Effects of Apolipoproteins on the Kinetics of Cholesterol Exchange†

Joan Y. Letizia and Michael C. Phillips*

Physiology and Biochemistry Department, Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129

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ABSTRACT: The effects of apolipoproteins on the kinetics of cholesterol exchange have been investigated by monitoring the transfer of [^{14}C]cholesterol from donor phospholipid/cholesterol complexes containing human apolipoproteins A, B, or C. Negatively charged discoidal and vesicular particles containing purified apolipoproteins complexed with lipid (75 mol % egg PC, 15 mol % dicetyl phosphate, and 10 mol % cholesterol) and a trace of [^{14}C]cholesterol were incubated with a 10-fold excess of neutral, acceptor, small unilamellar vesicles (SUV; 90 mol % egg PC and 10 mol % cholesterol). The donor and acceptor particles were separated by chromatography on DEAE-Sepharose, and the rate of movement of labeled cholesterol was analyzed as a first-order exchange process. The kinetics of exchange of cholesterol from both vesicular and discoidal complexes that contain apoproteins are consistent with an aqueous diffusion mechanism, as has been established previously for PC/cholesterol SUV. The addition of 2–3 molecules of apo A-I to a donor SUV does not significantly alter the half-time ($t_{1/2}$), which is 80 ± 9 min at 37 °C. However, addition of 5–12 apo A-I molecules progressively decreases $t_{1/2}$ from 65 ± 2 to 45 ± 4 min. This enhancement in the rate of desorption of cholesterol molecules is presumed to arise from the creation of packing defects at boundaries around the apoprotein molecules, which are intercalated among the phospholipid and cholesterol molecules in the surface of the donor SUV. Apolipoproteins A-I, A-II, reduced and carboxymethylated A-II, and B-100 present in SUV at the same lipid/protein (w/w) ratio all enhance the rate of cholesterol exchange to about the same degree. Cholesterol molecules exchange more rapidly from discoidal complexes; $t_{1/2}$ from a 150:1 (mol/mol) apo A-I/lipid disc is 14 ± 1 min compared to 45 ± 4 min for a SUV of the same composition. Generally, as the diameter of apoprotein/phospholipid/cholesterol discs decreases, $t_{1/2}$ for cholesterol exchange decreases. The apoprotein molecules at the edges of the discoidal complexes perturb the phospholipid/cholesterol interactions and facilitate desorption of cholesterol molecules from these areas. Since small bilayer discs have a relatively high ratio of boundary to face surface area, cholesterol molecules desorb more rapidly than from larger discs. The modulation of lipid packing by the apoprotein molecules present at the surface of lipoprotein particles affects the rate of cholesterol exchange from such particles.

An important aspect of cholesterol transport through the body is the exchange or transfer of cholesterol between cells and lipoproteins and between the different classes of lipoproteins in the plasma compartment. Cholesterol exchange has been observed between LDL[1] and HDL, between these lipoproteins and red blood cell membranes, and between lipoproteins and cells growing in culture [for reviews, see Bruckdorfer and Graham (1976), Bell (1978), Phillips et al. (1987), and Bittman (1988)]. There has been a great deal of interest in establishing both reliable rate constants and the molecular mechanism for these cholesterol movements. The most reliable information has come from studies using model membrane systems.

By use of model bilayer systems the mechanism for cholesterol exchange between small unilamellar vesicles has been determined to involve diffusion of cholesterol molecules through the aqueous phase (Phillips et al., 1980; McLean & Phillips, 1981; Backer & Dawidowicz, 1981; Bar et al., 1986). The bidirectional flux of cholesterol between cells and serum lipoprotein particles occurs by the same aqueous diffusion mechanism (Rothblat & Phillips, 1982; Johnson et al., 1986). The rate of cholesterol exchange can be analyzed in terms of a model where the desorption of the transferring molecule from the host bilayer occurs through a transition-state complex that is associated with a high free energy of activation (Aniansson et al., 1976; Pownall et al., 1983; McLean & Phillips, 1984a; Nichols, 1985). Therefore, factors that affect the free energy associated with the formation of the transition-state complex also modify the rate of cholesterol transfer. For instance, the nature of the host phospholipid affects the rate of cholesterol exchange, with the rate decreasing with increasing saturation (Bloj & Zilversmit, 1977; Nakagawa et al., 1979; McLean & Phillips, 1982). The decrease in the rate of cholesterol desorption out of saturated phospholipid bilayers reflects a tighter packing and an increase in the van der Waals interactions between the cholesterol and the host phospholipid molecules and therefore a higher free energy associated with the formation of the transition-state complex (Lund-Katz et al., 1988b).

Kinetic data for cholesterol exchange between human HDL and LDL are also consistent with a mechanism involving diffusion of cholesterol molecules through the aqueous phase (Lund-Katz et al., 1982). The rate of cholesterol exchange from serum lipoprotein particles is rapid relative to that from

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[1] Abbreviations: apo, apolipoprotein; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; GdnHCl, guanidine hydrochloride; $t_{1/2}$, half-time; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low density lipoprotein; PC, phosphatidylcholine; RCM-AII, reduced and carboxymethylated apolipoprotein A-II; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SM, sphingomyelin; SUV, small unilamellar vesicle(s).

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small unilamellar vesicles (SUV). For instance, the half-times for cholesterol exchange from 20 mol % cholesterol/egg PC SUV, human LDL, HDL_2, and HDL_3 are 149, 45, 4.2, and 2.9 min, respectively. In order to begin to understand these differences in transfer rate, the influence of the apolipoprotein molecules present at the surface of the lipoprotein particles must be elucidated. There have been no systematic investigations of the effects of protein molecules on the kinetics of cholesterol exchange. We employ well-defined model lipoprotein systems, in which the lipid and protein components are varied systematically, to study the effects of apoproteins on the kinetics of cholesterol flux.

Materials and Methods

Materials

Egg yolk L-α-phosphatidylcholine was purchased from Calbiochem (La Jolla, CA). Dimyristoyl L-α-lecithin, dicetyl phosphate, bovine brain sphingomyelin, egg yolk sphingomyelin, and cholesterol were obtained from Sigma Chemical Co. (St. Louis, MO). Glycerol tri[9,10(*n*)-^{3}H]oleate (1 Ci/mmol) and [4-^{14}C]cholesterol (50 mCi/mmol) were purchased from Amersham Corp. (Arlington Heights, IL) and Research Products International (Mount Prospect, IL), respectively. The purities of lipid samples were assayed by thin-layer chromatography on silica gel G plates (Analtech, Newark, DE) with three solvent systems: (1) chloroform/methanol/water [65:25:4 (v/v)] for phospholipids, (2) petroleum ether/diethyl ether/acetic acid [75:24:1 (v/v)] for triacylglycerol, and (3) diethyl ether for cholesterol. All samples were >99% pure.

