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Kinetics of Transfer of Pyrene and *rac*-1-Oleyl-2-[4-(3-pyrenyl)butanoyl]glycerol between Human Plasma Lipoproteins†

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ABSTRACT: Control of lipid transfer between plasma lipoproteins has been studied with pyrene and a pyrene-containing diglyceride analogue, *rac*-1-oleyl-2-[4-(3-pyrenyl)butanoyl]-glycerol. The rate constants for transfer of the fluorescent probes between high-density lipoproteins in dilute solutions containing ions of the Hofmeister series are correlated with the molal surface tension increments produced by this lyotropic series. Moreover, the rates of transfer correlate well with the aqueous solubilities of the hydrophobic compounds, which were increased in dilute solutions containing organic solvents. The rates of transfer are faster with an increase in the distribution coefficients of the probes between the lipoprotein donor and the aqueous phase, suggesting that the rate-determining process can be considered simply as a partitioning between two immiscible solvents. Rate constants for transfer of pyrene between high-density, low-density, and very low density lipoproteins depend on the ratio of the donor/acceptor lipoprotein concentrations and agree with those predicted from the distribution coefficients. In addition, the rate of transfer is inversely related to the lipoprotein radius, with a linear correlation observed between the log of the rate constant and 1/radius. By analogy with the evaporation of liquid droplets described by Kelvin's law, the transfer of a lipid from a lipoprotein or a membrane surface can be described as $k_d \alpha e^{-K\pi/r}$ where K is a constant, π is the interfacial surface pressure of a curved surface of a radius r, and k_d is the dissociation constant. These results show that the rate of transfer of lipids can be modified by changes in the solubility of the transferring species in the aqueous phase and imply that other factors, such as solubility of the transferring species in the membrane and the properties of the lipid–protein surface, may have important roles in regulating the kinetics of lipid transfer in biological systems.

Plasma lipoproteins function as circulating reservoirs for a wide variety of hydrophobic substances that have extremely low solubility in aqueous solutions (Smith et al., 1978; Scanu et al., 1979). These compounds include triglyceride, cholesterol, cholesteryl ester, and phosphatidylcholine as major components and minor amounts of lipid-soluble vitamins and environmentally derived contaminants.

The relatively constant compositions of the circulating lipoproteins result from five simultaneous processes: (1) synthesis and secretion, (2) passive transfer and exchange of lipid and apoprotein components, (3) active transfer involving specific plasma exchange proteins, (4) enzymatic changes in composition involving lecithin–cholesterol acyltransferase and lipoprotein lipase, and (5) removal from circulation by cellular uptake of lipid and apoprotein components. The dynamics and the contribution of each process to overall lipoprotein metabolism are poorly defined.

All lipoproteins contain proteins, cholesterol, and phospholipids in a surface film that surrounds a neutral lipid core of cholesteryl ester and triglyceride (Shen et al., 1977). Lipoprotein surfaces have the dynamic properties of a two-dimensional fluid, in which there are rapid noncovalent interactions between individual lipid and protein components, involving both rotational motion and translational diffusion in the surface (Lee, 1975; Thompson & Huang, 1978). Because the lipid components of lipoproteins are soluble in the aqueous solution to some extent, they also partition between the lipoprotein surface and the aqueous solution (Charlton et al., 1976, 1978a). Since some lipoprotein components transfer on time scales (milliseconds to hours) that are much shorter than the

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circulating lifetimes (3–5 days) of the most abundant lipoproteins (Havel et al., 1980), this spontaneous physical process is important in lipoprotein metabolism and requires further definition.

The transfer mechanism involves a rate-limiting dissociation of the lipophilic material into the aqueous phase, followed by rapid diffusion through the solution and uptake by an acceptor lipoprotein. This conclusion is based on our previous studies of the transfer of a fluorescent diglyceride analogue and of pyrene between high-density lipoproteins (HDL)[1] (Charlton et al., 1976, 1978a) and corroborated by studies of phospholipid transfer involving light-scattering techniques (Martin & McDonald, 1976; Duckwitz-Peterlein et al., 1977; Duckwitz-Peterlein & Moraal, 1978). Data from studies with the cholesterol analogue 3-pyrenylmethyl 3β-hydroxy-23,24-dinor-5-cholen-22-oate (Kao et al., 1977), 10-(3-pyrenyl)decanoic acid (Sengupta et al., 1976), 9-(3-pyrenyl)nonanoic acid (Doody et al., 1980), and phosphatidylcholine containing either the 2-[10-(3-pyrenyl)decanoyl] or 2-[9-(3-pyrenyl)nonanoyl] moiety (Galla et al., 1979; Roseman & Thompson, 1980; Massey et al., 1980) also support this mechanism of transfer. Similar studies have employed excitation energy transfer (Kano et al., 1981; Almgren, 1980) and reached the same conclusions regarding the mechanism of transfer.

Dilute solutions of pyrene emit from the lowest excited singlet state with one of its fluorescence maxima at 390 nm, with a relative intensity, I_M. At higher pyrene concentrations, an *excited dimer* (excimer) is produced by the transient association of an excited singlet state molecular with a ground-state pyrene molecule. At higher concentrations pyrene excimer emission with relative intensity, I_E at 470 nm, increases at the expense of the monomer emission, I_M (Birks, 1970). Since the ratio of these intensities, I_E/I_M, is directly proportional to the pyrene concentration (Pownall & Smith, 1973), I_E/I_M decreases when the probe transfers from a donor to an acceptor lipoprotein. The concentration-dependent fluorescence properties of pyrene and its derivatives allow quantification of the changes in mass as the result of transfer between lipoproteins and obviate the need to separate physically donor from acceptor particles. In this paper, we identify some of the factors that control the rate of transfer of these hydrophobic compounds between lipoproteins.

