region of the amphiphilic ligands.

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Registry No. OG, 29836-26-8; SDS, 151-21-3; TMAC, 4574-04-3; $diC_{10}PC$, 3436-44-0.

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