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Interactions of Serum Proteins with Small Unilamellar Liposomes Composed of Dioleoylphosphatidylethanolamine and Oleic Acid: High-Density Lipoprotein, Apolipoprotein A1, and Amphipathic Peptides Stabilize Liposomes†

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ABSTRACT: Small unilamellar liposomes composed to dioleoylphosphatidylethanolamine (DOPE) and oleic acid (OA) are stabilized by incubation with normal human serum or plasma [Liu, D., & Huang, L. (1989) *Biochemistry 28*, 7700-7707]. The present report describes a systematic study of interactions of purified serum proteins and lipoproteins with these liposomes. Albumin destabilized liposomes by extracting OA from the liposomes, whereas immunoglobulins and lipoproteins (HDL, LDL, and VLDL) had no effect. However, HDL and, to some extent, VLDL showed a rapid stabilization activity against the lytic effect of albumin. HDL added together with or shortly after the addition of albumin completely abolished the liposome leakage and aggregation effects induced by albumin. SDS-PAGE analysis of the HDL-stabilized liposomes revealed that apolipoprotein A1 was associated with liposomes. Purified apolipoprotein A1, but not a lipid mixture resembling the lipid composition of HDL, showed comparable liposome stabilization activity as HDL. Furthermore, synthetic peptides resembling the amphipathic helices found in apolipoprotein A1 also showed strong liposome stabilization activity. Peptides which were able to form amphipathic helixes of a wedge shape were more effective stabilizers than those which could not. These data indicate that HDL plays a major role in human serum or plasma for the liposome stabilization activity. HDL exerts its activity probably by the interactions of the amphipathic helices of apolipoprotein A1 with the hydrophobic voids found on the outer surface of the highly curved, small liposomes.

One of the crucial aspects of using liposomes as a drug delivery vehicle is to understand their interactions with plasma or serum components. Previous work has revealed that a number of serum proteins become associated with liposomes [for reviews, see Juliano and Lin (1980) and Senior (1987)]. For example, albumin, immunoglobulins, fibronectin, and other serum proteins are found to coat the surfaces of multilamellar liposomes composed of phosphatidylcholine (PC)[1] and cholesterol, and additionally containing phosphatidylserine or stearylamine (Juliano & Lin, 1980). Interaction of HDL with multilamellar liposomes composed of PC causes the lysis of liposomes and transfer of liposomal PC to HDL (Tall & Green, 1981; Kirby et al., 1980; Senior et al., 1983). Insertion of apolipoprotein(s) into the membrane of small unilamellar liposomes at or near the chain-melting temperature causes a rapid leakage of the entrapped contents (Weinstein et al., 1981; Scherphof et al., 1984). Activated complement components also induce liposome leakage by forming pores in the liposome membrane (Hexby et al., 1969). Some yet unidentified serum protein(s) is (are) likely to be opsonin(s), which promote(s) the uptake of liposome by macrophage, primarily those in liver and spleen (Ivanov et al., 1985).

It is well-known that liposomes composed of unsaturated PC are not stable in serum or plasma, unless cholesterol is

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[1] Abbreviations: DOPE, dioleoylphosphatidylethanolamine; OA, oleic acid; PC, phosphatidylcholine; BSA, bovine serum albumin; HDL, high-density lipoprotein(s); LDL, low-density lipoprotein(s); VLDL, very low density lipoprotein(s); PBS, phosphate-buffered saline; Apo A1, apolipoprotein A1; Igs, human immunoglobulins.

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included in the lipid composition (Gregoriadis & Senior, 1980). We have previously reported that small unilamellar liposomes (average diameter ≤200 nm) composed of DOPE and OA, unlike the same size liposomes composed of DOPC and OA, are not lysed by the serum. Instead, they are stabilized by some serum components (Liu & Huang, 1989a). The stabilized liposomes contain a number of serum proteins and lipids. Most of the proteins associated with the stabilized liposomes are probably not responsible for stabilization, because liposomes treated with trypsin which removed about 70% of the associated proteins are still stable. The stabilized liposomes show a significantly higher degree of membrane microviscosity, probably due to the insertion of serum protein into the liposome membrane (Liu et al., 1989). The serum-stablized liposomes stay in the circulation for a relatively long period as compared to other PC-based liposomes of similar size (Liu et al., 1989). Thus, the serum-stablized, PE-containing liposomes are potentially useful for drug delivery.

In order to further elucidate the interactions of this novel liposome with serum proteins, we have initiated a series of experiments using purified serum proteins, lipoproteins, Apo A1, and peptide analogues of Apo A1. Stability of liposomes is studied by measuring the release of an entrapped fluorescent dye, calcein, and by light scattering and electron microscopy. The results of these studies have been useful to identify the serum components which interact with and stabilize the PE-containing liposomes.

