Kinetics of Lipid–Protein Interactions: Effect of Cholesterol on the Association of Human Plasma High-Density Apolipoprotein A-I with L-α-Dimyristoylphosphatidylcholine†

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ABSTRACT: Apolipoprotein A-I (apoA-I) binds to cholesterol containing liposomes of L-α-dimyristoylphosphatidylcholine (DMPC) to give stable lipid–protein complexes which are readily isolated by gel filtration or by centrifugation in a density gradient of CsCl. The rate and affinity of apoA-I–DMPC association are sensitive functions of sterol content and temperature. ApoA-I spontaneously associates with cholesterol–DMPC liposomes when the cholesterol content of the liposomes is 24 mol % or less. In this range of lipid composition, complex formation is nearly quantitative and the lipid composition of the complex is similar to but not identical with that of the corresponding starting cholesterol–DMPC mixtures. At 33 mol % cholesterol a much lower yield of apoA-I–lipid complexes is obtained. Moreover, in the complexes which are formed, cholesterol composes only 24 mol % of the lipid. The protein–lipid complexes formed from apoA-I, cholesterol, and DMPC are much smaller than the starting liposomes as verified by chromatography on Sepharose CL-4B and by the large decrease in light scattering which accompanies the association of lipid and protein. The changes in light scattering are time dependent, and the rates vary with both temperature and cholesterol content. Between 0 and 33 mol % cholesterol apoA-I binds fastest to 12 mol % cholesterol in DMPC at all temperatures studied, and the rate of apoA-I association with cholesterol–DMPC mixtures was fastest at the transition temperature of DMPC (23.9 °C) regardless of the cholesterol content. The combined effects of cholesterol and temperature can alter the rates of reaction of apoA-I with DMPC by more than three orders of magnitude. We propose that apoA-I preferentially inserts into channel or hole defects at the boundary between coexisting gel and liquid crystalline phases. The greatest number of defects is formed in DMPC containing 12.5 mol % cholesterol at 23.9 °C, the transition temperature of the phospholipid. In our model the initial interaction of apoA-I with cholesterol–DMPC liposomes occurs at the interface of coexisting phases of 1:3 cholesterol–DMPC and pure DMPC.

Cholesterol is an important functional component of membrane and plasma lipoproteins and as such much interest has focused upon its relationship to phospholipids and proteins in real and artificial systems. In human plasma the esterification of cholesterol is mediated by lecithin:cholesterol acyltransferase (LCAT), an enzyme whose activity is stimulated by the major apoprotein of high-density lipoprotein (HDL), apoA-I (Soutar et al., 1975). The system apoA-I, PC,[1] and cholesterol contains the minimum number of components required for LCAT activity. It was therefore of particular interest to us to study the assembly and structure of apoA-I with mixtures of DMPC and cholesterol.

Addition of cholesterol to phosphatidylcholines is believed to produce a new lipid phase composed of up to 33 mol % cholesterol (Engelman & Rothman, 1972; Gent & Prestegard, 1974; Boggs & Hsia, 1972; Newman & Huang, 1975; Shimshick & McConnell, 1973; Estep et al., 1978; Mabrey et al., 1978). Relative to liposomes without cholesterol, cholesterol–phosphatidylcholine liposomes are more permeable to certain ions and hydrophobic molecules (Papahadjopoulas et al., 1973; Marsh et al., 1976; Blok et al., 1975; Tsong, 1975; LaBelle & Racker, 1977) both above and below T_c. While a great deal is known concerning the interaction of cholesterol and phosphatidylcholines, much less is known of the relationship between the structure of cholesterol–phosphatidylcholine liposomes and their rates of association with the human plasma apolipoproteins. In a previous paper (Pownall et al., 1978) we examined the kinetics of association of DMPC liposomes with apoA-I from human plasma HDL. Herein we report on the combined effects of cholesterol and temperature on the rate of association of apoA-I with DMPC.

Experimental Procedures

Materials. Cholesterol (Sigma) and [^{14}C]cholesterol were mixed and purified from the dibromide (Fieser, 1957), followed by column chromatography on silica gel eluted with isooctane–diethyl ether–acetic acid–ethyl acetate (75:25:2:15 v/v). The purified sterol eluted as a single spot on thin-layer plates of silica gel eluted with the same solvent system. The purity was verified by coincidence of radioactivity, iodine staining, and charring after spraying with concentrated sulfuric acid. Cholesterol was stored frozen in benzene until needed.

