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Spontaneous Phosphatidylcholine Exchange between Small Unilamellar Vesicles and Lipid-Apolipoprotein Complexes. Effects of Particle Concentrations and Compositions†

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ABSTRACT: We investigated the spontaneous exchange of phosphatidylcholine (PC) between model lipoproteins and small unilamellar vesicles. The model lipoproteins were recombinants of bovine apolipoprotein A-I, egg PC, and cholesterol. Vesicles contained egg PC and cholesterol in varying molar ratios. Lipid exchange was followed by incubating radiolabeled vesicles ([^{3}H]PC plus cholesteryl [^{14}C]oleate as a nonexchangeable marker) with unlabeled lipid-apolipoprotein complexes at a constant temperature of 37 °C. Incubation mixtures were fractionated on Bio-Gel A-5m columns at 5 °C, and the ^{3}H cpm/^{14}C cpm ratio under the vesicle peak was determined. Comparison of this ratio with that of vesicles before incubation was used to calculate the proportion of radiolabel transferred to complexes at various times. The results show that no net transfer of PC occurs under our experimental conditions; 70% of vesicle PC exchanges with complexes, indicating that only the outer monolayer of the vesicle is available for exchange; in contrast, all of the complex PC is exchangeable. Exchange is temperature dependent with an activation energy of 22.9 ± 2.0 kcal/mol. Under our experimental conditions the rate of PC exchange is linearly dependent upon both vesicle and complex PC concentrations with a rate constant of 6.9 ± 0.7 μM^{-1} h^{-1}, and the rate constant varies inversely with the cholesterol content of vesicles or complexes. These results imply that a complete kinetic model of spontaneous lipid exchange must account for the dependence of exchange rates not only on the concentration and composition of the donor particles but on the concentration and chemical nature of the acceptors, as well.

Exchange and transfer of protein and lipid components among lipoprotein classes or between lipoproteins and cell membranes are essential processes which determine the steady-state composition, structure, and metabolism of lipoproteins (Bell, 1978; Smith et al., 1978; Miller & Gotto, 1982). One aspect of these processes is the exchange and transfer of phospholipids, involving high-density lipoproteins (HDL)[1] (Scanu et al., 1982). Influx of phospholipids, particularly

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[1] Abbreviations: PC, phosphatidylcholine; HDL, high-density lipoproteins; apo A-I, apolipoprotein A-I; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; SDS, sodium dodecyl sulfate; DPPC, dipalmitoylphosphatidylcholine; SUV, small unilamellar vesicles.

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phosphatidylcholine (PC), into the plasma HDL pool occurs via nascent HDL particles (Norum et al., 1975; Hamilton et al., 1976; Tall & Small, 1978), from chylomicron and very low density lipoprotein surface components released during lipolysis (Havel et al., 1973; Redgrave & Small, 1979; Forte et al., 1979; Eisenberg, 1980) and from cell membranes (Reed, 1968; Blumenfeld et al., 1979). Losses of phospholipids from HDL occur via enzymatic degradation (Nilsson-Ehle et al., 1980) and by back-transfer to membranes and to other lipoprotein classes.

The mechanisms by which phospholipid transfers occur are not yet completely understood, but slow spontaneous and more rapid catalyzed transfers have been demonstrated by using plasma and purified model systems (Brewster et al., 1978; Damen et al., 1980). In the case of spontaneous transfer it appears that monomer lipids desorb from donor particles and reach acceptors via the aqueous medium (McLean & Phillips, 1981; Bojesen & Bojesen, 1982; Massey et al., 1982a). In the case of transfers stimulated by plasma phospholipid transfer factors, it is not clear whether monomer lipid solubilization or the formation of efficient transfer complexes (Ihm et al., 1982) is enhanced.