Human apolipoproteins A-I and A-II were isolated from the total HDL fraction by using procedures described previously (Ibdah et al., 1989). The apo A-I and apo A-II fractions gave single bands on sodium dodecyl sulfate–7.5% polyacrylamide gel electrophoresis (SDS–PAGE) when 50 μg of protein was applied under either reducing or nonreducing conditions. Reduced and carboxymethylated apo A-II (RCM-AII) was prepared as described previously (Ibdah et al., 1990). Human LDL (d = 1.019–1.063 g/mL) was isolated from plasma by sequential ultracentrifugation (Lund-Katz et al., 1988a), and the apo B-100 was extracted and purified by the method of Helenius and Simons (1971). The apo B-100 was about 90% pure by SDS–PAGE; some fragmentation products were present. Human VLDL (d < 1.006 g/mL) was used for the preparation of apolipoproteins C. Briefly, total apo C was obtained from VLDL by using an acetone extraction in conjunction with delipidation by using 3:2 (v/v) ethanol/diethyl ether (Holmquist & Carlson, 1977). The total apo C was about 80% pure and contained some albumin. Apo $C\text{-}III_1$ was separated by chromatofocusing with an equilibrated Polybuffer exchanger (PBE 94) eluted with Polybuffer 94 in 6 M urea (Knipping et al., 1984). Analytical isoelectric focusing indicated that the isolated apo $C\text{-}III_1$ was about 85% pure; small amounts of apo $C\text{-}III_2$ were also present.

Methods

Phospholipid–Apolipoprotein Complexes. Small unilamellar vesicles of phospholipid were prepared by sonication as described previously (McLean & Phillips, 1981). Lipid–protein vesicles were prepared by incubating the desired apoprotein A or C (freshly desalted from 4 M GdnHCl) with SUV at a specific lipid/protein ratio in buffer (10 mM Tris-HCl, 1 mM EDTA, and 1 mM NaN_3, pH 8) overnight near the gel–liquid crystal transition temperature of the lipid (Jonas et al., 1980; Yokoyama et al., 1984). The mixture was chromatographed on a Sepharose 4B-CL column (60 × 1.6 cm) and eluted with buffer (20 mM sodium phosphate, 1 mM EDTA, and 1 mM NaN_3, pH 6) at 15 mL/h to separate the lipid–protein SUV from free apoprotein. SUV in a narrow size range were obtained by selecting individual fractions from across the total SUV elution peak. Vesicles containing apo B-100 were prepared by the method of Walsh and Atkinson (1983); the lipid–protein complexes were exhaustively dialyzed to remove sodium deoxycholate and then fractionated by density gradient centrifugation (Redgrave et al., 1975). Discoidal particles of phospholipid and apolipoprotein A or C were prepared by the cholate dialysis method of Matz and Jonas (1982); the discoidal complexes were further fractionated by gel-filtration chromatography as described above for SUV. After a 48-h dialysis, <1% of the sodium cholate remained, and control experiments (not shown) showed that this had no effect on the kinetics of cholesterol transfer from the discoidal particles.

The lipid and protein contents of the various complexes were determined. Protein concentrations were assayed either by the method of Lowry (1951), as modified by Markwell et al. (1978), or by measuring tryptophan fluorescence (Jonas & Drengler, 1980). Lipids were extracted with chloroform and methanol by the method of Bligh and Dyer (1959). Phospholipids were analyzed for inorganic phosphorus after digestion with sulfuric and perchloric acids by the method of Goodwin et al. (1958), as modified by Sokoloff and Rothblat (1974). Cholesterol was determined by gas–liquid chromatography (Ishikawa et al., 1974), with coprostanol as an internal standard.

The particle diameters of the spherical lipid–protein SUV complexes were determined by either gel-filtration chromatography (Ibdah et al., 1990) or negative-stain electron microscopy (Collins & Phillips, 1982). The dimensions of the asymmetric discoidal lipid–protein complexes were determined by electron microscopy. The dimensions generated in this fashion were used in calculations of the volumes of the various vesicular and discoidal lipid–protein complexes. The numbers of lipid and protein molecules present in each particle were computed from this particle volume by using the partial specific volumes of the phospholipid, cholesterol, and apoprotein and the relative amounts of these components present.

The lipid/protein molar ratios determined as described above refer to the donor vesicular or discoidal particles before incubation with acceptor SUV. Apolipoproteins of the A and C classes can transfer between vesicles (Ibdah et al., 1990), so it is possible for the donor particles to become depleted of these apoproteins during the incubation to monitor the kinetics of cholesterol exchange. Using rate constants for apoprotein transfer measured in this laboratory (data not shown), we estimate that no significant transfer of apo A-I or apo A-II from the donor particles occurred under the conditions of our cholesterol exchange assays. SUV containing RCM-AII may have lost up to 10% of their protein content by the end of the incubation, while SUV containing apo C probably lost as much as 40% of the apoprotein. Any loss of protein did not lead to significant fusion or rearrangement of donor and acceptor particles, because the Stokes radii of the particles after incubation were the same as those of freshly prepared particles. In addition, there was no change with incubation time in the effectiveness of the separation of donor and acceptor particles by anion-exchange chromatography (see below).

Kinetics of Cholesterol Exchange. The rate of transfer of [^{14}C]cholesterol from negatively charged donor particles to neutral acceptor SUV was determined by using anion-exchange chromatography to separate the two populations as

described previously (McLean & Phillips, 1981). The donor particles contained [^{14}C]cholesterol at approximately 1.0 μCi/mg of donor lipid in order to trace the cholesterol movement; 15 mol % dicetyl phosphate was incorporated into the particle to confer a net negative charge. The lipid and protein composition of the donor particles was varied in order to determine how these constituents affect the rate of cholesterol exchange. The donor particles were incubated with a 10-fold excess of acceptor SUV containing [^{3}H]triolein at 0.1 μCi/mg of acceptor lipid as a nonexchangeable marker. The acceptor particle was a 90 mol % egg PC/10 mol % cholesterol SUV with a diameter of 200 ± 10 Å; the composition of the acceptor SUV was kept constant in all experiments. Incubations were carried out in buffer (20 mM sodium phosphate, 1 mM EDTA, and 1 mM NaN_3, pH 6) at donor cholesterol concentrations ranging from 0.001 to 0.01 mg/mL. Gel-filtration experiments were used to assess the stabilities of the donor and acceptor particles (each in turn containing [^{3}H]-triolein as a nonexchangeable marker) after a 24-h incubation at 37 °C in this medium. No significant aggregation or fusion of the discoidal complexes and vesicles was detected.