[^{3}H]Myristic acid was prepared by the method of Patel et al. (1976). The acid was converted to the anhydride by treatment with dicyclohexylcarbodiimide in chloroform and converted to DMPC by the method of Cubero Robles & van den Berg (1969). The product was purified on a Waters System 500 preparative liquid chromatograph. The sample was eluted through a silicic acid column with chloroform–methanol–water in a ratio of 60:30:4 v/v. The resulting

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[1] Abbreviations used: PC, phosphatidylcholine; DMPC, L-α-dimyristoylphosphatidylcholine; T_c, gel → liquid crystalline transition temperature; apoA-I, apolipoprotein A-I, the major protein of human high-density lipoproteins; HDL, high-density lipoproteins; ANS, 8-anilino-1-naphthalenesulfonate; Tempo, 2,2,6,6-tetramethylpiperidinyl-1-oxy.

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Table I: Properties of Complexes of ApoA-I, DMPC, and Cholesterol as a Function of Mole Percent of Cholesterol in the Initial Incubation Mixture

mol % cholesterol in starting mixture	density of complex (g/cm^3)	molar ratio of DMPC/ apoA-I in complex	mol % cholesterol[c] in complex
0	1.107	161	0 (0)
3		184	2.9 (–)
7[a]	1.100	174	5 (7)
12	1.089	151	12.1 (12)
17	1.085	177	16
24	1.080	100[b]	23 (19)
33	1.077		(24)

[a] Lipid to protein ratio is the average for the two partially resolved peaks. [b] Overlap of protein peak precluded an accurate estimate of the lipid to protein ratio. [c] The values in parentheses are those of complexes isolated by density-gradient centrifugation. The other values are from complexes isolated by chromatography on Sepharose CL-4B.

material gave a single spot on silica gel thin-layer plates eluted with chloroform–methanol–water (65:25:4 v/v) as visualized by iodine, by a phospholipid specific spray (Dittmer & Lester, 1964), and by charring. More than 99% of the radioactivity of the entire plate was in the spot visualized by iodine. Gas chromatographic analysis of the methyl esters obtained by transesterification of DMPC showed the ester composition to be >99% methyl myristoate. All buffer salts were obtained from Fisher Scientific or Schwarz/Mann. The specific activities of [^{125}I]apoA-I, [^{14}C]DMPC, and [^{3}H]cholesterol were 230 μCi/mmol and 18 and 2.0 μCi/μmol, respectively. The compositions of the isolated complexes were confirmed by absorbance for protein (ϵ_{280} = 28 500 M^{-1} cm^{-1}), by phosphorus analysis for DMPC (Bartlett, 1959), and with a commercial kit from Boehringer-Mannheim for cholesterol.

ApoA-I and [^{125}I]apoA-I were obtained as previously described (Pownall et al., 1978). The apoprotein gave a single band in sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Immediately prior to the turbidimetric, kinetic, and chromatographic experiments the required quantity of apoA-I was solubilized in 6 M quanidine hydrochloride and desalted on a Bio-Rad P-2 column.

Methods. Cholesterol–DMPC liposomes were prepared by cosolubilizing the desired quantity of each lipid in a small amount of chloroform which was evaporated to dryness under a stream of nitrogen. The residual traces of chloroform were removed by evaporation in vacuo. The dried lipids were dispersed by vortexing in 1 mL of buffer for a few minutes above the transition temperature of the phospholipid. The resulting mixture was diluted to the desired final concentration with a buffer composed of 8.5% KBr, 0.01% azide, 0.01% EDTA, and 0.01 M Tris, pH 7.4. Addition of KBr gave the solutions a density which was high enough to prevent settling of the PC liposomes. The chromatographic and kinetic procedures are the same as those given in the previous paper (Pownall et al., 1978). The procedure of density-gradient ultracentrifugation of the complexes was the same as given previously (Pownall et al., 1974) except that CsCl was substituted for KBr.

Results

Gel Filtration of ApoA-I–DMPC–Cholesterol Complexes. ApoA-I spontaneously associates with DMPC liposomes with and without cholesterol, giving small complexes which are optically clear. These complexes may be isolated by gel filtration on Sepharose CL-4B as illustrated in Figures 1A–F.

