Spontaneous Transfer of Monoacyl Amphiphiles between Lipid and Protein Surfaces

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ABSTRACT The kinetics of transfer of natural and fluorescent nonesterified fatty acids (NEFA) and *lyso*lecithins (*lyso*PC) from phospholipid and protein surfaces were measured. The kinetics of transfer of 12-(1-pyrenyl)dodecanoic acid, from liquid crystalline and gel phase single unilamellar phospholipid vesicles, very low, low, and high density lipoproteins, human serum albumin, and rat liver fatty acid-binding protein, were first-order and characterized by similar rate constants. The halftimes ($t_{1/2}$) of NEFA transfer from lipids and proteins were dependent on the acyl chain structure according to $\log t_{1/2} = -0.62n + 0.59m + 12.0$, where n and m, respectively, are the numbers of carbon atoms and double bonds. The structure of the donor surface had a measurable but smaller effect on transfer rates. The kinetics of NEFA and *lyso*PC transfer are slow relative to the lipolytic processes that liberate them. Therefore, one would predict a transient accumulation of NEFA and *lyso*PC during lipolysis and an attendant modulation of many metabolic processes within living cells and within the plasma compartment of blood. These data will be useful in the refinement of current models of membrane and lipoprotein function and in the selection of fluorescent NEFA analogs for studying transport in living cells.

INTRODUCTION

Nonesterified fatty acids (NEFA) and lysophosphatidylcholines (*lyso*PC), which are the products of lipolytic enzymes, regulate a broad spectrum of metabolic activities in living cells and in the plasma compartment of blood. *Lyso*PC, which is formed via the phospholipase A_2-mediated hydrolysis of oxidized LDL, down-regulates G-protein α subunits (Liao and Clark, 1995; Schakleford et al., 1995), cell function (Quinn et al., 1988; McMurray et al., 1993), and the expression of cell adhesion molecules (Kume et al., 1992), growth factors (Nakano et al., 1994; Ochi et al., 1995, Kume and Gimbrone, 1994; Jougasaki et al., 1992; Sakai et al., 1994), and signal transduction proteins (Zembowicz et al., 1995; Liao and Clark, 1995; Schakleford et al., 1995). Certain NEFA, particularly arachidonic acid and other long-chained NEFA, regulate intracellular processes such as signal transduction by interferon-α, (Hannigan and Williams, 1991) and ion channel activities (Ordway et al., 1989; Huang et al., 1992; Honore et al., 1994; Pepe et al., 1994). In plasma, NEFA inhibit the binding of low density lipoproteins (LDL) to the LDL-receptor (Bihain et al., 1989), and modulate the activities of lipoprotein lipase (Quinn et al., 1983; Clark and Quarfordt, 1985), which in turn affects the production of NEFA and *lyso*PC. NEFA affects the activities of several proteins involved in reverse cholesterol transport (Fielding and Fielding, 1995), including lecithin/cholesterol acyltransferase (Fielding et al., 1972) and lipid transfer proteins 1 and 2 (Sammet and Tall, 1985; Tall et al., 1985). Finally, in the postprandial state and in some metabolic disorders such as diabetes (Cistola and Small, 1991), changes in the molecular composition of the substrates and products of lipolytic enzymes (Morrisett et al., 1977) may alter lipoprotein metabolism to the extent that the kinetics of the redistribution of NEFA and *lyso*lecithin could be important determinants of lipoprotein turnover (Patsch et al., 1983; Tall, 1986).