Experimental Procedures

Materials. DOPE, egg PC, phosphatidylserine, phosphatidylinositol, phosphatidic acid, and sphingomyelin were purchased from Avanti Polar Lipids, Inc. Calcein, BSA, human γ-globulins, cholesterol, cholesteryl ester, glycerides, Ficoll, and oleic acid were obtained from Sigma Chemical Co. [^{14}C]Oleic acid (57 mCi/mmol) was purchased from New England Nuclear.

Isolation of Lipoproteins and Apolipoproteins. HDL, LDL, and VLDL were isolated from freshly collected normolipemic plasma in a potassium bromide density gradient using the ultracentrifugation procedure according to Chung et al. (1986). The individual lipoproteins were washed and reisolated employing the same gradient centrifugation method with a different density gradient. The appropriate fractions from each ultracentrifugation were pooled. Apo A1 was isolated from Apo-HDL by gel filtration and ion-exchange chromatography (Edelstein et al., 1972). The purified lipoproteins and apolipoproteins were then dialyzed against PBS (phosphate-buffered saline: 178 mM NaCl, 3.5 mM KCl, 2.0 mM KH_2PO_4, 1.3 mM Na_2HPO_4, and 1.3 mM EGTA). The protein concentration was determined by the Lowry assay method (Lowry et al., 1951).

Synthesis of Peptides. The peptides were synthesized by the solid-phase peptide synthesis procedure as previously described (Anantharamaiah et al., 1985). Release of peptides from the solid support was achieved by the modified HF procedure (Tom et al., 1979). Crude peptides were purified by reverse-phase HPLC. The purity was confirmed by sequencing and amino acid analysis. The sequence and characteristics of the peptides have been described (Anantharamaiah et al., 1985).

Preparation of Liposomes. Lipids containing DOPE and OA with a molar ratio of 2 to 1 were dried by using a N_2 stream, vacuum-desiccated for a minimum of 30 min, and hydrated for 3–5 h at room temperature in a calcein (50 mM) containing buffer. The osmolarity of this buffer adjusted with 10× PBS was equal to that of dialyzing buffer in lipoprotein preparation. A trace amount of hexadecyl [^{3}H]cholestanyl ether was included in lipids in order to monitor the lipid. The lipid suspension was sonicated with a bath sonicator for 10 min at room temperature (Laboratory Supplies, Hicksville, NY), and the pH was adjusted and maintained at pH 8.0 during sonication. The liposome suspension was left for 2 h at room temperature to facilitate the annealing process before free calcein was separated from liposomes by using BioGel A0.5M column chromatography equilibrated with PBS. The size of the liposomes used in the experiments was 90–120 nm as determined by quasi-elastic light scattering using a Coulter N4SD instrument.

Interaction of Serum Proteins with Liposomes. Liposomes (10 μL containing 5–15 μg of lipid) were added to a cuvette containing 1.99 mL of PBS for the fluorescence measurement with constant stirring. The serum protein was normally added at 2 min. The fluorescence intensity was measured with an excitation wavelength of 490 nm and an emission wavelength of 520 nm. The total fluorescence intensity was obtained by adding 50 μL of deoxycholate (5%) to lyse the liposomes. Release of the entrapped calcein from the liposomes was calculated with the equation:

$$\% \text{ release} = \frac{F_x - F_0}{F_t - F_0} \quad (1)$$

where F_0 is the fluorescence intensity of the liposomes at 1 min, i.e., before the addition of serum protein. F_t is the total fluorescence intensity of liposome after addition of deoxycholate. F_x is the fluorescence intensity at different times or the fluorescence intensity before the addition of deoxycholate in the presence of stabilizer. For the stabilization of liposomes by apolipoproteins and lipoproteins against the lysis of BSA, the apoproteins were added to the cuvette 1 min after the addition of liposomes and before the addition of BSA.

Removal of OA from Liposomes by BSA. Liposomes containing trace amounts of [^{14}C]OA were incubated with different amounts of BSA in the presence or absence of HDL at 37 °C for 1 h before passing through a BioGel A1.5M column. The fractions were counted for ^{14}C and ^{3}H. The percent removal of OA from liposomes was calculated by dividing the counts of ^{14}C from nonliposomal fractions with the total ^{14}C counts. Liposomes were eluted in the void volume fractions as monitored by both ^{3}H counts and calcein entrapment.

Electron Microscopy of Liposomes with Different Treatments. Liposomes treated with either PBS or BSA (in the presence or absence of HDL) for 1 h at 37 °C were passed through a BioGel A1.5M column to remove excess proteins. Liposomes were examined with negative-stain electron microscopy using 1% uranium acetate as a stain (Wang & Huang, 1984).