Materials and Methods

HDL, LDL, and VLDL were prepared from fresh, unfrozen human plasma from individual donors by ultracentrifugal flotation. The solution density was adjusted to 1.21 g mL^{-1} with solid KBr and centrifuged at 14 °C for 44 h at 45 000 rpm in a 60 Ti rotor (Havel et al., 1955). The lipoproteins were isolated by tube slicing and dialyzed against 0.15 M NaCl containing 0.02 M Tris-HCl, pH 7.4, 1 mM EDTA, and 1 mM NaN_3 before chromatography on a 6 × 60 cm column of Bio-Gel A-5M in the same buffer (Rudel et al., 1974). $VLDL_1$, $VLDL_2$, and $VLDL_3$ were prepared by cumulative flotation in a discontinuous salt gradient (Lindgren et al., 1972). Lipoprotein concentrations were based on lipoprotein protein content (Morrisett et al., 1975) as determined by the method of Lowry et al. (1951), modified by the inclusion of 0.1% (w/v) sodium dodecyl sulfate in all reagents. The sizes of these macromolecules, when required, were determined by dynamic laser light scattering (Morrisett et al., 1974). A triglyceride-rich dispersion (TRD), a model of VLDL, was prepared as described (Charlton et al., 1978b). *rac*-1-Oleyl-2-[4-(3-pyrenyl)butanoyl]glycerol (DG), a pyrene-containing analogue of a diglyceride, was prepared and purified as previously described (Charlton et al., 1978a). Lipoprotein solutions containing pyrene and DG were prepared by injection of an ethanolic solution of the fluorophore directly into the lipoprotein solution. A molar extinction coefficient of 50 000 M^{-1} cm^{-1} at 341 nm was used for pyrene and DG (Pownall & Smith, 1973). Organic solvents were reagent grade. All experiments were conducted in the standard buffer, which contained 50 mM Tris-HCl, pH 7.4, and 0.15 M NaCl, unless otherwise noted.

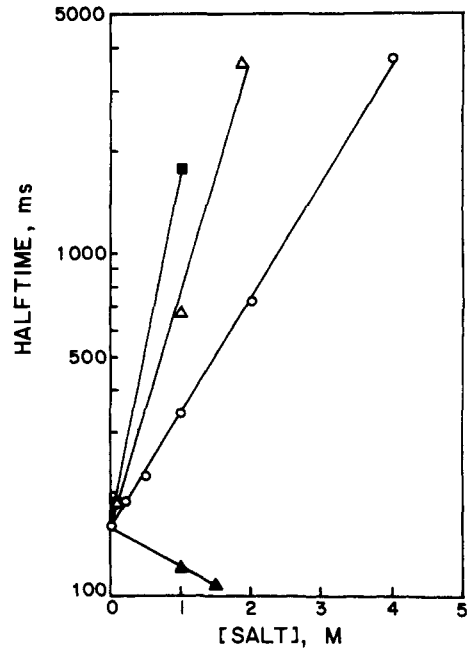

FIGURE 1: Effect of salt concentration on the half-time for transfer of DG between HDL. HDL, 0.27 mg mL^{-1}, containing 1% (w/w) DG in the indicated salt solution were mixed with a similar solution of HDL without fluorophore as described under Materials and Methods. (■) Na_2SO_4; (△) $(NH_4)_2SO_4$; (○) NaCl; (▲) NaSCN.

Fluorescence emission spectra were recorded on a Farrand Mark I spectrofluorometer with excitation at 328 nm. Calibrated gain controls were used to expand the spectrum in the excimer region. Kinetic measurements were obtained on a Durrum–Gibson stopped-flow spectrometer operated in the fluorescence mode. The sample was excited by radiation from a 150-W xenon arc lamp (331 nm, 1-mm slits). Monomer fluorescence was isolated with a 390-nm mirrored interference filter (9.85-nm half-bandwidth) and excimer fluorescence with a Corning 3-72 filter that transmitted wavelengths longer than 440 nm. Kinetic data were stored in a Biomation Model 805 wave form recorder. The output was passed through a log amplifier to give an analogue semilog plot from which reaction rates were calculated. Rate constants were obtained from the decrease in excimer fluorescence, except as indicated.

Results

Effects of Electrolyte Concentration on the Rate of DG Transfer. High concentrations of NaCl reduced markedly the rate of transfer of DG between HDL. At a final concentration of 4 M NaCl, the half-time for transfer was about 20 times

[1] Abbreviations: HDL, high-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low density lipoproteins; $VLDL_1$, $VLDL_2$, and $VLDL_3$, subclasses of VLDL with flotation values of S_f 100–400, S_f 60–100, and S_f 20–60, respectively (Lindgren et al., 1972); DG, diglyceride ether, *rac*-1-oleyl-2-[4-(3-pyrenyl)butanoyl]glycerol; TRD, triglyceride-rich dispersion, a protein-free, lipid model of VLDL; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; DMF, dimethylformamide.

longer than that observed in the absence of salt (Figure 1).