Investigations of the kinetics of spontaneous, labeled phospholipid exchange have shown that depending on the systems and on the experimental conditions, transfer rates dependent (Martin & MacDonald, 1976; Kremer et al., 1977; Jonas & Maine, 1979) or independent (Roseman & Thompson, 1980; McLean & Phillips, 1981; Massey et al., 1982a) of the acceptor particle concentration could be observed. Initially such results were thought to support either collisional or monomer desorption mechanisms of transfer, respectively. More recently, kinetic models based on the lipid monomer desorption idea have incorporated the on and off rates of the lipid monomer with respect to both donor and acceptor particles (Nichols & Pagano, 1981; Bojesen, 1982; Lange et al., 1983). Under appropriate conditions the transfer rates can be either dependent on or independent of the acceptor concentration.

In this study we use a simple model system, including synthetic nascent HDL analogues and small unilamellar vesicles (SUV), which allows us to vary the PC acceptor and donor particle concentrations as well as their compositions (in terms of PC to cholesterol ratios). We describe the kinetic effects of such variations in the system and discuss our results in terms of existing kinetic models.

Materials and Methods

Preparations. Egg PC (type III-E) and crystalline cholesterol were obtained from Sigma Chemical Co. Egg PC showed a single lipid spot on overloaded thin-layer plates of silica gel developed in a chloroform/methanol/water (65:25:4 v/v) solvent. Cholesterol also gave a single spot when chromatographed in petroleum ether/diethyl ether/acetic acid (90:10:1 v/v) or in cyclohexane/ethyl acetate (60:40 v/v) solvents. Radiolabeled cholesteryl [1-^{14}C]oleate and L-α-dipalmitoyl[*methyl*-^{3}H]PC (DPPC) were obtained from New England Nuclear Co. The radiolabeled lipids, checked for purity in the same chromatographic systems used for unlabeled lipids, gave single spots. The sodium cholate (98% pure) purchased from Sigma Chemical Co. was used without any treatment.

Bovine apolipoprotein A-I (apo A-I) was isolated by a modification of the method described previously (Jonas, 1975). Delipidated bovine HDL was fractionated on a column of Sephacryl 200 (85 × 2.5 cm) in 0.1 M Tris-HCl, pH 8.2, 3 M guanidine hydrochloride, and 0.005% EDTA, at 4 °C. Fraction II, containing apo A-I (Jonas, 1975), was pooled, dialyzed against 0.05% EDTA, lyophilized, and stored at −20 °C. The apolipoprotein was judged pure by polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) under reducing conditions. Before use, apo A-I preparations were solubilized in 3 M guanidine hydrochloride and were dialyzed against buffer. All experiments were performed in 10 mM Tris-HCl, 150 mM NaCl, 1 mM NaN_3, and 0.01% EDTA, pH 8.0 buffer, unless otherwise stated.

Concentrations of apo A-I were determined from its extinction coefficient at 280 nm, $E^{1\%} = 1.25 \times 10^3$ g^{-1} cm^2 (Jonas, 1975) and by the procedure of Lowry et al. (1951) modified to include 1% SDS (Markwell et al., 1978) for samples containing lipids.

Complexes of egg PC, cholesterol, and bovine apo A-I were prepared by using a modification of the sodium cholate dispersion and dialysis technique described previously (Matz & Jonas, 1982). Usually, 14.4 mg of egg PC and 2.4 mg of cholesterol (PC:cholesterol, 3:1 mol/mol) were mixed in chloroform and dried under N_2 to give a thin film. Sodium cholate (sodium cholate:PC, 2:1 mol/mol) and buffer were added such that the sodium cholate concentration was 14 mM. The mixture was vortexed and cooled to −5 °C before addition of 5.0 mg of apo A-I. The final incubation mixture had a PC:apo A-I ratio of 100:1, mol/mol, and a sodium cholate concentration around 12 mM. The mixture was incubated 12 h at 5 °C, followed by dialysis against several changes of buffer over 2.5 days to remove cholate. Several identical incubations were performed simultaneously and pooled prior to dialysis. Dialyzed preparations were fractionated on a Bio-Gel A-5m (2.9 × 73 cm) column at 4 °C. Those fractions on the trailing side of the peak having similar PC:apo A-I ratios were pooled and concentrated by dialysis against polyethylene glycol. Before use, complexes were dialyzed overnight against buffer.