Given that many different processes are regulated by *lyso*PC and NEFA concentrations, it is important to know how fast they are transported from their sites of production to the tissue sites or plasma compartments where they elicit their physiologic effects. Previous data have suggested that NEFA transfer occurs by rate-limiting desorption into the surrounding aqueous phase followed by diffusion-controlled uptake on another lipid or protein surface (Doody et al., 1980; Massey et al., 1982, 1984, 1985; McLean and Phillips, 1984; Patton et al., 1985; Pownall et al., 1983). These and other data suggested that acyl chain length and unsaturation might be important determinants of NEFA transfer (Massey et al., 1982; Pownall et al., 1991; Zhang et al., 1996). Although these studies have been useful in understanding the transfer mechanism, they were less useful in a physiologic context because they used fluorescent lipids or donor particles that do not occur naturally. In addition, it has become important to identify more reliable fluorescent

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Abbreviations used: NEFA, nonesterified fatty acid; an-HSA, anthraniloyl-HSA; apoA-I, apolipoprotein A-I; BSA, bovine serum albumin; CETP, cholesteryl ester transfer protein; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; $DNPS_2A$, dinitrophenyl distearylamine; DPPC, 1–2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPC-ether, 1,2-O-dipalmityl-*sn*-glycero-3-phosphocholine; FABP, rat liver fatty acid binding protein; HDL, high density lipoproteins; HSA, human serum albumin; LDL, low density lipoproteins; *lyso*palmitoyl PC, 1-palmitoyl-*sn*-glycero-3-phosphocholine; *lyso*PC, 1-acyl-*sn*-glycero-3-phosphocholine; *lyso*stearoyl PC, 1-stearoyl-*sn*-glycero-3-phosphocholine; PDA, 12-(1-pyrenyl)dodecanoic acid; PHDA, 16-(1-pyrenyl)hexadecanoic acid; PNA, 9-(1-pyrenyl)-nonanoic acid; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; SUV, single unilamellar vesicles; VLDL, very low density lipoproteins.

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NEFA analogs that can be used in the study of lipid transport in living cells in real time (Pownall and Smith, 1989). Herein we report the rates of transfer of a series of naturally occurring NEFA and *lyso*PCs, develop a quantitative model for their transfer, and characterize a fluorescent NEFA analog that has properties that are similar to some physiologically occurring NEFA.

EXPERIMENTAL

Materials

Palmitic, stearic, behenic, oleic, linoleic, and linolenic acids, *p*-nitrophenyl anthranilate, and fatty acid-free HSA were obtained from Sigma Chemical Co. (St. Louis, MO). POPC, DPPC, and *lyso*PCs were from Avanti Polar Lipids (Birmingham, AL). 1,2-O-Dihexadecyl-*sn*-glycero-3-phosphocholine was from Bachem Biosciences (Philadelphia, PA). PNA, PDA, PHDA, 11-(dansylamino)undecanoic acid, and 7-(9-anthroyloxy)stearic acid (7-an-stearic acid) were from Molecular Probes (Eugene, OR). [^{3}H]Oleic acid was purchased from New England Nuclear (Boston, MA). The nonexchangeable fluorescence quencher, dinitrophenyldistearylamine, was isolated from the reaction of dinitrofluorobenzene (Aldrich Chemical Co., Milwaukee, WI) and dioctadecylamine (Pfaltz and Bauer, Inc., Stamford, CT) as previously described (Plant et al., 1983). All other salts and solvents were from commercial vendors. Protein concentrations were determined by the procedure of Lowry et al. (1951), and phospholipid concentrations by the method of Bartlett (1959). A standard buffer composed of 0.1 M NaCl, 1 mM NaN_3, 1 mM EDTA, and 0.01 M Tris, pH 7.4, was used throughout.

Anthraniloyl-HSA was prepared as described by Hagag et al. (1983). FABP prepared from rat liver (Takikawa and Kaplowitz, 1986) was homogeneous by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and exhibited a binding isotherm for 11-(dansylamino)undecanoic similar to that described by Wilkinson and Wilton (1987). To prepare HSA, an-HSA, or FABP containing a specific NEFA or *lyso*lecithin, an aliquot of the lipid in ethanol or dimethylsulfoxide was injected into a buffered protein solution where the amount of ethanol added was <1% of the volume of buffer. Although the binding of lipids by some carrier proteins is modified by small amounts of ethanol, FABP is not affected (Schroeder et al., 1995). In addition, for the kinetics studies stock solutions of donor species were diluted 2- to 10-fold before mixing with acceptors, which produced at least an additional 2-fold decrease in concentration. As a consequence, the amount of organic solvent remaining is very small and its effects on transfer rates is expected to be minimal. VLDL, LDL, and HDL isolated by sequential flotation (Havel et al., 1955) were labeled similarly.