At 1 M concentrations, Na_2SO_4 was more effective than NaCl; the rates of transfer were 11- and 2-fold slower, respectively, than that observed in standard buffer containing 0.15 M NaCl. Increasing concentrations of NaSCN had the opposite effect; the rate of transfer was increased about 25%. The salting-in–salting-out effects of various electrolytes and their relative effectiveness in changing the solubility of nonpolar compounds in aqueous solution have been summarized (Long & McDevit, 1952; Aquan-Yuan et al., 1979, and references therein). In general, the experimental data fit the empirical Setschenow equation

$$\log (\gamma/\gamma_0) = \log (S_0/S) = K_s c_s \quad (1)$$

where γ_0 and γ are the activity coefficients of the solutes in water and electrolyte solution, respectively, S_0 and S are the mole fraction solubilities of the solutes in water and electrolyte solution, respectively, K_s is the salting-out coefficient, and c_s is the molar concentration of the electrolyte solution. Therefore, if the rate depends on the solubility of the transferring species in the solution, a plot of the logarithm of the transfer rate of DG should be a linear function of the salt concentration. With the salts tested, such a relationship was found (Figure 1).

The effects of neutral salts on hydrophobic interactions in salting-out phenomena can be related quantitatively to the nonpolar contact area of the interacting species and the molal surface tension increment produced by the salt (Paul, 1952; Melander & Horvath, 1977). Neutral salts increase the surface tensions of aqueous solutions γ in a linear fashion:

$$\gamma = \gamma^0 + \sigma m \quad (2)$$

where γ^0 is the surface tension of pure water, m is the molality of the salt, and σ is a constant, the molal surface tension increment. It can be shown that there is a linear relationship between the ln (S/S_0) and σ (Melander & Horvath, 1977). Similar treatment of the rates of transfer of DG between HDL in the presence of these lyotrophic salts illustrated that a linear relationship existed (data not shown).

Effect of Organic Solvents on the Rate of DG Transfer. When labeled HDL were mixed with unlabeled HDL in solutions that contained small amounts of organic solvents, the transfer rates were faster than those observed in the absence of solvent. For example, ethanol (10% v/v) increased the rate of DG transfer 3-fold; in DMF (10% v/v), the rate was 8-fold greater than that found in buffer alone.

Distribution Coefficients of DG and Pyrene between HDL and Aqueous Solution. If the lipid region of a lipoprotein is considered to be a water-immiscible phase, the solubilities of the probes may be expressed in terms of a distributiion coefficient between these two phases, the lipoprotein and the aqueous solution, by the relationship

$$f = [P]_{aq}^{\infty}/[P]_{LP}^{\infty} \quad (3)$$

where f is the distribution coefficient, $[P]_{aq}^{\infty}$ is the equilibrium concentration of the probe in water, and $[P]_{LP}^{\infty}$ is the equilibrium concentration of the probe in the lipoprotein. When saturating amounts of the partitioning compound are present, the distribution coefficient, f, is the ratio of the limiting solubilities of the compound in the two phases (Karger et al., 1973).

The distribution coefficients of the fluorescent probes can be measured directly by sequential dilution of the labeled lipoprotein. A lipoprotein solution is chosen with a lipoprotein concentration sufficiently high that the fluorescent probe is associated almost entirely with the lipoprotein and the fluorescence of the probe in the aqueous solution is negligible. The ratio of excimer to monomer fluorescence measured for the initial solution is designated $(I_E/I_M)^0$. The lipoprotein solution is then diluted sequentially until $(I_E/I_M)^0/(I_E/I_M)^n$ is approximately 2, where n is the number of dilutions. Since the total amount of probe P_t present is constant, then

$$P_t = [P]_{LP} v_{LP} + [P]_{aq}(v_t - v_{LP}) \quad (4)$$

The volume of lipoprotein per milliliter of solution, v_{LP}, is $[LP]/\rho \times 10^{-3}$ L/L, where [LP] is the lipoprotein concentration in milligrams per milliliter and ρ is its density in grams per milliliter (Lindgren et al., 1972). Therefore

$$P_t = [([LP]/\rho \times 10^{-3})[P]_{LP} + [P]_{aq}]v_{aq} \quad (5)$$

where v_{aq} is the volume of solution. At a much higher concentration, $[LP]^0$, the amount of probe in the aqueous compartment can be ignored, so that

$$P_t = [([LP]/\rho \times 10^{-3})[P]_{LP} + [P]_{LP} f]v_{aq} \quad (6)$$

Prior to dilution, the amount of the probe in the aqueous solution is negligible so that

$$P_t = P_t^0 \simeq ([LP]^0/\rho \times 10^{-3})[P]_{LP}^0 v_t^0 \quad (7)$$

Dividing eq 6 by eq 7, rearranging, and remembering that, for a fixed mass of lipoprotein, the product of $[LP]v_{aq}$ is constant gives

$$[P]_{LP}^0/[P]_{LP}^n = 1 + (\rho f + 10^3)/[LP] \quad (8)$$

Since I_E/I_M is a measure of probe concentration in the lipoprotein, then

$$(I_E/I_M)^0/(I_E/I_M)^n = 1 + \rho f/[LP] \times 10^3 \quad (9)$$

ignoring the monomer fluorescence of the aqueous probe.