The fraction of labeled cholesterol appearing in the acceptor SUV after a given incubation period was calculated by using the counts of [^{14}C]cholesterol per minute that appeared in the eluate from the DEAE-Sepharose chromatographic separation. The fraction of [^{14}C]cholesterol appearing in the eluate at any time point is given by

$$X_e = X_a R + X_d L \quad (1)$$

where X_e, X_a, and X_d are the fractions of [^{14}C]cholesterol present at any time in the eluate, acceptor SUV, and donor particle, respectively. R represents the fraction of acceptor SUV recovered in the eluate, and L represents the fraction of the donor particles that leaks into the eluate due to incomplete separation. The fraction of the donor particles that leaked was determined from the fraction of [^{14}C]cholesterol that appeared in the eluate at time $t = 0$ so that $L = X_0$; this assumes that there is no pool of rapidly transferring cholesterol. The fraction of [^{14}C]cholesterol remaining in the donor particles at any time is given by

$$X_d = 1 - X_a \quad (2)$$

Substitution of eq 2 into eq 1 gives

$$X_a = \frac{X_e - X_0}{R - X_0} \quad (3)$$

By using the experimental counts per minute, this expression becomes

$$X_a = \frac{{}^{14}C_t/{}^{14}C_{mix} - {}^{14}C_0/{}^{14}C_{mix}}{{}^{3}H_t/{}^{3}H_{mix} - {}^{14}C_0/{}^{14}C_{mix}} \quad (4)$$

In eq 4, $^{14}C_t$, $^{14}C_0$, and $^{14}C_{mix}$ are the ^{14}C counts per minute appearing in the eluate at time t and 0 and in an aliquot of the unseparated incubation mixture, respectively. $^{3}H_t$ and $^{3}H_{mix}$ are the ^{3}H counts recovered in the eluate at time t and in the unseparated incubation mixture, respectively. This equation corrects for leak of donor particles and for recovery of the acceptor SUV in the eluate from the DEAE-Sepharose column. Equation 4 differs from the equation described by McLean and Phillips (1981); they assumed that the ^{14}C counts appearing in the eluate at time $t = 0$ were due to a rapidly exchanging pool of cholesterol. In eq 4, the counts appearing at time $t = 0$ represent the leak of donor particles into the eluate due to incomplete retention on the anion-exchange column.

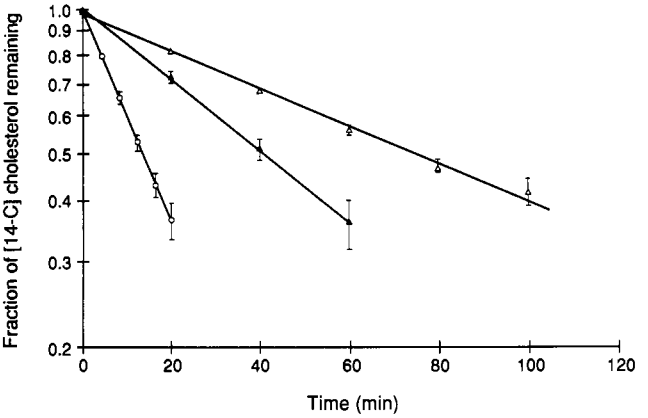

FIGURE 1: Rates of cholesterol exchange from egg PC/cholesterol/apo A-I vesicles and discs of similar composition. Donor particles (SUV or discs) composed of 75 mol % egg PC, 15 mol % dicetyl phosphate, and 10 mol % cholesterol, [^{14}C]cholesterol at 1.0 μCi/mg of lipid, with or without apo A-I at a lipid/protein mole ratio of 150 were incubated at 37 °C with a 10-fold excess of acceptor SUV whose composition was 90 mol % egg PC and 10 mol % cholesterol with [^{3}H]triolein at 0.1 μCi/mg of lipid. The acceptor and donor particles were separated by DEAE-Sepharose anion-exchange chromatography, and the rate of transfer of [^{14}C]cholesterol to the acceptor was analyzed as described under Methods. △, lipid SUV; ▲, lipid + apo A-I SUV; ○, lipid + apo A-I disc.

Rate constants and half-times ($t_{1/2}$) for [^{14}C]cholesterol exchange were calculated from the values of X_a at various time points by the method of McKay (1938) for isotope exchange reactions in a homogenous system as described previously (McLean & Phillips, 1981). All cholesterol exchange experiments were performed by using a minimum of duplicate preparations of each type of donor particle and taking triplicate measurements at five or more time points. All $t_{1/2}$ values are presented ± standard deviation and are compared by using Student's t-test.

Results

Cholesterol exchange between SUV has been shown to occur by an aqueous diffusion mechanism (Phillips et al., 1980; McLean & Phillips, 1981; Backer & Dawidowicz, 1981; Bar et al., 1986), where a cholesterol molecule desorbs from its host phospholipid bilayer, diffuses through the aqueous phase, and adsorbs onto an acceptor particle. It is well established that cholesterol exchange from phospholipid/cholesterol SUV follows first-order kinetics, and in the case of the egg PC/cholesterol vesicles described in Figure 1, $t_{1/2}$ = 80 min. In this system, most of the cholesterol is in this kinetic pool, although multiple pools have been reported for SUV formed from other phospholipids [cf. Bar et al. (1987) and Nemecz et al. (1988)]. In the presence of sufficient acceptor particles, the rate-limiting step is the transfer of the cholesterol molecule from the donor bilayer into the aqueous phase. The rate of this first-order desorption step for cholesterol molecules is independent of SUV concentration and is not influenced by formation of transient vesicle–vesicle complexes (in contrast to the situation for phospholipid molecules) (Jones & Thompson, 1990).

It is apparent from Figure 1 that addition of apo A-I to the donor SUV at a lipid/protein molar ratio of 150:1 (12 apoprotein molecules per SUV) enhances the rate of cholesterol exchange. The [^{14}C]cholesterol equilibrates between the donor and acceptor SUV, indicating that it forms a single kinetic pool with a $t_{1/2}$ of about 40 min. Gel-filtration chromatography indicated that the size of the donor SUV was not affected significantly by the adsorption of any of the A or C apolipoproteins used in this study (data not shown).

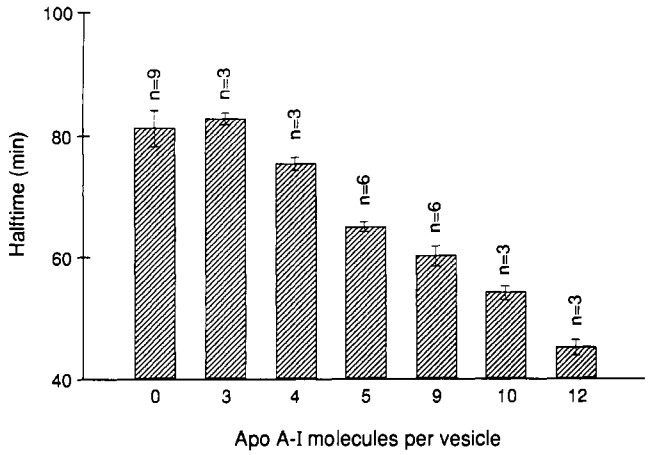

FIGURE 2: Effect of apo A-I content on the half-time of cholesterol exchange from SUV at 37 °C. Donor vesicles containing an increasing number (0–12) of apo A-I molecules on their surfaces were incubated with a 10-fold excess of acceptor SUV as described in the legend to Figure 1.