Association of Serum Proteins with Liposomes. Liposomes containing 2.5 mg of lipids were incubated with HDL or Igs with a ratio of 0.2 (protein to lipid ratio) for 1 h at 37 °C. The mixture was then mixed with a Ficoll solution (in PBS, pH 8.0) to obtain a final Ficoll concentration of 20% (w/w) and placed into a 15-mL COREX centrifuge tube. Six milliliters of 15% (w/w) Ficoll was overlayed, followed by 0.5 mL of PBS. The mixture was centrifuged in a Sorvall HB4 swinging-bucket rotor for 1 h at 5000 rpm at room temperature. The liposomes floated to the PBS and 15% Ficoll interface were collected. The liposomes were floated 1 more time with an identical procedure before SDS–PAGE analysis. SDS–PAGE was done using a 15% acrylamide gel under reducing conditions (Laemmli, 1970).

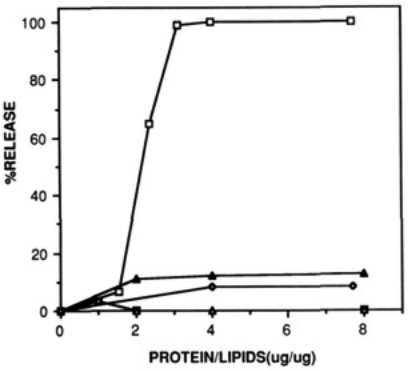

FIGURE 1: Effect of serum proteins on liposome stability. Ten microliters of liposomes containing 15 μg of lipid was added at time zero to 1.99 mL of PBS in a cuvette with constant stirring. Proteins were added at 1 min. Percent release was calculated according to eq 1 (see Experimental Procedures). The fluorescence intensity at 9 min was used as F_x to calculate the release. (□) BSA; (■) HDL; (▲) LDL; (△) VLDL; (◊) Igs.

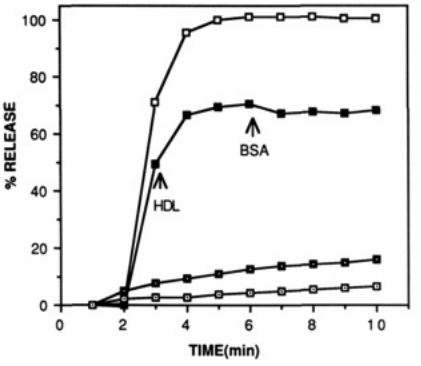

FIGURE 2: Effect of HDL on BSA-induced lysis of liposome. Ten microliters of liposomes containing 15 μg of lipids was added to 1.99 mL of PBS in a cuvette with constant stirring. Proteins were added at 2 min. (□) BSA only (50 μg/mL); (⊡) HDL only (1.5 μg/mL); (■) BSA (50 μg/mL) plus HDL (1.5 μg/mL); (■) BSA (50 μg/mL) followed by HDL (1.5 μg/mL) and then more BSA was added as indicated by arrows (final concentration of BSA was 2 mg/mL).

RESULTS

Effect of Serum Proteins on Liposome Stability. In order to study the interaction between liposomes and serum proteins, we have examined the effects of BSA, HDL, LDL, VLDL, and Igs, which were selected according to their abundance in plasma and known functions on liposomes (Senior, 1987). As can be seen in Figure 1, none of these proteins except BSA caused any significant release of calcein at concentrations tested. On the other hand, BSA induced a complete calcein release from the liposomes. Thus, the destabilization activity of liposome is rather unique to the albumin, probably related to its strong affinity to oleic acid.

Stabilization of Liposomes against the Lytic Effect of Albumin. Although most of the proteins investigated above had no lytic activity to liposomes, some of them showed a striking effect of stabilization against the lysis induced by BSA. Figure 2 shows an example with HDL. When BSA was added at 2 min, it caused a rapid and complete release of calcein ($t_{1/2}$ ~40 s). If BSA and HDL were both added at 2 min, no rapid liposome lysis was observed. Even if HDL was added 1 min after the addition of BSA, a partial protection of liposome against the BSA-induced lysis was seen. Addition of more BSA (at 6 min) to the protected liposomes no longer produced any calcein release. This experiment clearly indicated the liposome stabilization effect of HDL against the lysis by BSA.

Destabilization of liposomes induced by BSA was accompanied by a rapid increase in turbidity as measured by 90° light scattering (data not shown). The rapid increase in light scattering, which was BSA concentration dependent, was followed by a slower decrease. This is due to the formation of very large lipid aggregates which gradually settled out of the solution. Addition of both BSA and HDL to liposomes had completely blocked the liposome aggregation (data not shown).