An example of this analysis is shown in Figure 2. On the basis of eq 9, a plot of $(I_E/I_M)^0/(I_E/I_M)^n$ against 1/[lipoprotein] gives a line, from the slope of which f can be obtained. Typical values for DG distribution coefficients between HDL and buffer, HDL and ethanol (10% v/v), and HDL and dimethylformamide (10% v/v) were 0.88×10^{-6}, 280×10^{-6}, and 875×10^{-6}, respectively. The plot of the distribution

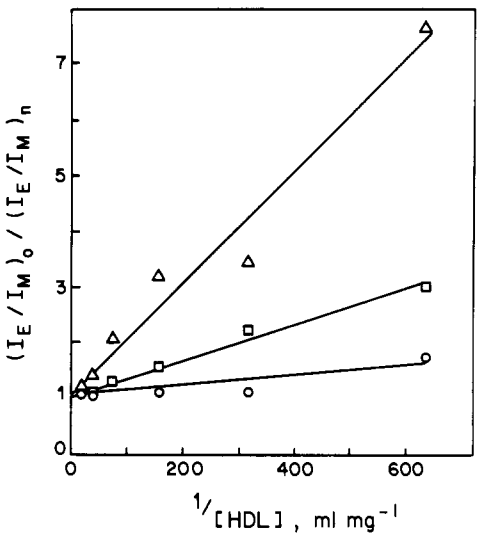

FIGURE 2: Effect of dilution on the I_E/I_M ratio of DG in HDL. Spectra of HDL, 0.1 mg (mL of protein)$^{-1}$, containing 3% (w/w) DG were recorded after sequential dilution of the initial solution with either buffer (○) or buffer containing the appropriate amount of the indicated organic solvent: (□) ethanol (10% v/v); (△) dimethylformamide (10% v/v). The distribution coefficients were calculated from the slope of $(I_E/I_M)^0/(I_E/E_M)^n$ plotted against the HDL dilution.

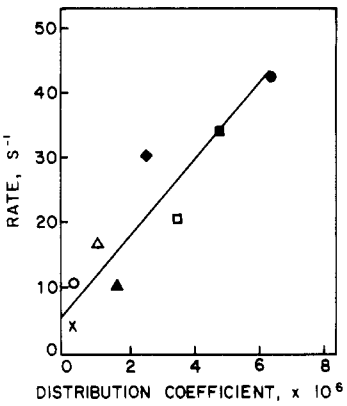

FIGURE 3: Dependence of DG transfer rate on partition coefficient. Partition coefficients in the indicated solvents were obtained as described in Figure 2. (×) Buffer; (○) dimethyl sulfoxide (10% v/v); (△) ethanol (10% v/v); (▲) 0.5 M tetramethylurea; (□) tetrahydrofuran (10% v/v); (♦) dioxane (10% v/v); (■) dimethylformamide (10% v/v); (●) 1 M tetramethylurea.

Table I: Thermodynamic Quantities for Transfer of Pyrene and DG within a Lipoprotein Class[a]

system	probe	rate constant (s^{-1})	E_a	$\Delta G^{\ddagger}$ (kcal mol^{-1})	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$\Delta S^{\ddagger}$ (eu)
HDL + HDL	DG	4.0	12.2	16.3	11.6	−15.9
HDL + HDL	DG	3.4	11.6	16.4	11.0	−18.3
VLDL + VLDL	DG	0.13	10.1	18.3	9.5	−29.9
HDL + HDL, 10% ethanol	DG	8.4	10.7	15.9	10.1	−19.6
HDL_3 + HDL_3	pyrene	281.0	8.3	13.8	7.7	−20.9
HDL + HDL	pyrene	250.0		13.9		
LDL + LDL	pyrene	66.5		14.7		
VLDL + VLDL	pyrene	12.6	10.6	15.6	10.0	−19.1
VLDL + VLDL	pyrene	14.8		15.5		
$VLDL_1$ + $VLDL_1$	pyrene	10.8		15.7		
$VLDL_2$ + $VLDL_2$	pyrene	15.7		15.5		
$VLDL_3$ + $VLDL_3$	pyrene	21.4		15.3		

[a] Duplicate entries are from separate lipoprotein preparations from different individuals. From the measured rate constants over a temperature range of 25 °C, values for the activation energies, E_a, were obtained. $\Delta G^{\ddagger}$ of the activated state was calculated from eq 25 where $kT/h = 6.11 \times 10^{12}$ at 20 °C. The enthalpy of activation $\Delta H^{\ddagger}$ was calculated from $\Delta H^{\ddagger} = E_a - RT$; entropy of activation $\Delta S^{\ddagger}$ was calculated from $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$.

coefficients as a function of the observed rate of DG transfer between HDL in these solvents showed a clear relationship (Figure 3). The rate of transfer increased as the partition coefficients became larger. Similar experiments were attempted with pyrene as the probe. No reliable data were obtained, since the rate of transfer in the presence of some of the salts and the organic solvents exceeded the resolution limit of the stopped-flow instrument.

Transfer of Pyrene and DG within a Lipoprotein Class (Homogeneous Transfer). Rates of transfer of pyrene and DG have been summarized for HDL, LDL, VLDL, $VLDL_1$, $VLDL_2$, and $VLDL_3$ in Table I. The reaction rates differed considerably, ranging from 280 s^{-1} for transfer of pyrene between HDL to 10.8 s^{-1} between $VLDL_1$.