The rates of cholesterol exchange from SUV containing different numbers of human apo A-I molecules on their surface were examined. Figure 2 illustrates that the addition of up to 4 molecules of apo A-I to the surface of donor SUV has little or no effect on the rate of cholesterol exchange. In all experiments there is a slight increase in $t_{1/2}$ when 2–3 apo A-I molecules are added to the SUV surface; however, this increase is not statistically significant. The addition of increasing amounts of apo A-I to the surface of SUV increases the rate of cholesterol exchange, giving $t_{1/2}$ values of 65 ± 2, 60 ± 3, 54 ± 3, and 45 ± 4 min when 5, 9, 10, and 12 apo A-I molecules, respectively, are present on the vesicle surface. These values are all significantly different ($p < 0.001$) from $t_{1/2}$ for cholesterol transfer from a SUV without apo A-I on its surface. Apo A-I has also been shown to enhance the rate of transfer of [^{14}C]cholesterol from mitochondria to lipid vesicles (Van Heusden et al., 1989). Cholesterol transfer rates from SUV containing low levels of apo A-II and apo C were also studied. The changes in the rates of cholesterol transfer from vesicles containing these apoproteins exhibit trends similar to those for apo A-I. The addition of 4 molecules of apo A-II and 7 molecules of apo C slightly decreases the rate of cholesterol transfer relative to SUV without protein (addition of apo A-II and apo C increases $t_{1/2}$ from 80 ± 9 to 85 ± 2 and 88 ± 4 min, respectively).

Table I compares the rate of cholesterol desorption from SUV containing the same masses of apo A-I, apo A-II, RCM-AII, apo C, and apo B-100. At a lipid/protein weight ratio of 10, both apo A-I and apo A-II (monomer and dimer) cause a statistically significant ($p < 0.001$) decrease in $t_{1/2}$ for cholesterol exchange in these SUV. Apo C does not cause a statistically significant difference ($p < 0.05$) in the rate of cholesterol exchange. The effect of apo B-100 is compared to apo A-I at a lipid/protein weight ratio of 4 (corresponding to 1 apo B-100 molecule per vesicle). At this ratio, apo B-100 causes a significantly greater increase in the rate of cholesterol exchange than apo A-I. The rate of cholesterol exchange from SUV containing apo C on their surface is slower than that from vesicles which initially contained the same mass of the various apo A molecules (Table I). This is probably due to the transfer of apo C molecules between vesicles, resulting in changes in the surface composition of the donor SUV during the incubation with acceptor vesicles (see Methods).

Figure 1 also provides information on the rate of cholesterol exchange from a discoidal complex. It is apparent that $t_{1/2}$ for transfer of [^{14}C]cholesterol from an apo A-I/phospholipid disc is 14 ± 1 min compared to 45 ± 4 min for a SUV of the same composition. The $t_{1/2}$ value of 14 min is similar to the value of 22 min for a discoidal apo A-I/palmitoyloleoyl-PC complex (Massey, 1984). The fact that the exchange of cholesterol from all of the donor particles described in Figure 1 obeys first-order kinetics is consistent with the same molecular mechanism applying in all cases. Several kinetic criteria that apply to the aqueous diffusion mechanism of exchange of cholesterol between membranes are now well established [for a review, see Phillips et al. (1987)]. In addition to the rate of cholesterol exchange being first-order with respect to the concentration of cholesterol in the donor particle, the following three conditions apply: (1) The rate of cholesterol exchange is strongly temperature dependent because of the high energy of activation associated with the desorption step. (2) The rate of exchange is zero-order with respect to the acceptor particle concentration when excess acceptor particles are present. (3) The rate of cholesterol exchange is influenced by the nature of the host phospholipid present in the donor particle. These conditions are known to apply to cholesterol exchange from SUV, and the following experiments were performed to see if they also apply to exchange from discoidal complexes.

Table I: Comparison of Cholesterol Exchange Rates from Egg PC Vesicles Containing Various Apolipoproteins at 37 °C[a]

apolipoprotein	lipid/protein (w/w)	$t_{1/2}$[b] (min)	n
none		80 ± 9	9
A-I	10	65 ± 2	6
A-II	10	63 ± 2	6
RCM A-II	10	63 ± 1	6
C	10	74 ± 4	4
A-I	4	45 ± 4	6
B-100	4	39 ± 3	4

[a] The donor vesicle lipid composition was 75 mol % egg yolk phosphatidylcholine, 15 mol % dicetyl phosphate, and 10 mol % cholesterol containing [^{14}C]cholesterol at 1.0 μCi/mg of lipid. [b] The half-times for cholesterol exchange from vesicles containing apo A-I, apo A-II, and RCM A-II are significantly different from $t_{1/2}$ for vesicles with no apoprotein ($p < 0.001$). $t_{1/2}$ for vesicles containing total apo C is not significantly different ($p < 0.05$).

The rate of [^{14}C]cholesterol transfer from DMPC/cholesterol/apo A-I discs (75 mol % DMPC, 15 mol % dicetyl phosphate, and 10 mol % cholesterol, [^{14}C]cholesterol at 1.0 μCi/mg of lipid, lipid/apo A-I mole ratio = 200:1) to acceptor SUV (90 mol % egg PC and 10 mol % cholesterol) was measured at various concentrations of acceptor SUV at 37 °C. The donor particle concentration was kept constant at 0.13 μg of cholesterol/mL, and the acceptor concentration was varied to give acceptor/donor cholesterol ratios in the range of 1–20. At acceptor/donor cholesterol ratios of 5 (acceptor/donor particle ratio of 10) and above, the rate constant for cholesterol exchange was independent of the acceptor particle concentration. This condition holds for all of the lipid–protein particles studied. All of the experiments presented in this paper were conducted at acceptor/donor cholesterol ratios of at least 10 so that any variations in the rate constant for cholesterol exchange can be attributed to changes in the structure of the donor particle. The temperature dependences of cholesterol exchange from an egg PC/apo A-II [150:1 (mol/mol)] disc containing 10 mol % cholesterol and a similar DMPC/apo A-I [200:1 (mol/mol)] disc were determined. The activation energies for cholesterol exchange from these particles were 16 and 20 kcal/mol, respectively. To test the effect of the host phospholipid, the rates of cholesterol exchange from a series of discs containing 10 mol %

Table II: Rates of Cholesterol Exchange from Egg PC/Apo A-I and Dipalmitoyl-PC/Apo A-I Discoidal Complexes with Different Lipid/Protein Ratios and Sizes[a]

lipid/protein (mol/mol)	diameter[b] (nm)	$t_{1/2}$[c] (min)
	Egg PC/Apo A-I	
196	17 ± 1	86 ± 5
147	15 ± 1	74 ± 10
108	12 ± 1	57 ± 1
64	11 ± 1	50 ± 2
	Dipalmitoyl-PC/Apo A-I	
267	23 ± 2	127 ± 6
253	19 ± 1	95 ± 8
118	16 ± 2	77 ± 4
62	11 ± 3	34 ± 4

[a] The disc lipid composition was 75 mol % PC, 15 mol % dicetyl phosphate, and 10 mol % cholesterol containing [^{14}C]cholesterol at 1.0 μCi/mg of lipid. The errors in the lipid/protein stoichiometries of the egg PC and DPPC complexes are within ±12% and ±14%, respectively. [b] Disc diameters were determined by electron microscopy. [c] The half-times are for 25 and 45 °C for the egg PC and dipalmitoyl-PC systems, respectively; both types of PC form bilayers in the liquid crystal phase under these conditions. $t_{1/2}$ values are reported ± standard deviation, with triplicate time points from two and three experiments, respectively, for the egg PC and dipalmitoyl-PC systems.