Isolated complexes were characterized by a variety of chemical and physical methods. The composition of complexes was determined by the Markwell et al. (1978) assay for protein content using bovine apo A-I as a standard, and the Chen et al. (1965) procedure for phospholipid content. Cholesterol was quantitated by a colorimetric assay using a kit purchased from Boehringer Mannheim GMBH. Isolated complexes were subjected to density gradient centrifugation in a linear gradient of NaBr from 1.025 to 1.165 g/mL. Centrifugation was carried out in a Beckman SW41 Ti rotor at 39 000 rpm, 20 °C for 84 h. Intrinsic tryptophan fluorescence spectra, circular dichroism measurements, and electron micrographs of isolated complexes were obtained as described previously (Matz & Jonas, 1982). The physical and chemical properties of the complexes, stored at 5 °C, remained stable for at least 1 month. By gel filtration on a calibrated Bio-Gel A-5m column the complexes corresponded in size to complexes with molecular weights of 2.4×10^5.

Small unilamellar vesicles of egg PC and cholesterol were prepared by sonication (Huang, 1969). The PC:cholesterol molar ratio was 2:1 unless otherwise stated. A typical preparation consisted of 15.0 mg of egg PC, 3.7 mg of cholesterol, (4–6) × 10^7 cpm [^{3}H]DPPC, and (3–5) × 10^7 cpm cholesteryl [^{14}C]oleate (1% mol/mol) which were mixed in chloroform/methanol (2:1 v/v). This mixture was dried under N_2 to give a thin film and suspended in 6.0 mL of buffer. Before sonication aliquots were counted for ^{3}H radioactivity to determine working specific activities. Sonication was carried out by using a Heat Systems Ultrasonics Inc. sonifier, Model W185, at a power of 60 W, using 4-min bursts over a 45-min time period. During sonication samples were kept under a N_2

atmosphere and at a temperature not exceeding 25 °C. The vesicles were centrifuged at 18 000 rpm, 5 °C for 1 h, and the supernatant was fractionated on a Sepharose CL-4B column (2.2 × 45 cm) at 25 °C. Those fractions corresponding to SUV (Huang, 1969; Newman & Huang, 1975) were pooled and concentrated by using a Millipore CS-30 immersible ultrafiltration unit. Typically there was a 10–15% loss of cholesteryl [^{14}C]oleate in the isolated vesicles compared to [^{3}H]DPPC. The isolated vesicles were stored at 4 °C and were used within 2 weeks. PC concentrations were determined by the method of Chen et al. (1965) or from ^{3}H cpm and the specific activity.

Lipid Exchange and Kinetic Analysis. Most incubations were carried out at 37 °C, the mixtures containing 2.7 mM complex PC and vesicle PC in the range from 0.13 to 0.54 mM. To facilitate analysis, PC concentrations are given in molar terms as though they were distributed homogeneously in solution. After the appropriate incubation period, samples were placed on ice and were fractionated on Bio-Gel A-5m columns (1.6 × 52 cm) at 4 °C. Fractions were analyzed by measuring intrinsic tryptophan fluorescence (excitation 280 nm, emission 335 nm) and by double-label scintillation counting of ^{14}C- and ^{3}H-labeled lipids in 400-μL aliquots. The ^{3}H cpm:^{14}C cpm ratio was determined for those fractions under the vesicle peak which were uncontaminated by complexes, and the average ratio was determined. In virtually all cases the standard deviation of the average was less than 5%.

Kinetic data were analyzed according to the isotope exchange kinetics treatment (McKay, 1938; Frost & Pearson, 1961) applied to previous work in our laboratory (Jonas & Maine, 1979). Since cholesteryl [^{14}C]oleate is nonexchangeable in this system (data not shown), the transfer of [^{3}H]DPPC can be followed by monitoring the ^{3}H cpm:^{14}C cpm ratio of the vesicle fraction. The fraction of labeled PC transferred, F, is given by

$$F_t = 1 - [(^{3}\text{H cpm}:^{14}\text{C cpm})_t/(^{3}\text{H cpm}:^{14}\text{C cpm})_0]$$