Transfer of Pyrene between Dissimilar Lipoproteins (Heterogeneous Transfer). The amount of information that could be obtained was limited when the donor and acceptor lipoproteins were the same, because transfer rates were independent of either the lipoprotein concentration or the probe concentration. Consequently, transfer between donor and acceptor lipoproteins of different density classes was investigated. Pyrene-labeled LDL, HDL, and VLDL were used as donor lipoproteins, with HDL, LDL, and HDL, respectively, as unlabeled acceptors. The rates of transfer as a function of donor and acceptor lipoprotein concentrations and probe concentrations are shown in Tables II and III.

Table II: Distribution Coefficients of Pyrene between Water and Lipoproteins

lipoprotein	distribution coefficient	dissociation constant[a] (s^{-1})	association constant[b] (s^{-1})
HDL	12.2×10^{-6}	250	21×10^6
LDL	10.6×10^{-6}	66.5	6.3×10^6
VLDL	10.8×10^{-6}	14.8	1.4×10^6

[a] From Table I. [b] Pseudo first order. Association requires the collision of a probe with a lipoprotein particle and is a second-order reaction (Almgren, 1980). However, since the lipoprotein is normally present in excess, the pseudo-first-order rate constant, k_a, is used.

The kinetic behavior of the probes in heterogeneous systems was different from that found in homogeneous systems. The observed rates of probe transfer in the heterogeneous systems had intermediate values, compared to those found for transfer between homogeneous lipoproteins, and differed by 20–30% over a 10-fold range of lipoprotein concentrations. There were no changes in transfer rates with different pyrene concentrations in the donor lipoproteins. However, the rate for transfer between HDL and LDL measured by the increase in excimer fluorescence was often faster than that determined by the decrease in monomer fluorescence. While the I_E/I_M ratio was a linear function of probe concentration under all conditions in a particular lipoprotein, differences in lipid and protein compositions, as well as in organization of the nonpolar region of the lipoproteins (Soutar et al., 1974), precluded quantitative comparison of the changes in relative intensities of the excimer and monomer fluorescence in different lipoproteins (Tachiya & Almegren, 1981).

Reaction rates were predicted from the following kinetic scheme for transfer of a probe molecule. This model assumes a simple two phase system in which the lipoprotein is considered to be an immiscible organic phase in a continuous aqueous phase:

$$P_1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} P_{aq} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} P_2 \qquad (10)$$

where P_1 is the donor lipoprotein with a concentration of milliliters of L_1 per milliliter of solvent, P_2 is the acceptor lipoprotein with a concentration of milliliters of L_2 per milliliter of solvent, and P_{aq} is the probe in the aqueous solution.

The kinetic equations are

$$dP_1/dt = -k_1P_1 + k_2P_{aq} \qquad (11)$$

$$dP_2/dt = -k_4P_2 + k_3P_{aq} \qquad (12)$$

$$dP_{aq}/dt = k_1P_1 + k_4P_2 - (k_2 + k_3)P_{aq} \qquad (13)$$

The equations had previously (Frost & Pearson, 1961a,b) been considered to be independent. They are, in fact, linked by conservation of mass:

$$P_1L_1V + P_2L_2V + P_{aq}V = P_t \qquad (14)$$

where P_t is the total mass of the probe and V is the volume of solution.

Rearranging and substituting for P_{aq} in eq 11 and 12 give

$$dP_1/dt = -[(k_1 + k_2L_1)P_1 + k_2L_2P_2] + k_2P_t/V \qquad (15)$$

$$dP_2/dt = -[(k_3L_1P_1 + k_4 + k_3L_2)P_2] + k_3P_t/V \qquad (16)$$

Table III: Pyrene Transfer between HDL and LDL as a Function of Lipoprotein and Probe Concentrations[a]

donor lipoprotein	protein (mg mL^{-1})	pyrene (wt %)	acceptor lipoprotein	protein (mg mL^{-1})	wavelength (nm)	obsd rate constant (s^{-1})	calcd rate constant (s^{-1})
HDL	2.87	1	LDL	1.2	470	155	113
HDL	2.87	1	LDL	0.12	470	149	73
HDL	0.29	1	LDL	1.2	470	183	203
HDL	0.29	1	LDL	0.12	470	151	111
HDL	0.29	1	LDL	0.012	470	159	72
HDL	0.029	1	LDL	0.12	470	180	202
HDL	0.029	1	LDL	0.012	470	164	109
HDL	0.29	3	LDL	0.12	470	133	
					390	250	112
					470/390	144	
HDL	0.29	1	LDL	0.12	470	150	112
HDL	0.29	0.3	LDL	0.12	470	140	112
					390	250	
LDL	1.2	1.8	HDL	2.87	470	133	
					390	76	
					470/390	112	113
LDL	1.2	1.8	HDL	0.29	470	226	203
LDL	0.12	1.8	HDL	0.29	470	111	112
					390	79	
LDL	0.12	1.8	HDL	0.029	470	180	202
LDL	0.012	1.8	HDL	0.029	470	90	109
					390	78	
LDL	0.12	5	HDL	0.29	470	113	112
					390	85	
LDL	0.12	1.8	HDL	0.29	470	111	112
					390	79	
LDL	0.12	0.5	HDL	0.29	470	123	112
LDL			LDL			66.5	

[a] Experimental procedures are described under Materials and Methods.