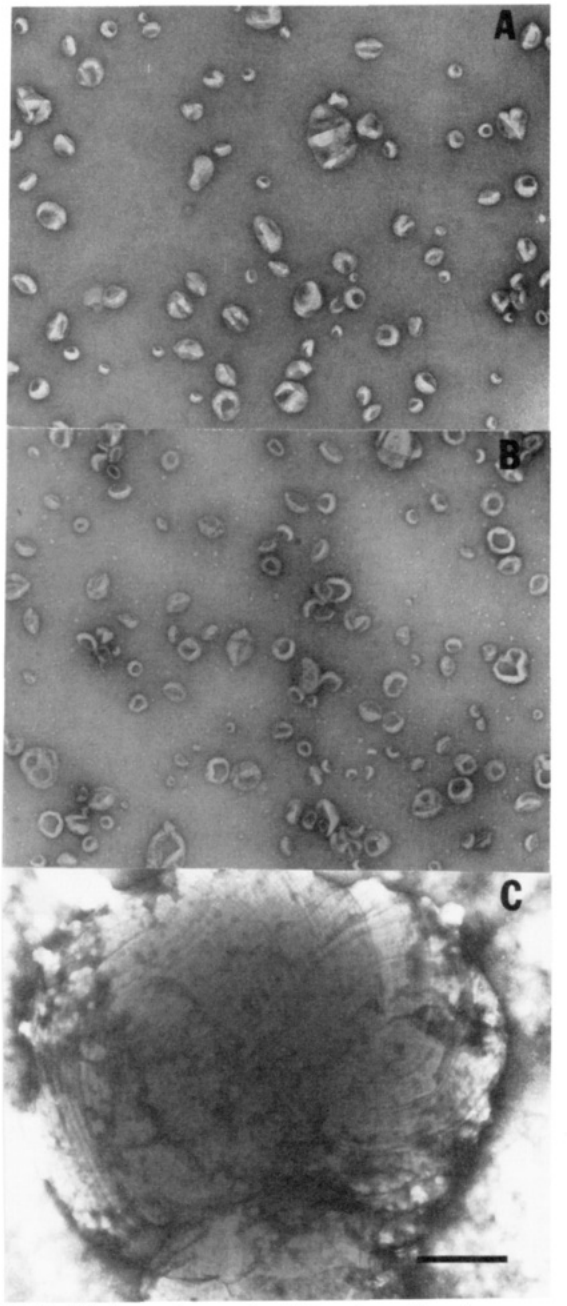

FIGURE 3: Electron micrographs of liposomes. Samples were negatively stained with 1% uranyl acetate. Bar is 0.5 μm. (A) Liposomes treated with PBS. (b) Liposomes treated with BSA in the presence of HDL. BSA/lipid ratio = 22.5, HDL/lipid ratio = 0.34. (C) Liposomes treated with BSA (BSA/lipid ratio = 22.5) in the absence of HDL. All ratios are weight ratios.

The protection effect of HDL against the BSA-induced lysis of liposomes can also be seen by negative-stain electron microscopy (Figure 3). Liposomes prepared by sonication were unilamellar and nonaggregative with an average diameter of 120 nm (Figure 3A). Addition of BSA had caused the formation of large lipid aggregates such as the one shown in Figure 3C. These aggregates were greater than 1 μm in size and often showed a fine structure of parallel cylinders (Figure

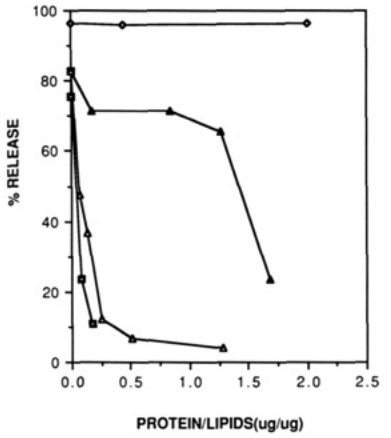

FIGURE 4: Block of BSA-induced release by plasma proteins. The BSA concentration used in this experiment was 0.2 mg/mL which gave a ratio of BSA to lipids of 26 (μg/μg). The maximum release was plotted. (■) HDL; (▲) LDL; (△) VLDL; (◇) Igs.

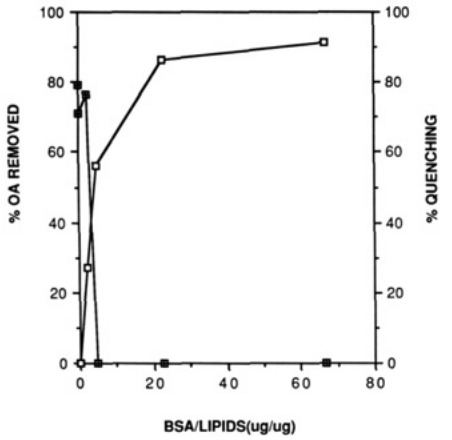

FIGURE 5: Removal of OA from liposomes by BSA. (□) Percent OA removed; (⊞) percent fluorescence quenching.