SUV containing a specific NEFA (3 mol %), *lyso*lecithin (1 mol %), or the fluorescence quencher, $DNPS_2A$ (5 mol %), were prepared by mixing the lipids in chloroform, removing the chloroform under a stream of nitrogen, and then in vacuo. The sample was suspended in buffer, sonicated under nitrogen for 30 min using a Branson sonifier, and isolated by ultracentrifugation (Barenholz et al., 1977).

Methods

Equilibrium partitioning

The partition coefficient (K_p) for the distribution of NEFA between phospholipid vesicles and albumin or the fatty acid binding protein is given by

$$K_p = (\text{mol lipid-boundFA/mol phospholipid})/(\text{mol protein-boundFA/mol protein}) \quad (1)$$

and was determined by chromatographic or titration methods.

In the first method, the protein and phospholipid vesicles containing a radioactive or fluorescent NEFA were mixed, incubated for 1 h to permit the NEFA to equilibrate, and passed over a Sepharose Cl-4B column (1.6 × 40 cm). Radioactivity was quantified by liquid scintillation counting; fluorescent NEFA concentrations were determined by measuring the fluorescence intensity of a 0.1 ml aliquot diluted with 2.0 ml ethanol. At the albumin and SUV concentrations that were used, the recoveries of radioactivity were >95%. The partition coefficient was calculated from

$$K_p = A/B \times (\text{mol protein/mol PC}) \quad (2)$$

where A and B are the respective fractions of NEFA bound to the vesicles and protein.

The second method is a fluorescence titration. This method assumes there are two NEFA compartments; one of these is the protein compartment, in which the labeled lipid is fluorescent, and the other, composed of lipid vesicles and the fluorescence quencher, $DNPS_2A$, in which the PDA fluorescence is quenched. Equation 2 was validated by titration of POPC vesicles containing PDA with POPC/$DNPS_2A$ SUV, where the fluorescence intensity was directly proportional to the mol fraction of vesicles containing the quencher. Following titration of the protein with bound NEFA with POPC/$DNPS_2A$ SUV, the data were analyzed according to

$$1/\Delta F = (1/K_p)(1/\Delta F_{max})(\text{mol HSA/mol PC}) + 1/\Delta F_{max} \quad (3)$$

where ΔF was the difference between the initial intensity of NEFA bound to the protein and the fluorescence intensity at a given molar ratio of protein to lipid, and ΔF_{max} was the maximal fluorescence intensity change. In this experiment, the fluorescence change represents the amount of NEFA bound to the vesicle. For titration of the NEFA in POPC/$DNPS_2A$ SUV with albumin, where the initial fluorescence was quenched, the data were analyzed by Eq. 4:

$$1/\Delta F = (K_p)(1/\Delta F_{max})(\text{mol PC/mol HSA}) + 1/\Delta F_{max} \quad (4)$$

where ΔF was the difference between the fluorescence intensity in the presence of a given protein to phospholipid molar ratio and that of the NEFA bound to vesicles, and ΔF_{max} was the maximal fluorescence intensity change. The initial NEFA/albumin molar ratio was 1.0. A free energy for the partitioning of NEFA between proteins and lipids was calculated as $\Delta G = -RT \ln K_p$.