Boundary conditions are these. At zero time, all of the probe is in the donor lipoproteins, so that

$$P_1^0 = \frac{k_2 P_t}{V(k_1 + k_2 L_1)} \quad (17)$$

and

$$P_2^0 = 0 \quad (18)$$

At infinite time, the equlibrium distribution prevails with concentrations

$$P_1^\infty = \frac{k_2 k_4 P_t}{V(k_1 k_4 + k_2 k_4 L_1 + k_1 k_3 L_2)} \quad (19)$$

and

$$P_2^\infty = \frac{k_1 k_3 P_t}{V(k_1 k_4 + k_2 k_4 L_1 + k_1 k_3 L_2)} \quad (20)$$

The particular solution for these conditions is

$$P_1 = \frac{k_2 P_t}{V(k_1 k_4 + k_2 k_4 L_1 + k_1 k_3 L_2)}\left[k_4 + \frac{k_1 k_3 L_2}{(k_1 + k_2 L_1)(\lambda_p - \lambda_q)}(\lambda_p e^{-\lambda_q t} - \lambda_q e^{-\lambda_p t})\right] \quad (21)$$

$$P_2 = \frac{k_1 k_3 P_t}{V(k_1 k_4 + k_2 k_4 L_1 + k_1 k_3 L_2)}\{1 + [\lambda_p(\lambda_q - k_1 - k_2 L_1)e^{-\lambda_q t} - \lambda_q(\lambda_p - k_1 - k_2 L_1)e^{-\lambda_p t}]\} \quad (22)$$

where the rate constant

$$\lambda_p = \{(k_1 + k_4 + kL_2 + k_3 L_2) - [(k_1 + k_4 + k_2 L_1 + k_3 L_2)^2 - 4(k_1 k_4 + k_1 k_3 L_2 + k_2 k_4 L_1)]^{1/2}\}/2 \quad (23)$$

and the rate constant

$$\lambda_q = \{(k_1 + k_4 + k_2 L_1 + k_3 L_2) + [(k_1 + k_4 + k_2 L_1 + k_3 L_2)^2 - L_1(k_1 k_4 + k_1 k_3 L_2 + k_2 K_4 L_1)]^{1/2}\}/2 \quad (24)$$

Two points merit discussion. First, the time course is predicted to be biexponential with rate constants of λ_p and λ_q. Moreover, the ratio of the amplitudes of these two exponentials is inversely related to their magnitude. Substitution of typical values of k_1, k_2, k_3, and k_4, for transfer within an HDL population, shows λ_p to be 250 s^{-1} and λ_q 0.13 × 10^6 s^{-1}. The amplitude of λ_p, the more rapid component, is calculated from $250/0.13 \times 10^6$ and is 0.02 of that of λ_q, the slower component. For practical purposes then, the time course is predicted to be monoexponential.

Second, for the special case of homogeneous transfer, where $k_1 = k_4$ and $k_2 = k_3$, λ_p reduces to k_1 and λ_q to $k_1 + k_2(L_1 + L_2)$. The sum of λ_q is that observed in relaxation experiments and probably corresponds to reestablishment of equilibrium of the probe between water and the lipoprotein. If this is indeed the case, then λ_p is the rate constant corresponding to transfer of the probe between lipoproteins, and λ_q describes reestablishment of equilibrium with the solvent. For the homogeneous transfer situation, the observed rate is that of dissociation of the probe molecule from the donor lipoprotein.

It is possible to predict the observed rate with values for the rate constants k_1, k_2, k_3, and k_4. The dissociation rate constants k_1 and k_4 are directly measurable from homogeneous transfer measurements. Association rate constants k_2 and k_3 are available indirectly from distribution coefficient measurements via $k_1/k_2 = f$ and $k_2 = f/k_1$. The measured distribution coefficients of pyrene between water and HDL, LDL, and VLDL are found in Table II.

By use of these measured and derived rate constants, the following properties of the observed rate are predicted: (1) the value should be between the two dissociation constants, k_1 and k_4; (2) the observed rate depends only on the ratio of donor/acceptor lipoprotein concentrations; (3) it is unchanged if the roles of donor and acceptor lipoproteins are reversed and

Table IV: Pyrene Transfer from VLDL to HDL

VLDL[a] protein (mg mL^{-1})	VLDL[a] pyrene (wt %)	HDL[a] protein (mg mL^{-1})	wavelength (nm)	obsd rate constant (s^{-1})	calcd rate constant[a] (s^{-1})
0.3	0.3	2.87	470	18.9	32.2
0.3	0.3	0.72	470	20.6	71.1
0.3	0.3	0.29	470	32.4	117
0.22	0.3	2.87	470	19.2	27.8
0.22	0.3	0.72	470	16.8	59
			390	18.1	
0.11	0.3	0.29	470	18.5	67
			390	17.1	
0.03	0.3	2.87	470	17.5	16.7
			390	10.6	
0.03	0.3	0.72	470	15.6	22.1
			390	10.7	
0.17[b]	0.024	0.25[b]	470/390	40.8	37.2

[a] The transfer rates between VLDL were 15 s^{-1} and between HDL, 240 s^{-1}. [b] Transfer rates for these lipoprotein preparations were 20.6 s^{-1} for VLDL and 269 s^{-1} for HDL.

the concentrations remain the same; and (4) it is independent of the probe concentration.

Examination of Table III, which contains data for pyrene transfer between HDL and LDL, shows that agreement of calculated rates with experimental data is very good and that all of the predictions are accurate. In Table IV, however, pyrene transfers from VLDL to HDL do not show such good agreement. The trends in the data are correctly predicted, that is, the rate increases with increasing donor VLDL/acceptor HDL, but the rates are overestimated by a factor of 2–3. The calculated rates can be made to agree very well with these determined experimentally if the association rate constant with VLDL is decreased 5-fold. We have no explanation for this discrepancy.