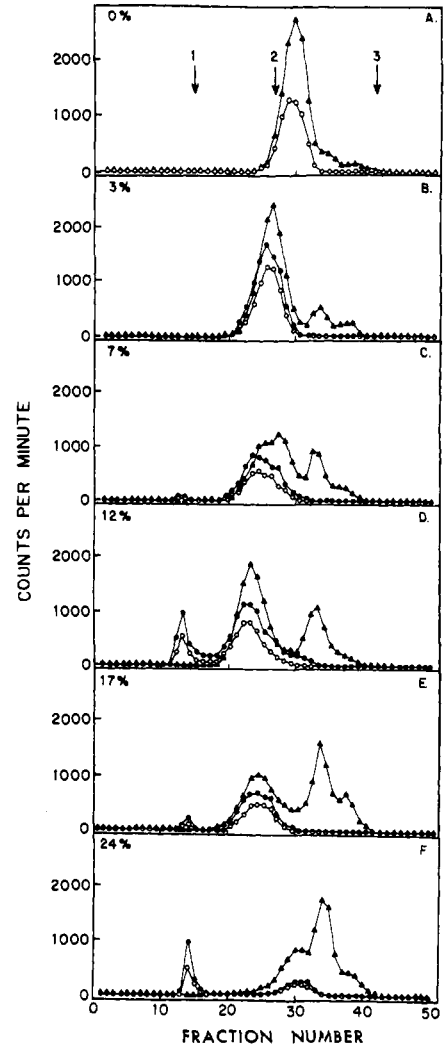

FIGURE 1: Gel filtration profiles of apoA-I–DMPC–cholesterol complexes as a function of the mole percent of cholesterol. All samples were 1 mL, containing 10.5 nmol of apoA-I, 2.2 μmol of DMPC, and various mole percents of cholesterol as follows: (A) 0; (B) 3.0; (C) 7.0; (D) 12; (E) 17; (F) 24. All samples were incubated for 12 h at 24 °C before applying to a 1.6 × 40 cm column of Sepharose CL-4B whose temperature was maintained at 24 °C. [^{3}H]DMPC (○), [^{14}C]cholesterol (●), and [^{125}I]apoA-I (▲) were monitored by scintillation counting of the lipid radioactivity and γ counting for the protein. Protein recovery was greater than 90% in all samples. Recovery of DMPC and cholesterol was >85% except in E and F where recovery was ~50 and ~20%, respectively. Mixtures containing 33 mol % cholesterol did not elute from the column. Arrows 1, 2, and 3 indicate the elution volumes of DMPC multilamellar liposomes, DMPC single bilayer vesicles, and [^{14}C]glucose, respectively.

The compositions of the isolated complexes are collected in Table I. The results may be summarized as follows. First, the cholesterol contents of the complexes were, within experimental error, identical with those of the starting cholesterol–DMPC mixtures. In cholesterol–DMPC mixtures containing 33 mol % cholesterol no association of apoA–I could be detected by changes in turbidity nor were there any complexes observed by Sepharose CL–4B chromatography. Secondly, the DMPC/apoA-I molar ratios in the isolated complexes produced from different cholesterol–DMPC mixtures were the same regardless of the cholesterol content; this value was 168 ± 12. (This value does not include the ratio observed at 24 mol % since there was a large overlap of the complex peak with that of the free protein.) In the absence of 8.5% KBr the reported DMPC/apoA-I ratio of isolated complexes is about 100:1 (Jonas et al., 1977). Evidently,

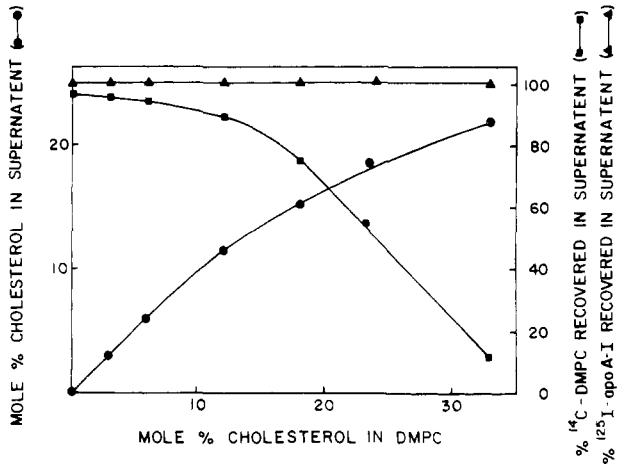

FIGURE 2: Composition and percent recovery of soluble complexes of apoA-I, cholesterol, and DMPC. ApoA-I (51 nmol) was mixed with liposomes of DMPC (5.1 μmol) to which various amounts of cholesterol had been added. These were incubated at 24 °C for 24 h. These were analyzed by scintillation counting and γ counting of aliquots before and after low-speed centrifugation. The recoveries of lipid and protein represent the ratio of the counts obtained after centrifugation to those obtained before centrifugation. The mole percent of cholesterol was calculated by comparison of the cholesterol specific activity and ^{3}H counts with the corresponding values for [^{14}C]DMPC.

increasing the ionic strength with KBr increased the lipid/protein ratio of isolated complexes but we have not pursued this effect in detail. The yield of complexes decreased as the mole percent of cholesterol in the starting mixtures was increased. This was assigned to adsorption to the column rather than to a specific lipid–lipid or lipid–protein interaction since our recoveries of lipid were much poorer in the mixtures having higher cholesterol contents. Finally, there were significant differences in the sizes of the complexes formed. Initially, an increase in the mole percent of cholesterol in DMPC that was mixed with apoA-I resulted in the formation of a particle which eluted much earlier, suggesting that a higher cholesterol content increased the size of the protein–lipid complex. However, above 12 mol % cholesterol the size of the complexes decreased.