3C) which could be related to the hexagonal (H_{II}) phase of DOPE. Treatment with both BSA and HDL showed that liposomes remained unilamellar and small in size (average d = 120 nm) (Figure 3B). Numerous HDL particles can also be seen in Figure 3B.

The stabilization activity was not limited to HDL; VLDL had a similar but somewhat weaker activity (Figure 4). LDL only showed a partial stabilization activity at very high concentrations. Igs were without any activity in the concentration range tested.

Removal of OA by BSA from Liposomes in the Presence or Absence of HDL. Albumin is the most abundant protein in serum. It also has a strong affinity to bind amphipathic molecules such as free fatty acids. Removal of OA from liposomes was investigated by including [^{14}C]OA in the liposomes. Using gel filtration to separate [^{14}C]OA associated with liposomes and BSA, it is shown in Figure 5 that at 5 μg of BSA/μg of lipid, approximately 57% OA was removed from liposomes which had caused the complete release of all entrapped calcein, i.e., 0% fluorescence quenching. Higher amounts of BSA removed more OA from liposomes. Thus, the interaction of albumin with small unilamellar liposomes composed of DOPE and OA is to extract OA from liposomes, leading to a rapid destabilization of the liposomes.

One possibility of the stabilization activity of HDL is to prevent the removal of OA from liposomes by BSA. It is clear from the data shown in Figure 6 that HDL did not prevent the removal of OA from liposomes by BSA. Approximately

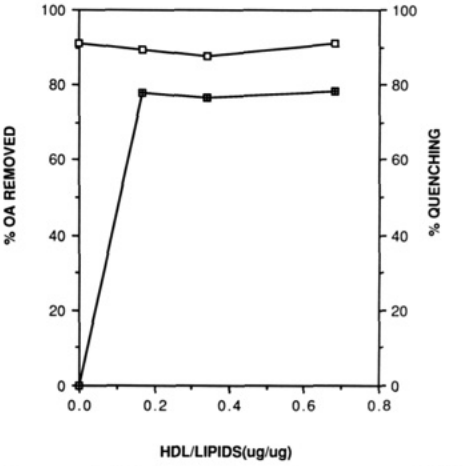

FIGURE 6: Removal of OA by BSA in the presence of HDL. Liposomes containing trace amounts of [^{14}C]OA were incubated with BSA (BSA/lipid ratio = 22.5) in the presence of different amount of HDL for 1 h at 37 °C, and a BioGel A1.5M column was used to separate BSA-extracted OA from liposome-associated OA. (□) Percent OA removed from liposome; (⊞) percent quenching of fluorescence.

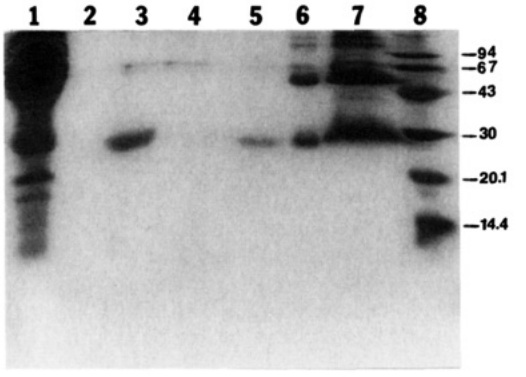

FIGURE 7: SDS–PAGE analysis of liposome-associated proteins. Lane 1, whole human plasma; 2, HDL alone floated; 3, HDL; 4, liposomes floated; 5, liposomes treated with HDL and then floated; 6, immunoglobulins; 7, liposomes treated with immunoglobulins and then floated; 8, molecular weight standards.

90% of liposomal OA was still transferred to BSA in the presence of HDL which had completely protected the liposomes against the lytic activity of BSA. The liposomes, with only about 10% of the original OA remaining, stayed stable, showing 80% calcein fluorescence quenching.

Association of Serum Proteins with Liposomes. Strong liposome stabilization activity of HDL could be due to the interaction of apolipoproteins with the liposome membrane. To examine this possibility, we have isolated liposomes after incubation with HDL using a Ficoll gradient floatation protocol (see Experimental Procedures). The protein composition of the stabilized liposomes was examined by SDS–PAGE using 15% acrylamide under reducing conditions. Figure 7 shows the electrophoretic patterns of liposomes. It is clear from lane 5 of the figure that Apo A1 was associated with the HDL-stabilized liposomes. It should be noted that Apo A1 is the major apolipoprotein of HDL. Under the electrophoretic conditions used in the experiment, Apo A1 was the only apolipoprotein seen on the gel; no other smaller molecular weight apolipoproteins such as Apo A2 were seen (lane 3). It is also clear that HDL itself did not float in the Ficoll gradient; hence, no protein bands could be detected in the floated HDL sample (lane 2). Since the light-scattering measurements (data not shown) suggest that Igs may be weakly interacting with liposomes, we have also examined the liposomes reisolated by