where the isotope ratios in the vesicle peak are obtained initially (time 0) and at time t. The fraction of labeled PC transferred, F_t, can then be used in the first-order rate equation

$$-\ln (1 - F_t/F_\infty) = [R/(ab)](a + b)t$$

where F_∞ refers to the fraction of radiolabel transfer occurring at infinite time, R is the constant rate of lipid exchange, labeled and unlabeled, a is the concentration of PC in the acceptor species, b is the concentration of PC in the donor species, and t is time. R is a constant for any particular experiment but can be a function of a and b and dependent on the mechanism of exchange. Generally R can be expressed as

$$R = ka^m b^n$$

In this equation k represents the true rate constant. The only assumption made in the derivation of these equations is the absence of net transfer. The dependence of R on a and b can be determined empirically by carrying out exchange reactions in which either a or b is kept constant, i.e., making the reaction pseudo first order with respect to one of the reactants.

Results

During the egg PC-cholesterol-apo A-I complex preparation, essentially 100% of the apo A-I eluted off the preparative column in complexes, together with 70% of the egg PC and 55% of the cholesterol. However, since only the trailing edge of the complex peak was pooled, only 50% of the original apo A-I is recovered in concentrated, isolated complexes. The complexes contained 74 ± 7 mol of egg PC and 16 ± 2 mol of cholesterol for each mole of apo A-I and had an average

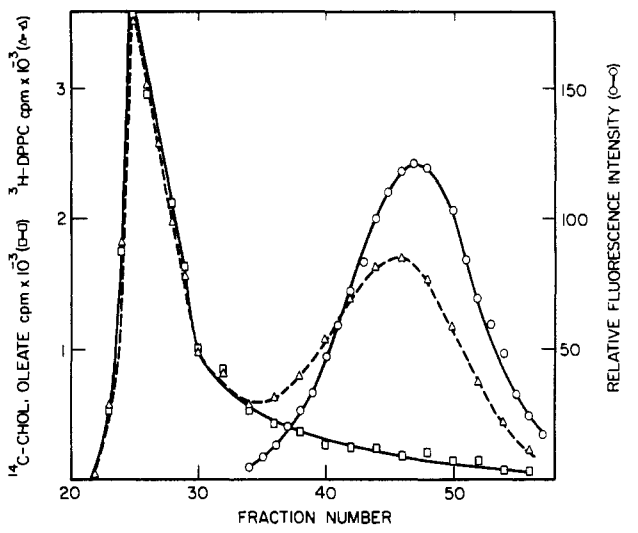

FIGURE 1: Gel filtration elution profile of a typical vesicle and complex mixture on a Bio-gel A-5m column (1.6 × 52 cm). The incubation was carried out at 37 °C for 24 h, and fractionation was performed at 4 °C. The reaction mixture contained 0.16 mM vesicle PC and 1.66 mM complex PC in 0.41 mL. Column fractions were 1.4 mL; 0.4 mL of each fraction was used for scintillation counting.

hydrated density of 1.085 g/mL. Electron micrographs showed disk-shaped particles with a diameter of 101 ± 15 Å and a width of 43 ± 3 Å.

Structural changes in apo A-I upon complex formation were followed by intrinsic fluorescence and circular dichroism measurements on the free protein and on the isolated complexes. There was a 6-nm blue shift of the maximum fluorescence wavelength of apo A-I going from the free (336 nm) to the lipid-bound state (330 nm); the percentage of α-helix content increased concomitantly from 37% to 75%.

Figure 1 shows a typical elution profile of radiolabeled vesicles incubated with complexes. Good separation between the two reactants was achieved, with only 10% of the vesicle radioactivity trailing beneath the complex peak. Contamination of the vesicle peak by complexes was never seen. The possibility of net transfer was investigated by using a larger Bio-Gel A-5m column (1.8 × 72 cm) on which base-line separation of vesicles and complexes could be achieved. Scaled up incubations of vesicles and complexes (0 and 24 h, 37 °C) were fractionated on this column, and the amount of PC in each particle was determined (data not shown). No net transfer of PC was detected. Judging by their gel filtration characteristics, complexes, as well as the SUV, remained stable over all the incubation periods, at 37 °C, used in this work.