Density Gradient Centrifugation of ApoA-I–DMPC–Cholesterol Complexes. [^{125}I]ApoA-I was added to liposomes of [^{14}C]DMPC containing various amounts of [^{3}H]cholesterol and the mixture incubated at 24 °C for 24 h. At the end of this time interval samples containing 0, 7, and 12 mol % cholesterol were optically clear; those containing 17 and 24 mol % cholesterol were slightly turbid; and that sample containing 33 mol % cholesterol was very cloudy. The samples were analyzed before and after centrifugation at low speed (5 min in a Beckman Microfuge B). The results are plotted in Figure 2 in terms of the percent of [^{14}C]DMPC and [^{125}I]-apoA-I retained in the clear supernatant and the mol % of cholesterol in the supernatant. In the samples containing up to 24 mol % cholesterol, the lipid composition of the supernatant was identical with that of the starting lipid mixture, and 90–100% of the [^{125}I]apoA-I, [^{14}C]DMPC, and [^{3}H]-cholesterol counts remained in the supernatant. At 33 mol % cholesterol only 14% of the [^{14}C]DMPC counts remained in the supernatant, and the lipid composition of the supernatant was only 24 mol % cholesterol. Density-gradient centrifugation of these samples (Figure 3) demonstrated that a stable lipid–protein complex was formed in all experiments. The lipid compositions of these complexes are compared with those isolated by gel filtration in Table I. The main differences in

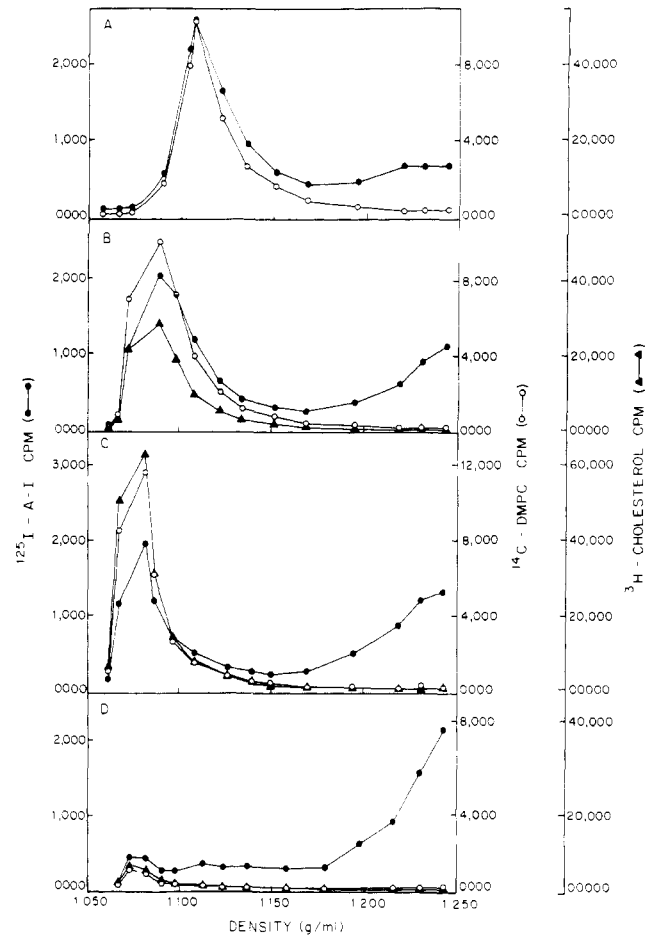

FIGURE 3: Distribution of apoA-I, DMPC, and cholesterol in a density gradient of CsCl. The supernatant from Figure 2 was mixed with increasing concentrations of CsCl with a Buchler Densi-Flow. After centrifugation at 25 °C for 72 h at 40 000 rpm in a Beckman SW 50.2 rotor the samples were fractionated with a peristaltic pump and a Buchler Densi-Flow. Each fraction was analyzed for composition which was plotted here against density. (A) 0 mol % cholesterol; (B) 12 mol % cholesterol; (C) 24 mol % cholesterol; (D) 33 mol % cholesterol.

the two methods appear at or above 24 mol % cholesterol. Recoveries of cholesterol, DMPC, and apoA-I were nearly quantitative in the density gradient, whereas the recoveries were very poor by chromatography (Figure 1F). In all experiments conducted with 24 mol % or less cholesterol the clearing of lipid turbidity correlated with the formation of a lipid–protein complex which could be isolated by centrifugation. At 33 mol % cholesterol no clearing was observed and a complex was formed in low yield. There appears to be some loss of apoA-I from all samples during centrifugation since the more dense fractions contain apoA-I without [^{3}H]-cholesterol or [^{14}C]DMPC. The difference in the recoveries obtained by gel filtration and ultracentrifugation may be due to the lower stability of the complexes having higher cholesterol contents.