Table III: Rates of Cholesterol Exchange from Discoidal Complexes of Egg PC and Apolipoproteins A-II and C-III$_1$ with Different Lipid/Protein Ratios and Sizes at 25 °C[a]

lipid/protein (mol/mol)	diameter[b] (nm)	$t_{1/2}$[c] (min)
	Egg PC/Apo A-II	
371	20 ± 1	172 ± 6
205	16 ± 2	109 ± 1
109	12 ± 1	71 ± 4
	Egg PC/Apo C-III$_1$	
65	24 ● 2	240 ± 4
54	20 ± 1	185 ± 9
35	17 ± 3	154 ± 1

[a] The disc lipid composition was 75 mol % egg PC, 15 mol % dicetyl phosphate, and 10 mol % cholesterol containing [^{14}C]cholesterol at 1.0 μCi/mg of lipid. The errors in the lipid/protein stoichiometries of the apo A-II and apo C-III$_1$ complexes are within ±19% and ±12%, respectively. [b] Disc diameters were determined by electron microscopy. [c] $t_{1/2}$ values are reported ± standard deviation, with triplicate time points from two experiments.

cholesterol and apo A-I at a lipid/protein mole ratio of 100:1 were determined. The $t_{1/2}$ values at 37 °C for discs containing egg PC, DMPC, egg SM, and bovine brain SM were 14 ± 1, 111 ± 8, 82 ± 5, and 120 ± 1 min, respectively. It should be noted that the above host phospholipids contained 15 mol % dicetyl phosphate to confer a negative charge on the donor discoidal particles. These data for cholesterol exchange from discoidal particles are consistent with equivalent data for SUV systems where it has been shown that the rate decreases with increasing saturation of the acyl chain and an increase in the content of SM [for a review, see Phillips et al. (1987)]. Taken together, the above kinetic data provide convincing evidence that the mechanism of cholesterol exchange from vesicular and discoidal phospholipid/apolipoprotein complexes is identical.

To determine whether the rate of cholesterol desorption from discs is sensitive to the composition and size of the disc, exchange experiments were conducted with egg PC/apo A-I and DPPC/apo A-I discs of various stoichiometries and sizes. Tables II lists the results from cholesterol exchange experiments with both types of discs of diameters ranging from 11 to 23 nm. Donor discs were incubated with a 10-fold excess of acceptor SUV at 25 and 45 °C for the egg PC and DPPC systems, respectively. $t_{1/2}$ of cholesterol exchange decreases with decreasing size of the donor disc particles. It should be noted that an increase in the protein content of apo A-I/egg PC SUV and discs is associated with a decrease in $t_{1/2}$ in both cases (cf. Figure 2 and Table II). However, the dependence of $t_{1/2}$ on the protein content of the donor particles is greater for discs than SUV; the additional effect in the disc system is the decrease in particle size that accompanies the reduction in lipid/protein ratio. Cholesterol exchange rates from egg PC discs containing apo A-II and apo C-III$_1$ were also measured (Table III); the $t_{1/2}$ values exhibit a pattern similar to those seen in discs containing apo A-I. The kinetics of cholesterol exchange are the same for discs containing either total apo C or purified apo C-III$_1$ (data not shown). Generally, as the diameter of the phospholipid/apoprotein disc decreases, the rate of cholesterol exchange increases.

Discussion

Effects of Apolipoproteins on Cholesterol Exchange from Vesicles. The data presented in Figure 2 show that, while addition of up to 3 molecules of apo A-I to a SUV does not decrease and perhaps even slightly increases $t_{1/2}$ for cholesterol exchange, addition of 5 or more molecules of apo A-I causes a progressive decrease in $t_{1/2}$. These effects on the rate of cholesterol desorption imply that the presence of a few apo A-I molecules does not lead to a significant perturbation of the packing and interactions of cholesterol molecules present in the donor lipid–water interface. An increase in the surface concentration of apo A-I molecules to 4 or more per SUV reduces the interactions of cholesterol molecules in the lipid–water interface so that they are able to form the transition state for desorption more readily [cf. Phillips et al. (1987)], which is reflected in the reduced $t_{1/2}$ for exchange. Since the addition of 3 apo A-I molecules to a SUV does not cause a change in particle size, the penetration of the apoprotein molecules into the SUV surface must condense the lateral packing of the PC and cholesterol molecules [cf. Ibdah and Phillips (1988)]. The change in the average molecular packing density of phospholipid and cholesterol on the surface of a vesicle due to the addition of apo A-I can be estimated as follows. An egg PC SUV containing 10 mol % cholesterol with an outer radius of 110 Å (surface area = 150 000 Å^2) contains 1533 molecules of phospholipid and 170 molecules of cholesterol on its surface (Newman & Huang, 1975). This assumes that $^2/_3$ of the phospholipid molecules are present in the outer monolayer of the bilayer vesicle. The average surface area occupied by these molecules is 88 Å^2 per molecule. The area taken up by a fully α-helical protein lying completely in the surface is close to 15 Å^2 per amino acid (Phillips & Sparks, 1980). Therefore, 3 apo A-I molecules (243 amino acid residues per molecule) occupy a total area of approximately 11 000 Å^2. Consequently, the change in the average packing density of the lipid at the surface is about 6.5 Å^2 per molecule. Lund-Katz et al. (1988b) showed that increases in the lateral packing density in the lipid–water interface of bilayers decreases the rate of cholesterol desorption, so a decrease of 6.5 Å^2 per molecule would be expected to reduce the rate of desorption of cholesterol molecules. However, the data in Figure 2 show that the addition of 3 molecules of apo A-I to a SUV has no effect on the rate of cholesterol exchange. One possible explanation of this apparent anomaly is the occurrence of a compensating effect that offsets the compression of PC and cholesterol molecules induced by the adsorbed apo A-I molecules.