Figure 2 shows the percent of [^{3}H]PC transferred from vesicles to complexes with time. The theoretical equilibrium value for a one-to-one exchange, based on the PC mass ratio in vesicles and complexes, is 91%; the extrapolated equilibrium value of 78 indicates the presence of a nonexchangeable pool. Previous studies (Jonas & Maine, 1979) indicated that only the PC present in the outer monolayer of the vesicle, i.e., 69% of the total PC, was exchangeable. Since in the present experiments equilibrium is not reached, even after 72 h, the extrapolated equilibrium value is subject to considerable error (±15%); therefore, an equilibrium value of 69% exchange has been assumed.

The time dependence of [^{3}H]PC transfer from complexes to vesicles is shown in Figure 3. The extrapolated equilibrium value is 105% compared with the calculated value of 91%. While the extrapolated value is somewhat high, it indicates that 100% of the complex PC is exchangeable. In all kinetic analyses we used only the exchangeable PC concentrations,

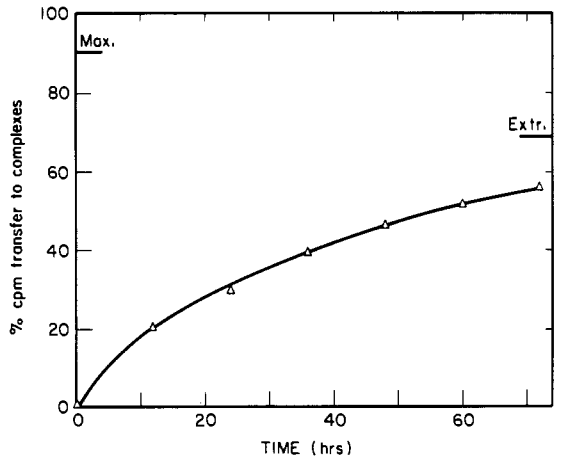

FIGURE 2: Percent of [^{3}H]PC transferred from vesicles to complexes with time. The reaction mixture of 6.4 mL contained 2.31 mM complex PC and 0.23 mM vesicle PC. Incubations were performed at 37 °C; 0.8-mL aliquots were removed at appropriate times and were fractionated as in Figure 1. The experimental curve is essentially exponential and gives an R value of 3.49 μM/h.

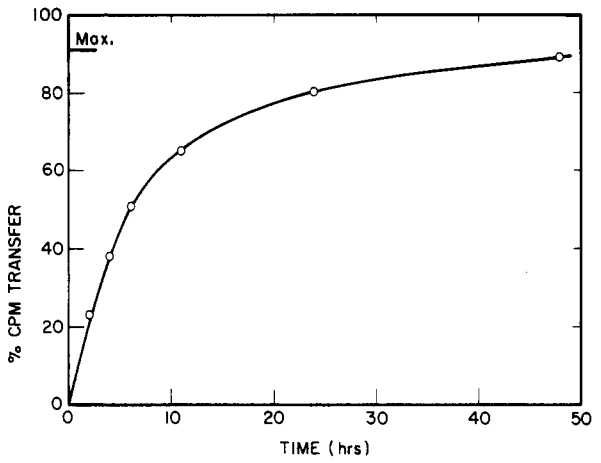

FIGURE 3: Percent of [^{3}H]PC transferred from complexes to vesicles with time. The reaction mixture of 6.1 mL contained 2.35 mM vesicle PC and 0.23 mM complex PC. Incubations were performed at 42 °C; 0.8-mL aliquots were removed at appropriate times and fractionated as in Figure 1. The experimental curve is exponential and gives an R value of 18.0 μM/h.

i.e., 69% of the vesicle and 100% of the complex PC, and assumed that [^{3}H]DPPC and egg PC molecules are equivalent in the exchange process.

Temperature and concentration dependence experiments were carried out by using 24 h incubation periods. The original data and calculated R values are shown in Table I. The linear Arrhenius plot of ln k vs. $1/T$ is not shown; the slope of the plot gave an activation energy for PC exchange of 22.9 ± 2.0 kcal/mol.