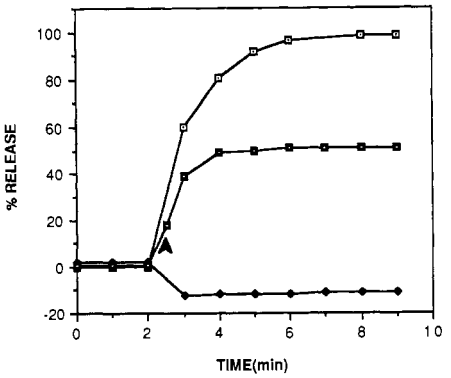

FIGURE 8: Block of BSA-induced release by Apo A1. The conditions used in this experiment were the same as those described in Figure 2. (⊡) BSA only (0.2 mg/mL); (♦) ApoA1 plus BSA (Apo A1/lipid ratio = 0.2 μg/μg); (■) Apo A1 was added 0.5 min after addition of BSA (arrowhead).

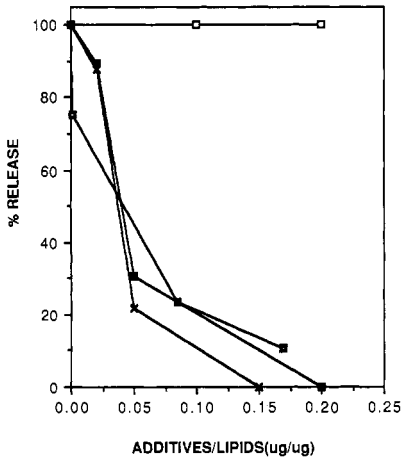

FIGURE 9: Comparison of stabilization effect of liposome by HDL or HDL components against BSA (0.2 mg/mL)-induced lysis. The assay was carried out in the same way as described in Figure 4 except that the lipid components of HDL were mixed and the organic solvent was evaporated in a N_2 gas and hydrated for a few hours before an intensive sonication (30 min). The lipid mixture was incubated with Apo A1 for 3 h at room temperature before the stabilization assay. (■) HDL only; (×) Apo A1; (□) lipid components of HDL (see text for composition); (■) mixture of Apo A1 and lipid components of HDL.

the floating protocol after incubation with Igs (lane 7). It is also evident that Igs were associated with the liposomes. Free Igs did not float in the gradient (data not shown); hence, the bands shown in lane 7 were due to liposome association.

Stabilization Effect of Apo A1 against the Lytic Activity of BSA. The results of the experiment shown in Figure 7 strongly suggest that Apo A1 of HDL is responsible for the liposome stabilization activity of HDL. We have thus tested the stabilization of liposomes with purified Apo A1. To rule out the possibility of lipid involvement in the stabilization process, we have also used a mixture of purified lipids resembling the lipid composition of HDL (Charles, 1981). The mixture contained the following lipids (all weight percent): cholesterol oleate (32.4%), cholesterol (6.8%), glycerides (13.4%), egg PC (35.2%), lysoPC (1.4%), sphingomyelin (6.3%), phosphatidylserine (0.04%), phosphatidylinositol (1.1%), DOPE (1.5%), and phosphatidic acid (1.0%). Apo A1 added together with BSA blocked the liposome lysis induced by BSA alone (Figure 8). Even added 0.5 min after the addition of BSA, Apo A1 still partially protected the liposomes against the lysis of BSA. The action of Apo A1 completely resembled that of HDL (see Figure 2). The stabilization activity of Apo A1 was as strong as that of HDL,

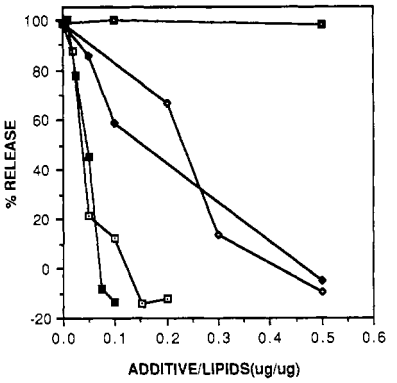

FIGURE 10: Block of BSA-induced release by Apo A1 and peptides. The conditions were the same as described in Figure 4. (⊡) Apo A1; (♦) 18A; (■) 18R; (◇) 37AA; (■) 37PA.

giving a complete stabilization at protein/lipid ratios of about 0.15 or higher (Figure 9). A lipid mixture resembling the lipid composition of HDL had no stabilization effect at the concentration range tested. It also did not interfere with the activity of Apo A1 when it was premixed with the apolipoprotein before addition to the liposomes. These results strongly indicate that Apo A1, not the lipids, is the molecular component in HDL which gives rise to liposome stabilization activity.