The packing of lipid molecules in the "boundary" layer immediately adjacent to the apo A-I molecules is perturbed. In studies on the lipid–protein interactions of model HDL particles, it has been proposed that cholesterol is rigorously excluded from the boundary layer of phospholipid that is

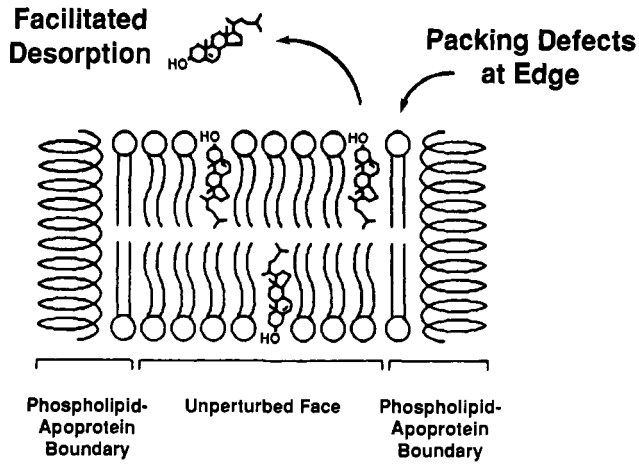

FIGURE 3: Model for cholesterol desorption from phospholipid/cholesterol/apolipoprotein discoidal particles. Discoidal particles contain a "boundary" region consisting of the apoprotein molecules at the edge of the disc and the phospholipid molecules immediately adjacent to the protein molecules. In addition, there is a "face" region consisting of unperturbed phospholipid molecules. It is assumed that the desorption of cholesterol molecules from the boundary region is faster than from the face region (see text for further details).

adjacent to apo A-I (Tall & Lange, 1978). Others have proposed that there is preferential association of the phospholipid with the protein surface and that cholesterol is not excluded from these regions but rather comes into contact with the protein molecule with a lower frequency (Massey et al., 1985). In both these models, phospholipid molecules interact strongly with protein molecules, so that the intermolecular interactions of the cholesterol and phospholipid in these boundary regions are probably weaker. It follows that desorption of cholesterol molecules will occur more readily from the boundary regions. As the apoprotein concentration at the SUV surface is increased, there is an increase in the surface packing density and a decrease in the intermolecular interactions between cholesterol and phospholipid due to the boundary layer effect. With 3 or 4 apo A-I molecules per SUV, these effects cancel out and there is no change in the cholesterol desorption rate. When the apo A-I content of vesicles is increased further, the contribution of the lipid–protein boundary effects overcomes the contribution of the increase in overall surface packing density and the cholesterol exchange rate rises (Figure 2).

Equal masses of apolipoproteins of the A, B, and C classes present in SUV exert similar effects on $t_{1/2}$ for cholesterol exchange (Table I). This implies that the general nature of the lipid–protein interaction of these apoproteins are similar and that specific interactions with phospholipids or cholesterol are not involved. Consistent with this concept, apolipoproteins A and C interact with lipid–water interfaces primarily by insertion of segments of amphipathic α-helix [for reviews, see Atkinson and Small (1986) and Pownall et al. (1987)]. The interaction of apo B-100 with lipids is not so well understood, but the data in Table I suggest that this apoprotein exerts an effect similar to that of apo A-I on cholesterol packing in SUV.

Cholesterol Exchange from Apolipoprotein/Phospholipid Discoidal Complexes. Transformation of a vesicle into a disc without a change in overall composition increases the rate of cholesterol desorption by a factor of about 3 (Figure 1). A hypothesis to explain this rate enhancement involves the existence of packing defects at the edge of the disc particle (Figure 3). Tall and colleagues (1977) have proposed that there is a boundary of phospholipid molecules associated with protein molecules located at the edge of the bilayer disc. Phospholipid molecules in this location do not undergo the hydrocarbon chain melting characteristic of unperturbed phospholipid molecules and packing defects occur at the disc edge. These packing defects may reduce the cholesterol–phospholipid interaction energy at the edge of the disc and facilitate the desorption of cholesterol molecules from these areas.

If the above ideas are correct, then the dependence of cholesterol desorption rate on disc diameter can be explained in terms of a model where cholesterol exists in two rapidly equilibrating pools within a disc particle. One pool is associated with the phospholipid in the face of the disc particle and desorbs with rate constant k_f. The second pool is found at the edge of the disc in the boundary region consisting of phospholipid, cholesterol and apoprotein; the desorption of this pool of cholesterol has a rate constant k_b. If the cholesterol–phospholipid interaction energy in these boundary regions is lower than in the face region, then $k_b > k_f$. The fraction of [^{14}C]cholesterol appearing in acceptor SUV represents the cholesterol desorbing in parallel from both the boundary and the face regions of the disc. The flux of cholesterol from both the boundary region and the face region of the disc contributes to the rate constant k_{obs} of cholesterol exchange measured in these experiments (Figure 3). It follows that, for large discs where the area of the face is large relative to the area of the boundary, the rate constant for cholesterol exchange (k_{obs}) will be relatively small. This effect is seen qualitatively in the data listed in Table II and III, where the rate of cholesterol exchange decreases with increasing size of donor disc particles.

The model described above and in Figure 3 for cholesterol transfer from discoidal particles can be used to examine the quantitative relationship between k_{obs} and the disc major radius. If X_b is the mole fraction of cholesterol in the boundary region of the disc, it follows that $k_{obs} = k_b X_b + k_f(1 - X_b)$. Since $k_b > k_f$, $k_{obs} \approx k_b X_b$ when X_b is a significant fraction. Thus, k_{obs} should be proportional to X_b for any series of discoidal complexes of varying size for which k_b and k_f are constants. X_b cannot be measured directly, but we assume that cholesterol molecules partition between the boundary and unperturbed face regions in proportion to the fraction of the total face area of the disc that each domain occupies. On this basis, $X_b \propto A_b/A_t$, where A_b is the area of the boundary region and A_t is the total surface area of the two faces of the disc (i.e., the area of the disc edge is excluded). For a disc of major radius r, $A_t = 2\pi r^2$. The area of the unperturbed face can be estimated by subtracting 2 nm from r [1 nm for the α-helix of the apoprotein at the edge of the disc and 1 nm for a perturbed layer one phospholipid molecule thick immediately adjacent to it (Figure 3)]. A_b is then derived by subtracting this area of the unperturbed face from A_t. By using these values of A_t and A_b, which are expressed in terms of r, to derive X_b, it can be shown that to a first approximation k_{obs} should be inversely proportional to r. It is apparent from Figure 4 that much of the data in Tables II and III fits this relationship.