Concentration-dependence experiments were performed by varying the concentration of one particle, vesicle or complex, while the concentration of the other was held constant. At least a 5-fold excess of complex PC was present in all incubations to allow significant radiolabel transfer to occur. The results are given in Table I. Plots of R vs. concentration (not shown) are linear functions of both vesicle and complex PC concentration; i.e., the rate of exchange is first order with respect to each concentration such that $R = kab$. The calculated second-order rate constant, k, varies somewhat depending on the particular complex preparation, its mean value being 6.9 ± 0.7 μM^{-1} h^{-1}.

Experiments were performed to determine the effect of varying vesicle or complex cholesterol content on the rate of PC exchange. The original data and calculated R values are summarized in Tables II and III. Cholesterol-free vesicles and those with PC:cholesterol molar ratios of 2:1, 4:1, and 8:1 were incubated at varying PC concentrations with complexes held at a constant PC concentration. Plots of the data showed that R is a linear function of the vesicle PC concentration for

Table I: Temperature and Concentration Dependence of PC Exchange between Vesicles and Complexes

[PC] (mM)		incubation		
vesicles	complexes	temp (°C)	F_{24}[a]	R (μM/h)[b]
0.162[c]	2.12[d]	45.7	46	6.81
0.162	2.12	36.8	22	2.49
0.162	2.12	24.0	6	0.57
0.162	2.12	14.8	1	0.14
0.160	0.699	37.0	9	0.77
0.160	1.17	37.0	12	1.11
0.160	2.33	37.0	20	2.17
0.160	4.66	37.0	34	4.31
0.284	2.11	37.0	19	3.45
0.142	2.11	37.0	19	1.74
0.095	2.11	37.0	18	1.11

[a] F_{24}, the fraction of radiolabel exchange in 24 h. [b] R, the rate of exchange, calculated from F_{24} and the corresponding PC concentrations in vesicles and complexes. [c] The exchangeable vesicle PC concentration, 69% of the total PC. [d] The exchangeable complex PC concentration, equivalent to the total PC.

Table II: Concentration Dependence of PC Exchange between Vesicles of Varying Cholesterol Content and Complexes[a]

vesicle PC:cholesterol (mol/mol)	vesicle [PC] (mM)	F_{24}[b]	R (μM h^{-1})[c]	k (μM^{-1} h^{-1})[d]
2:1	0.132[e]	32	3.32	7.0
2:1	0.199	32	4.75	
2:1	0.398	30	8.43	
4:1	0.132	37	4.11	9.0
4:1	0.199	37	5.94	
4:1	0.398	36	10.8	
8:1	0.132	38	4.21	10.2
8:1	0.199	38	6.33	
8:1	0.398	38	11.8	
cholesterol free	0.199	40	6.8	11.5
cholesterol free	0.398	41	13.1	

[a] Complex PC concentration was held constant at 2.88 mM. [b] F_{24}, the fraction of radiolabel exchange in 24 h, 37 °C. [c] R, the rate of exchange calculated from F_{24} and the corresponding PC concentrations in vesicles and complexes. [d] Second-order rate constant calculated from $R = kab$. [e] The exchangeable vesicle PC concentration, 69% of the total PC.

Table III: Concentration Dependence of PC Exchange between Complexes of Varying Cholesterol Content and Vesicles[a]

complex PC:cholesterol (mol/mol)	complex [PC] (mM)	F_{24}[b]	R (μM h^{-1})[c]	k (μM^{-1} h^{-1})[d]
4.5:1	0.810[e]	13	1.33	8.3
4.5:1	1.35	20	2.36	
4.5:1	2.70	31	4.37	
8:1	1.35	24	2.91	9.3
8:1	2.70	36	5.33	
cholesterol free	0.810	20	2.17	10.8
cholesterol free	1.35	26	3.20	
cholesterol free	2.70	38	5.79	