Effect of Temperature on the Turbidity of Mixtures of ApoA-I, Cholesterol, and DMPC. When apoA-I is added to highly turbid DMPC–cholesterol liposomes at 10 °C or less, the liposomal turbidity persists indefinitely (>12 h). As the temperature of the mixture is increased at a linear rate (12 °C/h), clarification of the turbidity is produced at a temperature which is dependent on the cholesterol content of the liposomes. This effect is illustrated in Figures 4A–F. In the absence of cholesterol the midpoint of the decrease in light-scattering intensity (absorbance) is at 24.5 °C. In similar

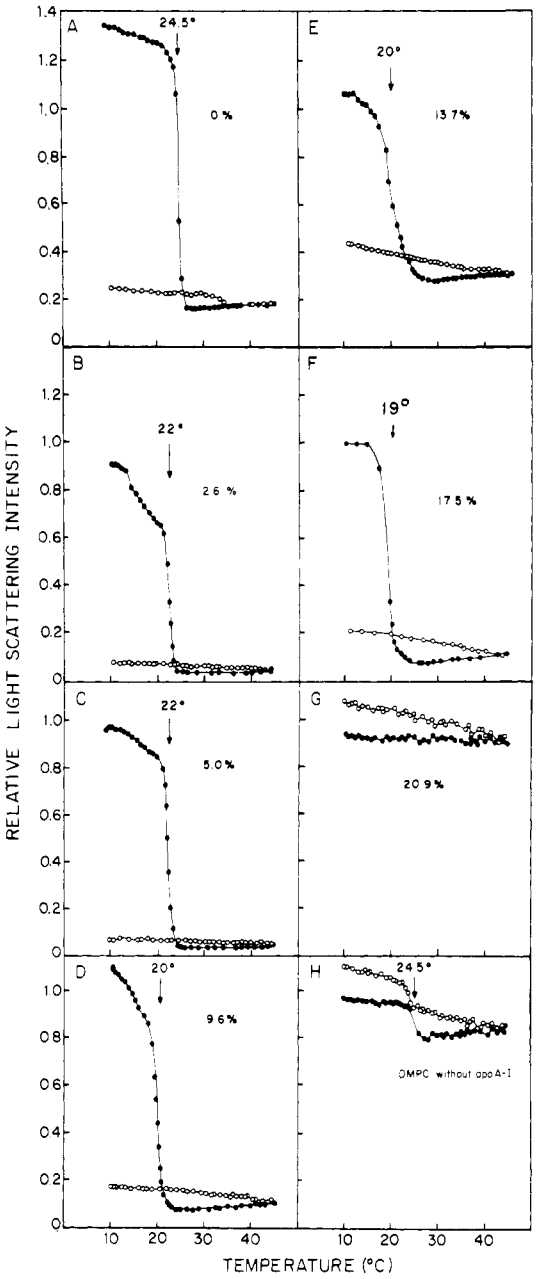

FIGURE 4: Turbidity vs. temperature profiles of apoA-I (10.5 nmol) with DMPC (2.2 μmol) containing various mole percents of cholesterol. The lipids and protein were combined at 10 °C, and the turbidity at 325 nm was measured as a function of increasing temperature (12 °C/h). The DMPC contained the following mole percents of cholesterol: (A) 0; (B) 2.6; (C) 5.0; (D) 9.6; (E) 13.7; (F) 17.5; (G) 20.9; (H) DMPC without cholesterol and apoA-I. Arrows indicate the midpoint of the curve where a rapid change in turbidity is observed. Closed and open circles designate values obtained with increasing and decreasing temperature, respectively.

experiments conducted with an increasing mole percent of cholesterol in the DMPC liposomes the midpoint of the decreased light scattering appeared at progressively lower temperatures. The lowest midpoint observed was at 19 °C in liposomes containing 17.5 mol % cholesterol (Figure 4F). At 20.9% cholesterol or greater no abrupt change in turbidity was observed over the ~5 h required for the experiment. Liposomes of pure DMPC exhibit a relatively small change in turbidity at 24.5 °C (Figure 4H).