The analysis summarized by Figure 4 lends support to the model (Figure 3) in which the measured rate of cholesterol exchange reflects the rates of cholesterol desorption from both the unperturbed face and the boundary areas of the discoidal PC/apolipoprotein complexes. The differences in slopes for the plots of k_{obs} against r for the complexes of egg PC and cholesterol with apolipoproteins A-I, A-II, and C-III$_1$ presumably reflect variations in lipid–protein interactions. It seems that k_{obs} for complexes containing the relatively small apo C-III$_1$ molecule is less sensitive to changes in r. For egg

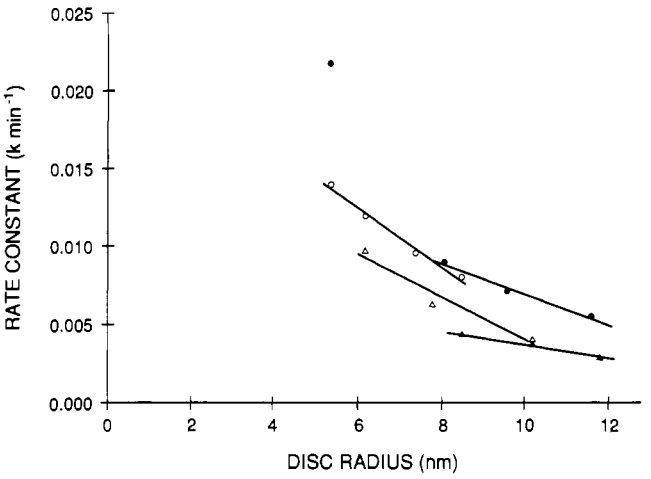

FIGURE 4: Variation of the rate constant for cholesterol exchange from phosphatidylcholine/cholesterol/apolipoprotein discoidal complexes with the disc major radius. The kinetic and particle size data are taken from Tables II and III. ○, egg PC/apo A-I; ●, DPPC/apo A-I; △, egg PC/apo A-II; ▲, egg PC/apo C-III$_1$.

PC/cholesterol discs with $r = 8$ nm, k_{obs} progressively increases as the molecular weight of the apoprotein increases. The reason for k_{obs} for the DPPC/cholesterol/apo A-I disc with $r \sim 6$ nm being so high and not obeying the linear relationship between k_{obs} and r is not clear. This discrepancy may reflect a structural change in this small particle relative to the larger DPPC discs so that k_b is increased.

Physiological Significance. This paper, which describes the effects of apolipoproteins on cholesterol exchange, extends previous work that elicited the influence of cholesterol–phospholipid interactions on the kinetics of cholesterol exchange [for a review, see Phillips et al. (1987)]. It is apparent that apolipoprotein molecules present at the surface of a donor particle can affect the rate of cholesterol desorption by modulating the molecular packing in the lipid–water interface.

The 6-fold enhancement in rate of cholesterol exchange for a discoidal apolipoprotein A or C/phospholipid complex relative to an apoprotein-free SUV of the same lipid composition partially explains the shorter $t_{1/2}$ previously observed for plasma HDL particles relative to SUV (Lund-Katz et al., 1982). However, $t_{1/2}$ at 37 °C for human HDL_3 is about 3 min, which is still significantly shorter thatn $t_{1/2}$ of about 14 min for an egg PC/cholesterol/apo A-I disc (Figure 1). The faster rate of desorption from the HDL_3 particle indicates that the cholesterol molecules interact relatively weakly with their neighbors in the surface of this particle. This effect may be due to (1) the highly curved HDL surface, because $t_{1/2}$ for cholesterol exchange decreases as the size of SUV decreases (McLean & Phillips, 1984b; Thomas & Poznansky, 1988), or (2) the high surface concentration of apoprotein in HDL_3; the phospholipid/apoprotein weight ratio is about 0.5 in HDL_3 and >3 for the egg PC/apo A-I disc and vesicle described in Figure 1.

This investigation of the effects of serum apolipoproteins has provided insights into the ways these protein molecules, which contain amphipathic helices that orientate parallel to the lipid–water interface in phospholipid monolayers and vesicles (Ibdah & Phillips, 1989), affect the cholesterol exchange process. It will be important to conduct similar investigations of proteins which contain α-helices that embed in a phospholipid bilayer vesicle with the long axes of the helices parallel to the lipid acyl chains. Such proteins may be important in modulating the rate of cholesterol transfer from cell plasma membranes to extracellular lipoprotein particles.

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Registry No. DMPC, 18194-24-6; DPPC, 63-89-8; dicetyl phosphate, 2197-63-9; cholesterol, 57-88-5.

REFERENCES

Aniansson, E. A. G., Wall, S. N., Almgren, W. M., Hoffmann, H., Dielmann, I., Ulbricht, W., Zana, R., Lang, J., & Tondre, C. (1976) *J. Phys. Chem. 80*, 905–922.

Atkinson, D., & Small, D. M. (1986) *Annu. Rev. Biophys. Biophys. Chem. 15*, 403–456.

Backer, J. M., & Dawidowicz, E. A. (1981) *Biochemistry 20*, 3805–3810.

Bar, L. K., Barenholz, Y., & Thompson, T. E. (1986) *Biochemistry 25*, 6701–6705.

Bar, L. K., Barenholz, Y., & Thompson, T. E. (1987) *Biochemistry 26*, 5460–5465.

Bell, F. P. (1978) *Prog. Lipid Res. 17*, 207–243.

Bittman, R. (1988) in *Biology of Cholesterol* (Yeagle, P. L., Ed.) pp 173–195, CRC Press, Boca Raton, FL.

Bligh, E. G., & Dyer, W. J. (1951) *Can. J. Biochem. Physiol. 37*, 911–917.

Bloj, B., & Zilversmit, D. B. (1977) *Biochemistry 16*, 3943–3948.

Bruckdorfer, K. R., & Graham, J. M. (1976) in *Biological Membranes* (Chapman, D., & Wallach, D. F. H., Eds.) Vol. 3, pp 103–152, Academic Press, London.

Collins, J. J., & Phillips, M. C. (1982) *J. Lipid Res. 23*, 291–298.

Goodwin, J. F., Thibert, R., McCann, D., & Boyle, A. J. (1958) *Anal. Chem. 30*, 1097–1099.

Helenius, A., & Simons, K. (1971) *Biochemistry 10*, 2542–2547.

Holmquist, L., & Carlson, K. (1977) *Biochim. Biophys. Acta 493*, 400–409.

Ibdah, J. A., & Phillips, M. C. (1988) *Biochemistry 27*, 7155–7162.

Ibdah, J. A., Lund-Katz, S., & Phillips, M. C. (1989) *Biochemistry 28*, 1126–1133.

Ibdah, J. A., Lund-Katz, S., & Phillips, M. C. (1990) *Biochemistry 29*, 3472–3479.

Ishikawa, T. T., MacGee, J., Merrison, J. A., & Glueck, C. J. (1974) *J. Lipid Res. 15*, 286–291.

Johnson, W. J., Bamberger, M. J., Latta, R. A., Rapp, P. E., Phillips, M. C., & Rothblat, G. H. (1986) *J. Biol. Chem. 261*, 5766–5776.