One interesting feature is that the model involves simple partitioning of a soluble phase between two immiscible phases by first-order processes. In all equations, however, k_2 always appears as k_2L_1 and k_3 as k_3L_2, exactly as if the uptake occurred by a second-order reaction.

By analogy with dialysis, the rate of transfer or the flux of the probe through the lipoprotein surface might be proportional to the total surface area. The total area, which is the product of the number of molecules and their surface area, is proportional to radius^{-1}. The predicted relationship, described by the dashed line in Figure 4, does not correlate with the experimental data. Better correlation was obtained between the log of the rate constant and radius^{-1}, shown by the solid line in Figure 4.

Discussion

The results of the experiments described here support the transfer mechanism in which the rate-limiting step in pyrene and DG transfer between lipoproteins is the dissociation of the probe molecules into the aqueous solution, followed by diffusion in the aqueous phase and rapid uptake into another lipoprotein (Charlton et al., 1976, 1978a). The increase by SCN^- and decrease by SO_4^{2-} and Cl^- can be interpreted quantitatively by the Setschenow equation in terms of salting-in or salting-out phenomena, with the electrolytes changing the solubility of the probe in the aqueous solution. The different rates of transfer found in the presence of electrolytes are in accordance with the effects predicted from the Hofmeister series of ions (Long & McDevit, 1952) and the molal surface tension increment produced by the salt (Melander & Horvath, 1977). Similarly, low concentrations of organic solvents increase the solubility of hydrophobic molecules in the aqueous

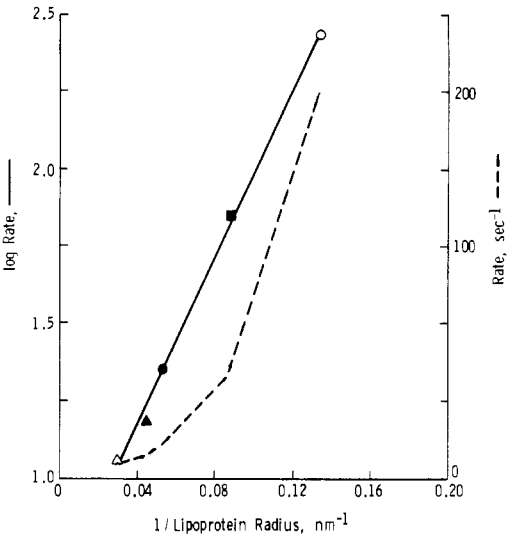

FIGURE 4: Relationship of the rate of pyrene transfer to lipoprotein size. Rate constants are given in Table I for HDL (○), LDL (■), $VLDL_3$ (●), $VLDL_2$ (▲), and $VLDL_3$ (△) with radii of 7.5, 11.3, 19, 22, and 29 nm, respectively, as determined by dynamic laser light scattering.

phase, demonstrated directly by measurement of the distribution coefficients. The data of Figure 3, in spite of considerable scatter, show a proportionality between the observed rates and the distribution coefficients. Differences in the values for the distribution coefficients are attributed only to a change in the aqueous phase solubility of the probe. This assignment is based on the assumption that the limiting solubility of the probe in the lipoprotein is relatively unaffected by the presence of organic solvents in the hydrocarbon phase and that the lipoprotein is not unduly perturbed by these concentrations of organic solvents.

Kinetic analysis of the transfer in heterogeneous systems containing different lipoproteins permits some predictions about the observed rates of transfer. The rate constants should be (a) independent of both donor and acceptor lipoprotein concentrations when the donor/acceptor ratio is maintained, (b) independent of the probe concentration, (c) identical in either direction, and (d) intermediate between those observed for dissociation of the probe from the individual lipoproteins. All of these predictions are reasonably well fulfilled, thereby providing convincing evidence that the proposed mechanism of transfer through the aqueous phase by simple partitioning is correct. A similar mechanism is suggested for transfer of slightly soluble molecules between polymer particles composing an oil-in-water emulsion (Ugelstad & Mork, 1980).

The calculations show that, in order for the predicted rate to reflect that measured experimentally, the possible range of the association rates is quite restricted. Association constants that accurately predict the rate of transfer in a system with dissimilar lipoproteins are the same as those calculated by dividing the dissociation rate constant by the distribution coefficient. Such agreement supports the postulate that transfer of hydrophobic compounds such as pyrene and the diglyceride analogue can be treated simply in terms of distribution between two immiscible solvents.

Inspection of Figure 4 and the data in Table I, which contain the rates of transfer of pyrene and DG in between a number of homogeneous reaction systems, reveals that the desorption rate from HDL is 20 times greater than that of VLDL; yet the distribution coefficients of pyrene between HDL, LDL, and VLDL are all very similar.

According to a current model (Shen et al., 1977), lipo-

proteins have a common architecture with an apolar core of triglyceride and cholesteryl ester and a surface shell of 2-nm thickness containing phospholipid, cholesterol, and apoproteins. If differences in the surfaces of the lipoproteins account for the observed differences in rate, the two features of greatest importance would be the composition and the radius of curvature. Although some apoproteins occur in more than one density range, there is no obvious gradation of protein composition from VLDL to LDL to HDL. The importance of minor lipid components as well as differences in the proportions of the major lipid components is not known. Pyrene transfer from a triglyceride-rich dispersion, with a diameter of 50 nm and containing only the lipid components of VLDL but no apoprotein (Charlton et al., 1978b), has a rate constant of 11.6 s^{-1}, very close to that of VLDL, 15 s^{-1}.