Kinetic Studies of the Association of ApoA-I with DMPC–Cholesterol Liposomes. The gel filtration data (Figure 1) show that the complexes of apoA-I, DMPC, and

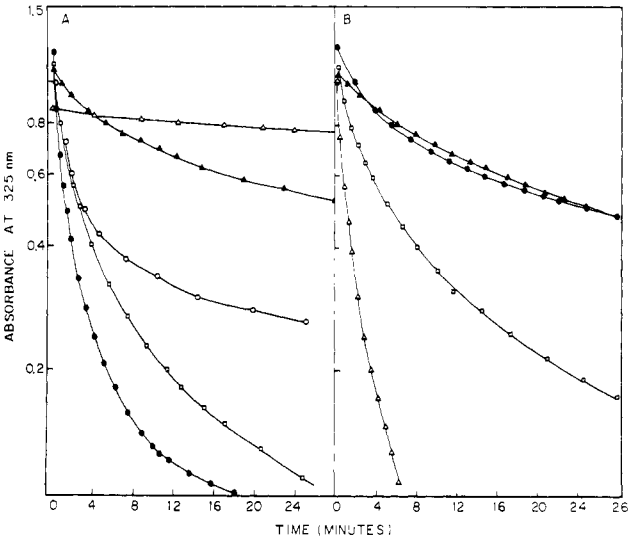

FIGURE 5: Time dependence of the changes in turbidity of DMPC–cholesterol mixtures induced by the addition of apoA-I. (A) Effect of cholesterol content (mol %) on the rate at 23 °C: 0 (△); 2.6 (▲); 5 (□); 13.6 (●); 17 (○). (B) Effect of temperature on the rate at 13.6 mol % cholesterol in DMPC: 20 (○); 24 (△); 27 (□); 30 °C (▲). In each experiment 10.5 nmol of apoA-I was mixed with 2.2 μmol of DMPC in a total volume of 3.15 mL plus the indicated mole percent of cholesterol.

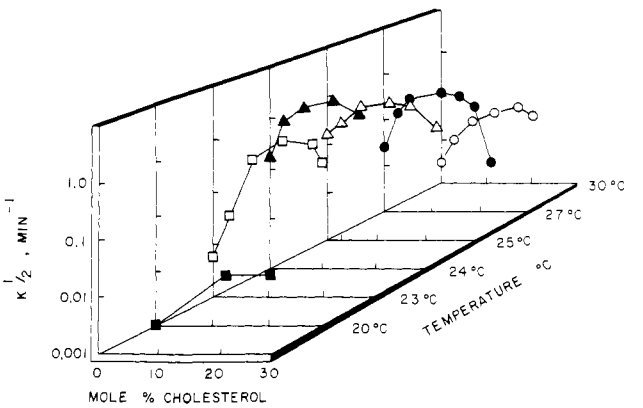

FIGURE 6: Effect of cholesterol on the rate of apoA-I-induced clearance of DMPC turbidity at various temperatures. See Figure 5 for details.

cholesterol were much smaller than those of the starting liposomes. Visual inspection of these complexes revealed no turbidity, whereas the starting liposomes were highly turbid. We have followed the rate of disappearance of liposome turbidity induced by the addition of apoA-I to the lipsomes to estimate the rate of complex formation. The effects of both cholesterol content and temperature on the rate of complex formation were determined. Representative curves illustrating the effect of these two variables are shown in Figure 5. For the sake of comparison these were plotted according to first-order kinetics although none of the rates obeyed a first-order rate law. In Figure 5A the effect of cholesterol on the rate of apoA-I-induced disappearance of liposomal turbidity at 23 °C is shown. In the absence of cholesterol the rate was very slow but was dramatically increased by increasing the mole percent of cholesterol in the DMPC liposomes. Figure 5B illustrates the effect of temperature on the rate of apoA-I-induced disappearance of turbidity of DMPC liposomes containing 13.6 mol % cholesterol. At this mole percent of cholesterol the rate was faster at $T = T_c$ (~24 °C) than at $T \neq T_c$. Since the reactions do not follow a simple rate law we defined a rate constant, $k_{1/2} = 1/\tau_{1/2}$, where $\tau_{1/2}$ represents the time required for disappearance of 50% of the

initial absorbance due to liposomal turbidity. The combined effects of temperature and cholesterol content of the DMPC liposomes on the rate constants are shown in Figure 6. The average mole percent of cholesterol, which gives the maximum rate of association of apoA-I with the lipid mixtures, was 12.5 ± 1.0 mol %. (The errors were higher at 20 and 30 °C where the rates were much slower; data collected at these two temperatures were not included in this calculation.) The combined effects of cholesterol content and temperature on the magnitude of $k_{1/2}$ are enormous; these vary from $k_{1/2}$ = 0.001 min^{-1} at 20 °C and 0 mol % cholesterol to $k_{1/2}$ = 2 min^{-1} at 12.5 mol % cholesterol at 24 °C (T_c), a total range of more than three orders of magnitude. Addition of cholesterol to DMPC only widens the temperature range over which the $k_{1/2}$ is relatively large; in DMPC liposomes containing 17.5 mol % cholesterol or less the value of $k_{1/2}$ was always maximal at the transition temperature of DMPC, 24 °C (lit. 23.9 °C; Mabrey & Sturtevant, 1976).