Jonas, A., & Drengler, S. M. (1980) *J. Biol. Chem. 255*, 2190–2194.

Jonas, A., Drengler, S. M., & Patterson, B. W. (1980) *J. Biol. Chem. 255*, 2183–2189.

Jones, J. D., & Thompson, T. E. (1990) *Biochemistry 29*, 1593–1600.

Knipping, G., Steyrer, E., Zecher, R., & Holasek, A. (1984) *J. Lipid Res. 25*, 86–91.

Lowry, O. H., Rosebrough, N. J., Farr, A. K., & Randell, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Lund-Katz, S., Hammerschlag, B., & Phillips, M. C. (1982) *Biochemistry 21*, 2964–2969.

Lund-Katz, S., Ibdah, J. A., Letizia, J. Y., Thomas, M. T., & Phillips, M. C. (1988a) *J. Biol. Chem. 263*, 13831–13838.

Lund-Katz, S., Laboda, H. M., McLean, L. R., & Phillips, M. C. (1988b) *Biochemistry 27*, 3416–3423.

Markwell, M. A. K., Haas, S. M., Bieber, L. L., & Tolbert, N. E. (1978) *Anal. Biochem. 87*, 206-210.
Massey, J. B. (1984) *Biochim. Biophys. Acta 793*, 387-392.
Massey, J. B., She, H. S., Gotto, A. M., & Pownall, H. J. (1985) *Biochemistry 24*, 7110-7116.
Matz, C. E., & Jonas, A. (1982) *J. Biol. Chem. 257*, 4535-4540.
McKay, H. A. C. (1938) *Nature 142*, 997-998.
McLean, L. R., & Phillips, M. C. (1981) *Biochemistry 20*, 2893-2900.
McLean, L. R., & Phillips, M. C. (1982) *Biochemistry 21*, 4053-4059.
McLean, L. R., & Phillips, M. C. (1984a) *Biochemistry 23*, 4624-4630.
McLean, L. R., & Phillips, M. C. (1984b) *Biochim. Biophys. Acta 776*, 21-26.
Nakagawa, Y., Inoue, K., & Nojima, S. (1979) *Biochim. Biophys. Acta 553*, 307-319.
Nemecz, G., Fontaine, R. N., & Schroeder, F. (1988) *Biochim. Biophys. Acta 943*, 511-521.
Newman, G. C., & Huang, C.-H. (1975) *Biochemistry 14*, 3363-3370.
Nichols, J. S. (1985) *Biochemistry 24*, 6390-6398.
Phillips, M. C., & Sparks, C. E. (1980) *Ann. N.Y. Acad. Sci. 348*, 122-137.
Phillips, M. C., Johnson, W. J., & Rothblat, G. H. (1987) *Biochim. Biophys. Acta 906*, 223-276.
Phillips, M. C., McLean, L. R., Stoudt, G. W., & Rothblat, G. H. (1980) *Atherosclerosis 36*, 409-422.
Pownall, H. J., Hickson, D. L., & Smith, L. C. (1983) *J. Am. Chem. Soc. 105*, 2440-2445.
Pownall, H. J., Massey, J. B., Sparrow, J. T., & Gotto, A. M. (1987) in *Plasma Lipoproteins* (Gotto, A. M., Ed.) pp 95-127, Elsevier, Amsterdam.
Redgrave, T. G., Roberts, D. C. K., & West, C. E. (1975) *Anal. Biochem. 65*, 42-49.
Rothblat, G. H., & Phillips, M. C. (1982) *J. Biol. Chem. 257*, 4775-4782.
Sokoloff, L., & Rothblat, G. H. (1974) *Proc. Soc. Exp. Biol. Med. 146*, 1166-1172.
Tall, A. R., & Lange, Y. (1978) *Biochim. Biophys. Acta 513*, 185-206.
Tall, A. R., Small, D. M., Deckelbaum, R. J., & Shipley, G. G. (1977) *J. Biol. Chem. 252*, 4701-4711.
Thomas, P. D., & Poznansky, M. J. (1988) *Biochem. J. 254*, 155-160.
Van Heusden, G. P. H., Van Schijndel, J. W. P. M., & Wirtz, K. W. A. (1989) *J. Lipid Res. 30*, 1357-1363.
Walsh, M. T., & Atkinson, D. (1983) *Biochemistry 22*, 3170-3178.
Yokoyama, S., Tajima, S., & Yamamoto, A. (1984) *J. Biochem. (Tokyo) 96*, 871-880.

Mechanism of Interaction of Vinca Alkaloids with Tubulin: Catharanthine and Vindoline†

V. Prakash‡ and Serge N. Timasheff*
Graduate Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02254-9110
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ABSTRACT: The interactions of the vinca alkaloid drugs catharanthine and vindoline with tubulin have been investigated and compared with those of vinblastine and vincristine. Both drugs were found to be less effective in bringing about the inhibition of tubulin self-assembly into microtubules than vincristine and vinblastine, the drug to protein molar ratio required being 3 orders of magnitude greater. An analytical ultracentrifuge study has shown that catharanthine can induce the self-association of tubulin into linear indefinite polymers with an efficacy that is 75% that of vinblastine or vincristine, the intrinsic dimerization constant for the liganded protein being $K_2 \simeq 1 \times 10^5$ M^{-1}. The effect of vindoline was marginally detectable. Binding studies of catharanthine using the gel batch and fluorescence perturbation techniques showed a polymerization-linked binding of one catharanthine molecule per tubulin α-β dimer with a binding constant of $(2.8 \pm 0.4) \times 10^3$ M^{-1}. For vindoline, binding to tubulin was marginally detectable by fluorescence spectroscopy, although addition of vindoline to tubulin did generate a difference spectrum. It was concluded that the binding of vinblastine/vincristine to tubulin and its consequences are determined by the interaction of the indole part of catharanthine with tubulin, the role of vindoline being that of an anchor.

The two major vinca alkaloids from the plant *Catharanthus roseus*, vinblastine (VBL)[1] (structure Ia, Chart I) and vincristine (VCR)[1] (structure Ib), are well-known effective mitosis-arresting reagents that are widely used in the treatment of neoplastic diseases (Dustin, 1978). Structurally these two drugs are "dimeric" molecules, as they comprise two domains, vindoline (VDN)[1] (lower half of VBL/VCR structure) and rearranged catharanthine (CTN)[1] (structure II). Both vincristine and vinblastine induce tubulin to self-associate in vitro

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‡ Present address: Biophysical Chemistry Unit, Central Food Technological Research Institute, Mysore 570013, India; Visiting Scientist at Brandeis University, 1989.

[1] Abbreviations: VBL, vinblastine; VCR, vincristine, CTN, catharanthine; VDN, vindoline; GTP, guanosine 5′-triphosphate; PG buffer, 10^{-2} M sodium phosphate, 1×10^{-4} M GTP, pH 7.0; PMG buffer, PG buffer to which is added 5×10^{-3} M $MgCl_2$.

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