Lipid transfer kinetics

The kinetics of transfer of lipids that had halftimes on the order of minutes were measured in an SLM 8000 spectrofluorimeter. Donors and acceptors were mixed manually and the fluorescence signal measured as a function of time, digitalized and analyzed by a nonlinear least squares program to obtain a first-order rate constant. When manual mixing was too slow, a hand-driven SFA-11 rapid kinetics stopped-flow accessory (Hi-Tech Scientific Limited, Salisbury, UK) was inserted into the cuvette holder of the fluorimeter and 200 μl aliquots of the donors and acceptors were mixed in <20 ms. The data were stored directly in an IBM-compatible computer and analyzed as described above.

The fluorescence quantum yields of pyrenyl fatty acids are essentially the same when bound to HSA, FABP, or POPC vesicles (0.1 mol %). However, within phospholipid vesicles containing $DNPS_2A$ (5 mol %), the fluorescence of pyrenyl fatty acids is reduced by 90%. Thus, the rates of transfer of pyrenyl fatty acids were determined from the change in fluorescence intensity (390 nm) following the mixing of pyrenyl fatty acids bound to HSA, FABP, or POPC vesicles with $DNPS_2A$/phospholipid vesicles.

To measure the rates of transfer of physiological NEFA, a system composed of donors, phospholipid SUV, and acceptors, an-HSA, was used. The fluorescence intensity of an-HSA is proportional to the amount of bound NEFA (Haggag et al., 1984). The transfer of physiologic NEFA or *lyso*lecithins from phospholipid SUV to an-HSA was followed by monitoring the change in the fluorescence of the anthraniloyl moiety (410 nm, excitation wavelength = 355 nm) that occurred on amphiphile binding to albumin (Hagag et al., 1983, 1984). Since the rate of association of NEFA

with albumin is very fast, the observed rates correspond to those for the desorption of the lipid from the surface of the donor particle. For the transfer of behenic and stearic acids from SUV to an-HSA, an-HSA (2 mg in 1.9 ml buffer) and SUV (2 mg in 0.1 ml buffer) were manually mixed in the fluorimeter at final concentrations of 40 μM NEFA and 15 μM albumin (molar ratio of 2.6 mol NEFA/mol albumin); the rates of transfer of *lyso*lecithins and unsaturated NEFA were measured using a rapid mixing device. The final concentrations after mixing were 15 μM an-HSA and 40 μM NEFA or 15 μM *lyso*lecithin. The rates of lipid transfer from an-HSA to HSA were also performed with a rapid mixing apparatus. The final concentrations were 15 μM an-HSA (containing 3 mol NEFA/mol an-HSA or 1 mol *lyso*lecithin/mol an-HSA) and 75 μM unlabeled HSA. The rate constants for transfer were determined by a computer program using the equation,

$$I_t = A + B_{exp}(-kt) \quad (5)$$

in which I_t is the transferred number of counts at time t, A is the value of I_t at infinity, B is a preexponential factor, k is the first-order rate constant, and t is the time in minutes. Activation energies were determined from Arrhenius plots of the rate constants (k). At each temperature, the rate constant was determined from the average of the rate constants for two to four individual traces. The enthalpies, entropies, and free energies of activation for transfer were calculated from absolute rate theory as previously described (Pownall et al., 1983).

RESULTS

Equilibrium partitioning of NEFA between vesicles and albumin

The partitioning of NEFA between phospholipid vesicles and albumin or FABP was measured to 1) compare fluorescent and physiologic NEFA, and 2) identify optimal conditions for the kinetic experiments with physiologic NEFA. Fig. 1 illustrates the chromatographic method for determining K_p for the partitioning of oleic acid between HSA and POPC SUV, and PDA between POPC/DNPS$_2$A SUV and FABP. These data and similar other data that are not shown were analyzed using Eq. 2. The partitioning of fluorescent fatty acids by the titration method shown in Fig. 2 was analyzed according to Eq. 3. These data are summarized in Table 1.