[a] Exchangeable vesicle [PC], i.e., 69% of total PC, was held constant at 0.186 mM. [b] F_{24}, the fraction of radiolabel exchange in 24 h, 37 °C. [c] R, the rate of exchange calculated from F_{24} and the corresponding PC concentrations in vesicles and complexes. [d] Second-order rate constant calculated from $R = kab$. [e] The exchangeable complex PC concentration, i.e., equivalent to the total PC.

each of the vesicle preparations. The second-order rate constants for the various vesicle PC:cholesterol molar ratios are 7.0, 9.0, 10.2, and 11.5 μM^{-1} h^{-1} for the 2:1, 4:1, 8:1, and cholesterol-free vesicles, respectively. Similarly, cholesterol-free complexes and those with PC:cholesterol molar ratios of 4.5:1 and 10:1 were incubated at varying PC concentrations with vesicles held at a constant PC concentration. Linear plots of R vs. complex PC concentrations gave k values of 8.3, 9.3, and 10.8 μM^{-1} h^{-1} for the 4.5:1, 10:1, and cholesterol-free complexes, respectively.

Discussion

The extent of radiolabeled PC transferred with time, reported in Figures 2 and 3, indicates the complete exchangeability of complex PC and the presence of a nonexchangeable PC pool within vesicles. Previous studies (Jonas & Maine, 1979) have shown that a nonexchangeable PC pool exists in egg PC/cholesterol (2:1 mol/mol) vesicles, representing 31% of the total PC. Huang (1969) and Newman & Huang (1975) demonstrated earlier that such small unilamellar vesicles have an inside–outside distribution of PC in the range of 26:74 to 32:68; the inner vesicle monolayer constitutes the nonexchangeable pool due to the extremely slow rate of PC "flip-flop" between the inner and outer monolayers (Johnson et al., 1975; Rothman & Dawidowicz, 1975). The complete exchangeability of complex PC indicates that, in the time frame of our experiments, all PC molecules are equivalent and exchangeable.

The slope of the Arrhenius plot (not shown) gave an activation energy of 22.9 ± 2.0 kcal/mol for PC exchange. While this is almost twice the value observed previously with preequilibrated HDL and vesicles (Jonas & Maine, 1979), it is identical with the activation energy reported for exchange of fluorescently labeled PCs between apolipoprotein–phospholipid complexes and vesicles (Massey et al., 1982a).

Following the original observations of lipid exchanges between erythrocytes and plasma, several decades ago, two mechanisms have been proposed for the spontaneous exchange of lipid: (1) monomeric lipid diffusion between donor and acceptor particles through the aqueous phase (Hagerman & Gould, 1951) or (2) lipid exchange during the formation of a transient, collisional complex between the donor and acceptor particles (Gurd, 1960). Frequently (Lawaczeck, 1978; Roseman & Thompson, 1980; McLean & Phillips, 1981; Massey et al., 1982a,b; De Cuyer, 1983) the dependence or independence of lipid exchange rate on acceptor concentration has been used to support one or the other mechanism. Exchange by aqueous diffusion would depend on the aqueous solubility of the lipid and would be independent of acceptor concentration, assuming rapid diffusion and uptake by the acceptor particles. A collisional mechanism would, by definition, depend on both donor and acceptor particle concentrations and would give exchange rates compatible with second-order kinetics. In the present work, exchange rates of PC between lipid–apolipoprotein complexes and vesicles vary linearly with both donor (vesicle) and acceptor (complex) concentrations (see Tables II and III). Although the second-order kinetic behavior might suggest a collisional mechanism of exchange, the evidence for the movement of lipid molecules through solution is solid (Roseman & Thompson, 1980; McLean & Phillips, 1981; Massey et al., 1982a,b). In fact two new kinetic models have been recently presented (Nichols & Pagano, 1981; Bojesen, 1982), which show that the independence of transfer rates on the acceptor concentration is not a necessary condition for a monomer diffusion mechanism. These two models take into account the on and off rates of monomer lipid with respect to both the donor and acceptor particles; thus, exchange rates depend not only on donor concentrations but also, under appropriate conditions, on acceptor concentrations. Physical and compositional differences between various acceptors and donors would be expected to affect the exchange rates of lipid due to the differences in monomer lipid "affinities" for the particles.