Discussion

Between 0 and 18 mol % cholesterol, gel filtration and ultracentrifugation both demonstrate the formation of DMPC–apoA-I complexes having the same average sterol/DMPC ratio as the starting lipid mixtures. Therefore, within this range of cholesterol content, apoA-I does not appear to displace cholesterol from DMPC nor does the apoprotein preferentially associate with lipid domains different from those of the total liposome ensemble. Above 18 mol % the sterol content of the isolated complexes was less than those of the starting lipid mixtures. Within this range it appears that apoA-I selectively associates with an ensemble of lipids that is preferentially enriched in DMPC. This effect may be attributed to what Tall & Lange (1978) assign to the association of apoA-I with a boundary of lipid from which cholesterol is excluded. Tall and Lange report much lower incorporation at low sterol content, which may be due to the fact the their experiments were conducted at pH 9 at 27 °C and ours were conducted at T_c and pH 7.4 where the reaction is much faster. The compositional heterogeneity of the complexes is verified by the density or elution ranges over which they are distributed in ultracentrifugal and chromatographic isolations, respectively. This result suggests that apoA-I, DMPC, and cholesterol do not form ternary complexes of constant composition. The addition of apoA-I to DMPC containing an increasing mole percent of cholesterol gives, initially, large complexes and then smaller complexes. At 7 mol % cholesterol in DMPC, apoA-I associates, giving two partially resolved peaks which correspond to the large and small complexes. At 12, 17.5, and 24 mol % cholesterol the size of the complex decreases. The initial increase, followed by a decrease in the size of the complex, probably represents the competing effects of cholesterol to combine with phospholipid, giving large sterol–phospholipid structures (Newman & Huang, 1975), and of apoA-I to combine with DMPC to give small lipid–protein complexes (Pownall et al., 1978; Jonas et al., 1977).

Addition of apoA-I to DMPC containing 33 mol % cholesterol produced little change in light scattering, and only 10–14% of the DMPC present in the mixture was incorporated into a lipid–protein complex. The cholesterol composition of this complex was much less (24 mol %) than that of the starting mixture (33 mol %). It is not clear on the basis of our data whether apoA-I interacts preferentially with a lipid domain having a lipid composition different from that of the total system or that apoA-I displaces cholesterol from DMPC. At greater than 25 mol % cholesterol Estep et al. (1978) suggest that a domain composed of 25 mol % cholesterol

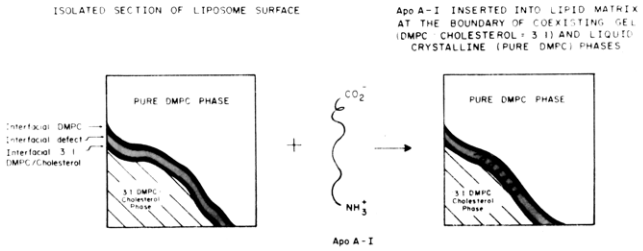

FIGURE 7: A hypothetical model for the mechanism by which apoA-I associates with a lipid matrix. On the left we show a section of the surface of a liposome in which a DMPC and a 1:3 cholesterol–DMPC phase coexist. Each phase is bounded by interfacial lipid, and the interfacial lipid of each phase is separated from that of the other phase by a hole or channel defect. ApoA-I may insert into this defect to give the initial lipid–protein intermediate on the right. We have drawn the apoA-I in the lipid matrix as a helical structure since it probably exists as such in HDL (Lux et. al., 1972) and in apoA-I–PC complexes (Jonas & Krajnovich, 1977).

coexists with a domain composed of up to 40 mol % cholesterol. We suggest that apoA-I spontaneously associates with the domain composed of 25 mol % cholesterol but not with the coexisting cholesterol-rich phase. Jonas & Krajnovich (1978) have also isolated complexes of apoA-I and DMPC containing 26 mol % cholesterol from a mixture whose initial sterol content was 33 mol %. They used sonicated vesicles of DMPC and cholesterol, whereas we have used liposomes as the lipid phase, but both systems give similar results. Therefore, the identity of the product formed by the association of apoA-I, DMPC, and cholesterol is independent of the physical form of the lipid. The rates of association, however, may vary considerably.