The K_p and ΔG_p for the partitioning of oleic acid between HSA and POPC SUV were independent of the NEFA/protein ratio. The mean value for K_p (=0.0025) was comparable to that reported for its partitioning between DMPC vesicles and BSA (K_p = 0.006; Daniels et al., 1985). Replacement of HSA with an-HSA gave a K_p that was ~4-fold higher; this difference corresponds to nearly 1 kcal greater affinity ($\Delta G = -RT$ ln 4) of oleic acid for HSA than for an-HSA. K_p and ΔG_p for the fluorescent fatty acids, PNA and PDA, were within the range of those of oleic acid. However, K_p values for 7-(9-anthroyloxy)stearate and PHDA were much higher, indicating that these NEFA preferentially associate with the lipid phase.

Transfer of fluorescent fatty acids

The kinetics of PDA transfer was studied over a time frame in which at least 85% transfer was observed; a typical trace

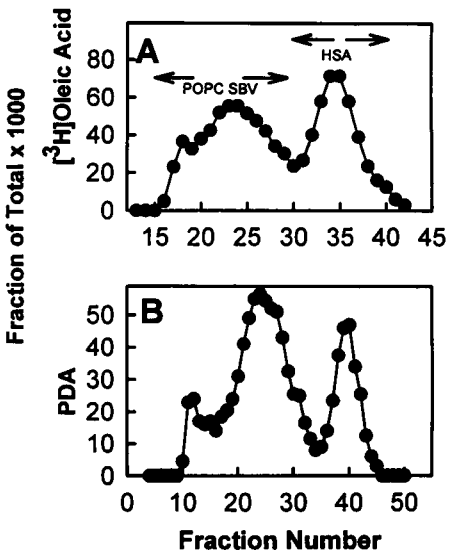

FIGURE 1 Partitioning of fatty acids followed by gel filtration chromatography over a column of Sepharose CL-4B (1.6 × 40 cm). (*A*) Separation of POPC SUV (5 mM) from HSA (10 μM) with a ratio of 1.5 mol [^{3}H]oleic acid/mol HSA. One ml of vesicles labeled with [^{3}H]oleic acid was mixed with 1 ml HSA, incubated at room temperature for 30 min, and applied to the column. The column effluent was monitored by liquid scintillation counting. Fractions 10 to 28 and 30 to 40, respectively, were used to calculate the fractions of fatty acid bound to the vesicles and albumin. The partition coefficient, determined from Eq. 2, was 0.0023 (Table 1). (*B*) Separation of POPC/DNPS$_2$A (1.2 mM) and FABP (5 μM) with an initial concentration of PDA of 1 μM. The experiment was performed as described above; however, the column dimensions and fraction sizes were slightly different. The PDA concentration was determined from the fluorescence (397 nm; excitation wavelenth = 327 nm) of a 0.1-ml aliquot and in 2 ml ethanol. Fractions 10 to 34 and 35 to 45, respectively, were used to calculate the fractions of PDA bound to the vesicles and FABP. The partition coefficient was 0.01 (Table 1).

for the transfer of PDA from HSA to POPC/DNPS$_2$A SUV is shown in Fig. 3 (*top*). The rates of transfer from HSA to POPC/DNPS$_2$A SUV were independent of the HSA, phospholipid, and NEFA concentrations (Table 2). The rates of PDA transfer between labeled and unlabeled SUV and between SUV and albumin were also measured. Similar rates were obtained irrespective of whether the quencher, DNPS$_2$A, was in the donor or acceptor (Table 3). The rate constants for the transfer from several different surfaces, which included fluid (POPC) and gel phase (DPPC-ether) phospholipid vesicles, plasma lipoproteins (VLDL, LDL, and HDL), and fatty acid-binding proteins (HSA, an-HSA, FABP) were determined (Table 3). The rate constants for PDA transfer from phospholipid vesicles and plasma lipoproteins were similar ($k \sim 0.2$ s^{-1}; $t_{1/2}$ = 3.5 s). The rate constants for transfer from HSA and an-HSA were slightly faster than those from the lipid matrices, whereas the rate constants for transfer from FABP and lipid matrices were similar. The range of rate constants for PDA transfer was small and extended from only 0.15 s^{-1} for donors of FABP to 1.13 s^{-1} for an-HSA donors. In contrast, the transfer of a longer-chained analog, PHDA, from phospholipid vesicles was nearly three orders of magnitude slower.