The rate of a reaction, $[P]_{LP} \rightleftarrows [P]^{\ddagger} \rightarrow [P]_{aq}$, is given by the Eyring theory as

$$k_d = \frac{kT}{h} e^{-\Delta G^{\ddagger}/(RT)} \quad (25)$$

where $\Delta G^{\ddagger}$ is the free energy of formation of the activated complex, k is Boltzmann's constant, h is Planck's constant, and T is the temperature in degrees K, and R is the gas constant (Glasstone et al., 1941). From the observed rate constants, $\Delta G^{\ddagger}$ can be calculated; from $\Delta G^{\ddagger} = -RT \ln K^{\ddagger}$, the equilibrium constant $K^{\ddagger}$ for formation of the activated complex is obtained. The calculated values for $K^{\ddagger}$ for pyrene transfer from HDL, LDL, and VLDL are 49×10^{-12}, 11×10^{-12}, and 2.4×10^{-12}, respectively. If the activated state is the probe in aqueous solution, $K^{\ddagger}$ would then be numerically equivalent to the distribution coefficient and the association rate would be encounter limited (10^9–10^{10} M^{-1} s^{-1}). The experimental value for $[P]_{aq}$ under these experimental conditions is 0.2×10^{-6} M (Charlton et al., 1976). The corresponding free-energy change for transfer of pyrene from HDL to buffer is 9.0 kcal mol^{-1}; consequently, the activated state of the probe must be more energetic than the state of the probe in buffer, since formation of the activated state for transfer of pyrene from HDL requires an additional 4.9 kcal more positive than that of pyrene in the aqueous solution.

The similarity of the distribution coefficients suggests that the lipid core (solvent)–solute interactions are energetically nearly identical in the three lipoprotein classes and that the free-energy barrier must lie physically between the core and the aqueous phase, presumably at the surface in the interfacial region. Additional support for penetration of the interfacial region as the kinetically limiting step is provided by the finding that the transition state pK_a of 9-(3-pyrenyl)nonanoic acid is about 4.5, rather than pH 7.1, the pK_a of the fatty acid in the phosphatidylcholine surface (Doody et al., 1980). Moreover, if the activated state corresponded to that of the probe in buffer, the transfer of the probe from buffer to lipoprotein would be diffusion controlled. The observed relationship between the transfer rates and the molal surface tension increment also supports the assignment of the kinetically limiting processes to the interfacial region. The transfer of a hydrophobic molecule from the lipoprotein into the aqueous solution requires the formation of a cavity in the aqueous solvent. The energy required for this process has been related in a number of systems (Melander & Horvath, 1977) to the surface area of the solute molecule and the surface tension of the aqueous solution. Consideration of activated state enthalpy $\Delta H^{\ddagger}$ and the corresponding entropy, $\Delta S^{\ddagger}$, listed in Table I, does not provide a clear indication as to whether or not the differences in rates of transfer between the lipoprotein classes have a single origin as, for example, the entropy of activation.

A conceptual basis for the rate dependence on the lipoprotein radius comes from an analogy to the evaporation of liquid droplets described by Kelvin's law

$$\ln P/P^0 = K\gamma/r \quad (26)$$

where K is a constant, P is the vapor pressure over a curved surface of radius r and the equivalent of k_d the dissociation constant, P^0 is the vapor pressure over a flat surface, and γ is the surface tension (LaMer & Gruen, 1952; DeFay et al., 1966). Thus, the transfer of a compound from a lipoprotein can be described by the relationship

$$k_d \alpha e^{-K\pi/r} \quad (27)$$

where π is the interfacial tension. Data presented in Figure 4, which are for lipoproteins that range in diameter from 15 to 60 nm, illustrate the clear exponential relationship between the log rate constant and the lipoprotein sizes. This equation has also been used to describe small molecule transfer between polymer particles in an oil-in-water emulsion (Ugelstad & Mork, 1980).

The amphipathic molecules that comprise the surface of the lipoproteins and membranes prevent extensive molecular interactions between bulk aqueous and apolar phases and reduce the interfacial tension to zero or to very low values (Gruen & Haydon, 1980; Tanford, 1979). Furthermore, the surfaces of biological membranes and lipoproteins are topographically heterogeneous (DePierre & Ernster, 1977), with the lateral movement of both lipid and proteins several orders of magnitude more rapid than the desorption process.

In summary, evidence has been presented to show that the transfer and the rate of transfer of pyrene and DG between lipoproteins can be treated simply as partitioning between two immiscible phases. The rate can be modified by changing the solubility of the transferring species in either phase. Radius of the donor lipoprotein has a large effect, which may be explained by a simple analogy with the evaporation of molecules from the surface of a liquid droplet. Transfer rates between different lipoprotein classes are intermediate between the homogeneous transfer rates. The probability of transfer of the lipophilic material from the aqueous solution to one of several individual lipoprotein surfaces is governed by both the number of lipoprotein macromolecules and their cross-sectional area. Thus small lipoproteins, such as HDL and LDL, because of the large number of particles, should have a promoting effect on the equilibration of lipids in the circulation.

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