Table IV: Variable Donor and Acceptor Concentrations with a Constant Concentration Ratio

[PC] (mM)[a]				
vesicles	complexes	F_{24}[b]	R (μM/h)	ϵ[c]
0.740	0.055	21.2	0.51	9.9
2.22	0.167	37.2	3.02	19.4
5.55	0.418	63.6	16.4	42.2

[a] The PC ratio of complexes:vesicles was constant at 9.2. [b] F_{24}, the fraction of radiolabel exchange in 24 h. [c] The time constant, ϵ, calculated according to Bojesen (1982).

We attempted to fit our data to these kinetic models, without success. For example, according to Bojesen's (1982) model a series of samples having different donor and acceptor concentrations, but identical concentration ratios, should give identical time constants (ϵ) for exchange. We performed an experiment (Table IV) where we used various dilutions of a donor/acceptor mixture; each gave a different ϵ value. According to Nichols & Pagano (1981), their monomer diffusion model is consistent with linear plots of inverse initial transfer rates vs. inverse donor or acceptor concentrations which have y-axis intercepts greater than zero. Furthermore, these authors state that a simple collisional mechanism would give a y intercept of zero. Interestingly the results we show in Table I and Table IV agree with such a collisional mechanism. It appears then that a more complex kinetic model is necessary to account for all of the lipid exchange data. Not only the on and off rates of the lipid but also the rates of lipid monomer diffusion and its collisional frequencies with particles may be involved (personal communications with M. C. Phillips and T. Steck).

In this study the rate constant for PC exchange varied inversely with the cholesterol content of the vesicles, decreasing 60% as cholesterol was varied from 0 to 33 mol %. Similar effects on PC exchange and transfer rates have been seen both in vitro (Allen, 1981; Damen et al., 1981) and in vivo (Tall, 1980) by using vesicles and HDL as donors and acceptors, respectively. With increasing cholesterol content, from 0 to 33 mol %, vesicle size also increases, from 210 to 250 Å in diameter (Newman & Huang, 1975); therefore, vesicle size and curvature could be contributing factors to the rate effects of vesicle cholesterol content. In vivo results show the inhibition of PC transfer and vesicle breakdown are due to cholesterol content alone, independent of vesicle size (Tall, 1980). We investigated vesicle size effects by incubation of large unilamellar vesicles of egg PC (Enoch & Strittmatter, 1979), 1100–1300 Å in diameter, with lipid–apolipoprotein complexes (results not shown). Exchange rates were extremely slow, approximately 30% of those obtained with the SUVs, 200 Å in diameter, containing egg PC alone. Changes in the exchange rates can, therefore, be primarily attributed to the cholesterol content and the cholesterol–PC interactions in the bilayer, with the possibility of minor effects due to changes in vesicle size and curvature.

We also showed that PC exchange rates vary with the composition of the acceptor particles. The rate constant for exchange varied inversely with the cholesterol content of the lipid–apolipoprotein complexes, decreasing 20% as cholesterol

was varied from 0 to 20 mol %. Comparison with previous experiments utilizing preequilibrated HDL as acceptors (Jonas & Maine, 1979) shows a 22-fold decrease in the rate constant for complexes vs. HDL with the same donor vesicles. This provides additional evidence for the importance of acceptor composition and possibly morphology in exchange processes.

In summary, under our experimental conditions, PC exchange between vesicles and lipid–apolipoprotein complexes follows second-order kinetics. A monomer exchange model, kinetically based only on lipid solubility, would predict absolute independence of exchange rates on acceptor concentration and properties. Clearly, our data show rate and activation energy dependence on both acceptor concentration and composition. The broader kinetic treatments of monomer exchange which take into account interactions of the monomer with both donor and acceptor particles, predict rate dependence on acceptor concentration and composition. However, these kinetic models do not account for our results as well as the simple radioisotope exchange approach with $R = kab$, implying that diffusion and collision effects may be important under certain conditions.

Registry No. Dipalmitoylphosphatidylcholine, 2644-64-6.

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