Stabilization Effect of Amphipathic Peptides against the Lytic Activity of BSA. Numerous studies have suggested that the amphipathic helical regions of Apo A1 are responsible for the interaction with the lipids in the HDL particles (Segrest et al., 1974; Anantharamaiah et al., 1990). We have tested if these amphipathic peptides could stabilize the liposomes against the lytic activity of BSA. As shown in Figure 10, peptide 37PA had a strong stabilization activity which was indistinguishable from that of ApoA1 on the basis of mass. However, on a molar basis, peptide 37PA was much more potent in liposome stabilization than Apo A1, since its molecular weight is much smaller. Peptides 37AA and 18A were less potent, requiring about 5-fold higher concentrations for a similar stabilization effect as that of peptide 37PA. Peptide 18R was ineffective over the concentration range tested.

DISCUSSION

It is known that albumin lyses the pH-sensitive liposomes containing DOPE and OA by extracting OA from the liposomes (Leventis et al., 1987). This lytic activity is at least partially responsible for the instability of large unilamellar liposomes in serum or plasma (Connor et al., 1986; Liu & Huang, 1989b). We have shown that inclusion of cholesterol in the large liposomes composed of DOPE and OA improves the stability of liposomes. The enhanced stability is not due to the absence of OA removal from liposomes, because the same rates of OA transfer are observed from liposomes with and without cholesterol (Liu & Huang, 1989b). The effect of serum or plasma on the small unilamellar liposomes ($d \leq$ 200 nm) of the same composition is quite different from that of the larger liposomes. The small liposomes, with or without cholesterol, are stabilized by some serum component(s) such that the liposomes are stable even after most of OA is extracted by albumin (Liu & Huang, 1989a). The molecular component(s) of the serum stabilizing factor was (were) not identified in the previous work. The present studies utilizing purified serum proteins have helped to elucidate the identity of the factors.

It is not surprising that BSA showed a rapid destabilization activity to the small liposomes composed of DOPE/OA

(Figures 1–4). It caused release of entrapped calcein (Figures 1, 2, and 5), extraction of OA (Figure 5), and formation of H_{II}-phase-like aggregates (Figure 3). These activities are best explained by the removal of OA from liposomes, which leaves DOPE by itself, and the transition of DOPE from a bilayer phase to the H_{II} phase. The temperature of the $L_\alpha \rightarrow H_{II}$ phase transition of pure DOPE is approximately 10 °C at pH 7 and physiological ionic strength (Cullis & de Kruijff, 1979). Thus, the equilibrium phase of DOPE at room temperature is the reverse-micellar, H_{II} phase. However, the presence of OA has stabilized the bilayer phase by providing a net negative surface charge (Straubinger et al., 1985) such that liposomes can be stabilized. Liposomes composed of DOPE and OA are acid-sensitive in that at mildly acidic pH rapid bilayer destabilization and vesicle fusion take place (Connor et al., 1984). This is the basis of using such liposomes for efficient cytoplasmic delivery following the endocytosis of liposomes by the target cells (Straubinger et al., 1983, 1985). Removal of OA would cause the liposomes to lose their negative surface charge and promote interliposome juxtaposition upon collision, which drives a rapid conversion into the H_{II} phase.

The emphasis of the present work is not about the liposome destabilization by BSA, but rather to use the BSA-induced liposome lysis as a method to study the stabilization effect of serum lipoproteins. We have used the experimental conditions mimicking the condition of serum incubation. The key aspect is to look for the stabilization event which proceeds at a rate much faster than that of the albumin-induced liposome lysis. Our data strongly indicate that HDL showed such stabilization activity (Figures 2–4). Liposomes treated with HDL and BSA did not release calcein (Figures 2 and 4) and stayed nonaggregated (Figure 3). The effect of HDL was concentration dependent (Figure 4) and proceeded at a very fast rate (Figure 2). Even added to liposomes 1 min after the addition of BSA, substantial amounts of liposomes were already stabilized (Figure 2). The stabilization activity of HDL is potent; at as low protein/lipid ratios as 0.1–0.3, which is roughly equivalent to 0.3–1.0 μg/mL in concentration, HDL had completely stabilized the liposomes. Note that the concentration of HDL in normal human serum is 3.6 (2.90–7.70) mg/mL (Altman & Katz, 1977) which is 3600–12 000-fold higher than the minimal concentration required for liposome stabilization. Thus, it is highly likely that HDL is a major serum component which stabilizes the small unilamellar liposomes composed of DOPE and OA, a peculiar phenomenon previously observed by us (Liu & Huang, 1989a).