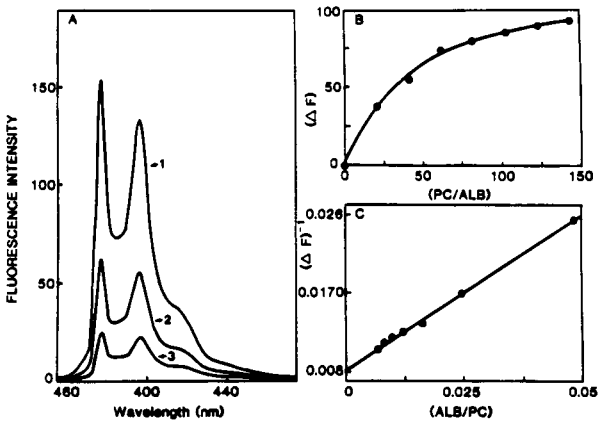

FIGURE 2 Fluorescence titration of PDA bound to HSA with POPC/$DNPS_2A$ at 37°C. (*A*) Fluorescence of PDA (10 μM) bound to HSA (10 μM) in the presence of 0 (*Curve 1*), 0.36 (*Curve 2*), and 0.88 mM POPC/$DNPS_2A$ SUVs (*Curve 3*). The addition of SUV/$DNPS_2A$ results in the transfer of PDA to the SUV/$DNPS_2A$ and the attendant quenching of fluorescence within the SUV. (*B*) Titration of PDA + HSA (1:1) with the SUV/$DNPS_2A$. The fluorescence change ($\Delta F_{397\ nm}$) is the difference between the fluorescence intensity of fatty acid bound to albumin and that observed at each POPC/$DNPS_2A$ concentration. The calculated partition coefficient (Eq. 3, Panel (*C*)) for the distribution of PDA between the SUV/$DNPS_2A$ and albumin was 0.020.

The activation energies for NEFA transfer were calculated from the temperature dependence of the rates. Fig. 3 (*bottom*) contains typical Arrhenius plots for the transfer of PDA from a fluid lipid matrix, POPC, a gel lipid matrix, DPPC-ether, and from HSA to either POPC or DPPC-ether matrices. For all the donor matrices used (Table 3), the Arrhenius plots were linear over the temperature range studied (10–45°C) except in the case of DPPC-ether, where a discontinuity in the Arrhenius plot appeared at 30°C. For the fluid lipid matrices, activation energies varied from 8.9 to 11.3 kcal/mol (Table 2), whereas those for transfer from the proteins and DPPC-ether ($T < 30$°C) were much higher, with values extending from 14.7 to 19.8 kcal/mol.

Transfer of physiologic NEFA and lysolecithin

The rates of transfer of NEFA and *lyso*lecithin from SUV to albumin were determined from the time-dependent changes in an-HSA fluorescence. Based on gel filtration studies similar to those given in Fig. 1, conditions were selected so that the NEFA were quantitatively associated with the protein. Under these conditions, addition of NEFA to an-HSA induced a 70% increase in the fluorescence intensity of the reporter group (Fig. 4). Moreover, titration of an-HSA with either palmitic acid or *lyso*palmitoyl PC added as a component of POPC SUV gave a linear relationship between fluorescence enhancement and the amount of NEFA or *lyso*lecithin added (Fig. 4, *inset*). Therefore, the fluorescence intensity is expected to accurately reflect the amount of amphiphile transferred. A representative exponential curve corresponding to the transfer of palmitic acid from POPC SUV to an-HSA is shown in Fig. 5 *A*. These data show that the calculated and experimental curves are nearly coincident. First-order rate constants were calculated from these data and other combinations of donors and acceptors. Using albumin acceptors, the rate constant was independent of the ratio of NEFA to POPC in the SUV donors (Fig. 5 *A*, *inset*). Similarly, the measured rate constant varied little with the ratio of *lyso*lecithin to albumin (Fig. 5 *A*, *inset*). At low molar ratios of NEFA or *lyso*lecithin to an-HSA, the rate constants increased slightly; however, at these molar ratios the small fluorescence changes were difficult to quantify.