Light Scattering and Kinetic Measurements. The clearing effect of apolipoproteins on phospholipids has been documented previously (Pownall et al., 1978; Tall et al., 1977; Träuble et al., 1974); with apoA-I and DMPC this effect is fastest at the T_c of the lipid (Pownall et al., 1978). We have observed a similar effect in the same system in which cholesterol has been incorporated into the liposomes. The gel filtration data of Figure 1 confirm the formation of small lipid–protein complexes; these are smaller than DMPC single bilayer vesicles and, therefore, scatter little light. This finding suggests that the rapid decrease in light scattering shown in Figure 2A–F must be due to the formation of a lipid–protein complex; thus the clearance of liposomal turbidity is a reliable indicator of apoA-I–DMPC–cholesterol complex formation. As the cholesterol content of the starting liposomes was increased, the midpoint for the decreased light scattering appeared at progressively lower temperatures. Since the addition of cholesterol can lower the T_c of PC (Estep et al., 1978) and apoA-I reacts fastest at T_c, the depression of the midpoint might be assigned to a melting point depression. Alternatively and probably more important is the increased reactivity of apoA-I with DMPC to which cholesterol has been added. The latter effect is more important because the time required for reaction of apoA-I with cholesterol–DMPC liposomes is competitive with the scan rate (12 °C/h) between 19 and 28 °C.

The combined effects of the cholesterol content of DMPC and temperature on the rate of lipid association with apoA-I are illustrated in Figure 6. There are several notable features of these data, which have been considered in the design of the hypothetical mechanism shown in Figure 7. First the maximum rate of association of apoA-I with cholesterol–DMPC liposomes is always at the transition temperature of pure DMPC liposomes regardless of their cholesterol content. Secondly, at a given temperature the maximum rate of as-

sociation of apoA-I and DMPC was at 12.5 mol % cholesterol. Finally, our previous report (Pownall et al., 1978) assigned the enhanced association of apoA-I with DMPC at T_c to the presence of coexisting gel and liquid crystalline phases. We suggested that defects in the lipid matrix produced at the boundary of gel and liquid crystalline phases are the sites of insertion of apoA-I. Therefore, the effect of cholesterol on the lipid matrix may be to produce more boundary lipid between two coexisting phases. Estep et al. (1978) have reported that cholesterol and DPPC form a homogeneous phase having a 1:3 sterol/PC molar ratio, and we have assumed for this model that DMPC and DPPC interact with cholesterol similarly. In lipid mixtures containing less than 25 mol % cholesterol a pure DMPC phase may coexist with a second phase composed of 25 mol % cholesterol and 75 mol % DMPC. If the sizes of the domains composing these phases are equal, the greatest number of boundary defects between unlike phases will exist when 50% of the lipid is in each phase. This requires that half of the lipid be in a pure DMPC phase and the other half in a 1:3 cholesterol–DMPC phase. The total composition of this system, 12.5 mol % cholesterol and 87.5 mol % DMPC, is identical with that at which the maximum rate of association of apoA-I with cholesterol–DMPC liposomes was observed. Also, consistent with this model is the fact that the rate is always maximal at T_c, suggesting that a pure DMPC domain is associated with the rate limiting step of this reaction.

This model is supported by other studies of DMPC, which show that the lipid matrix is more permeable at T_c and with 12 mol % cholesterol. That is, apoA-I and other molecules can penetrate DMPC via defects in lipid matrix. This mechanism is similar to those proposed by Marsh et al. (1976) for the enhanced passive permeation of a Tempo-choline through DMPC single bilayer vesicles at T_c, by Tsong (1975) for the facilitated binding of ANS to DMPC at T_c, and by Blok et al. (1975) for the passive leakage of ions through DMPC liposomes. This mole percent of cholesterol is identical wth the value reported by Blok et al. (1977) for the inhibition of water permeation through DMPC liposomes and by Tsong (1975) for the maximal rate of binding of ANS to cholesterol–DMPC mixtures.[2]

Biological Implications. Although there have been many studies on the association of cholesterol and phosphatidylcholines, few of these have been extended to the dynamics of more complex biological systems. Our studies show that the phase behavior of cholesterol–DMPC mixtures can be used to predict the rate of formation and stability of an apoA-I–DMPC–cholesterol complex. Because apoA-I is the activator protein for LCAT, there may be a correlation between LCAT activity and cholesterol content of the substrate, and this effect is now being investigated in greater detail. We further speculate that our rate data may be relevant to the in vivo movement of apoA-I among the HDL_2, HDL_3, or "nascent" HDL having different cholesterol contents; this suggestion, however, requires additional experimental support.

[2] In Figure 1 of this report (Tsong, 1975) the text and the curves indicate a maximum in the binding of ANS to DMPC containing 17 mol % cholesterol when the experimental points of that figure show the rate to be fastest between 11 and 13 mol % cholesterol.

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