VLDL showed a similar but somewhat weaker stabilization activity (Figure 4). However, the concentration of VLDL in normal human serum (0.1 mg/mL) is lower than that of HDL. Its contribution to the serum (or plasma) stabilization activity for liposomes is probably not as important as that of HDL. Igs also showed a weak stabilization activity when assayed by the enhancement of light scattering induced by BSA (data not shown).

The data shown in Figure 7 clearly indicate that Apo A1 of HDL had transferred to liposomes during the incubation. Data in Figures 8 and 9 also indicate that Apo A1, not the lipid components, of HDL is responsible for the liposome stabilization activity. The activity of Apo A1 was as potent as HDL, effective at protein/lipid ratio of about 0.15 or higher. Thus, Apo A1 is likely a major molecular species in the normal human serum or plasma which has brought about the liposome stabilization activity. Since Apo A1 is not found in VLDL (Charles, 1981) which has a similar stabilization activity as HDL (Figure 4), other apolipoproteins may also be involved in the stabilization reaction. This possibility will be investigated further.

Apolipoproteins contain amphipathic helices which interact strongly with phospholipids to form discoidal structures which resemble the structure of native lipoprotein particles. The peptide analogues of amphipathic helix in Apo A1 have been studied in detail (Anantharamaiah et al., 1985; Chung et al., 1985). It contains postively charged lysine and arginine residues at the interface between the hydrophilic and hydrophobic surfaces and negatively charged residues at the center of the hydrophilic face (Anantharamaiah et al., 1985). Due to the relatively bulky and hydrophobic side chains of lysine and arginine, the overall shape of the helix cylinder when lying on the surface of a phospholipid membrane looks like a wedge; i.e., the volume of the hydrophobic surface is bulky near the interfacial region, and the volume is relatively small near the center of the surface. It has been proposed that such a wedge-shaped amphipathic helix is essential for maximal interaction with phospholipids (Chung et al., 1985; Epand et al., 1989; Anantharamaiah et al., 1985).

DOPE contains a relatively small and weakly hydratable head group (Cullis & de Kruijff, 1979) and two cis-unsaturated, bulky acyl chains. The overall shape of the molecule is that of an inverted cone, i.e., a shape opposite to that of a wedge. This is the primary reason that DOPE has a high tendency to aggregate into the reverse-micellar, H_{II} phase. In small unilamellar liposomes of high curvature, DOPE is well-suited to locate in the inner monolayer, because it closely resembles the packing of the H_{II} phase. The lipids in the outer monolayer, on the other hand, are likely not packed well, resulting in exposures of hydrophobic surfaces to the aqueous environment. This energetically unfavorable situation is the major driving force for the aggregation and fusion of the small unilamellar liposomes incubated in a simple buffer at 37 °C (Liu & Huang, 1989a). On the other hand, a wedge-shaped amphipathic helix such as the ones found in Apo A1 can readily insert into the hydrophobic voids in the outer monolayer of the small unilamellar liposomes and reduce the free energy state of the system. The resulting proteoliposomes are stable even when OA is removed by BSA.

Data in Figure 10 support this model. Of the amphipathic peptides tested for liposome stabilization, 37PA showed the strongest activity. This peptide contains two amphipathic helices of 18 amino acids each, joined together with a proline residue to allow bending of two helices (Anantharamaiah et al., 1985). It resembles the amphipathic helices found in Apo A1 (Chung et al., 1985), and its stabilization activity is stronger than that of Apo A1 on a molar basis (Figure 10). Peptide 37AA is an analogue of 37PA (Lund-Katz et al., 1988). It contains two amphipathic helices but is connected by an analine residue which does not allow bending of the two helices. The activity of 37AA is considerably lower than that of 37PA (Figure 10), indicating that motional flexibility of the two joining helices is important for maximal interaction with the highly curved surface of a liposome. The activity of peptide 18A, a single amphipathic helix, is comparable to that of 37AA, but less than that of 37PA, suggesting some cooperativity between the two joining helices in the interaction with liposome membrane. Peptide 18R is a single amphipathic helix with the negatively charged residues located near the hydrophobic–hydrophilic interface and the positively charged residues at the center of the hydrophilic surface, a situation opposite to that of peptide 18A. The molecular shape of helix 18A is that of a wedge, but the shape of helix 18R is not. Data shown in Figure 10 clearly indicate that peptide 18R has no

liposome stabilization activity in the concentration range tested. Thus, the wedge shape of an amphipathic helix, which is shared by peptides 18A, 37AA, and 37PA, is important for the interaction with DOPE liposomes. This is consistent with the model described above.

This model also predicts that other amphipathic helices of wedge shape could also mediate the stabilization of liposomes. This could be the reason why the stabilization activity is also found in VLDL. The apolipoproteins in VLDL undoubtedly contain amphipathic helices which could be suitable for interaction with the small unilamellar liposomes described here.

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