As previously reported for pyrenyl lipids (Pownall et al., 1983; Massey et al., 1982, 1984), the rates of NEFA transfer from SUV were a sensitive function of the acyl chain length and unsaturation (Table 4). The transfer rate constant for oleic acid ($18{:}1_C$) was three orders of magnitude greater than that for behenic acid (22:0). Comparison of the data on all the NEFA in this study showed that increasing the acyl chain length decreased the rate constant by a factor of 8 per two methylene units and increasing the unsaturation increased the rate by a factor of 4 per double bond. Multiple linear regression on the data for the transfer of the *i*th NEFA from POPC (Table 4) gave the equations

$$\log k_i = 0.67n - 0.63m - 13.19 \quad (6a)$$

$$\log t_{1/2} = -0.62n + 0.59m + 12.0 \quad (6b)$$

where n and m, respectively, are the number of carbons and double bonds in the acyl chain, and the respective units for the rate constant (k_i) and halftime ($t_{1/2}$) are in s^{-1} and s. The transfer of *lyso*lecithin was slightly faster than the corresponding NEFA (Table 4). Changing the phospholipid matrix from a fluid matrix composed of POPC to a gel state matrix of DPPC altered the rate of transfer. For stearic and behenic acid, the rate was slower by a factor of 11 and 5, respectively, whereas for oleic acid, it decreased only by a factor of 2.

Lipid transfer from an-HSA to HSA

The time-dependent decrease in the fluorescence of the anthraniloyl group that occurred when an-HSA with bound NEFA or *lyso*lecithin was mixed with unlabeled fatty acid-free albumin was used to measure the transfer from albumin (Fig. 5 *B*). Analysis of the kinetic trace indicated that this was a first-order process. The rate for reequilibration of NEFA between albumins was only slightly slower (factor of 2 to 4) than for transfer from POPC vesicles (Table 3). Similarly, comparison of the data for palmitic acid and stearic acid showed that the rates of transfer increase with the acyl chain length.

Thermodynamics of lipid transfer

The temperature dependence of the rates of transfer of physiologic NEFA was used to calculate activation energies

TABLE 1 Equilibrium partitioning data for fatty acids between albumin and vesicles

Fatty Acid	Donor*	Acceptor	Ratio# PC/ALB	Ratio FA/ALB	K_p§ × 10^3	ΔG_a (kcal/mol)#
Oleic acid	HSA	POPC	500	0.04	2.0	3.73
	HSA	POPC	500	1.0	1.7	3.83
	HSA	POPC	500	1.5	2.3	3.64
	HSA	POPC	500	3.0	4.0	3.31
	AN-HSA	POPC	500	0.5	11.0	2.71
	AN-HSA	POPC	500	1.0	9.0	2.83
	AN-HSA	POPC	500	1.5	10.0	2.76
	AN-HSA	POPC	500	3.0	13.0	2.61
7-an-stearic acid	HSA	POPC	500	1.0	18.0	2.41
	HSA	POPC	100	1.0	28.0	2.15
PNA	HSA	POPC	500	1.0	5.7	3.10
	HSA	POPC	100	1.0	5.5	3.12
	HSA	POPC/$DNPS_2A$	85	1.0	7.4	2.94
	HSA	DPPC-ether/$DNPS_2A$	—	—	(11.0)	2.71
	DPPC-ether/$DNPS_2A$	HSA	—	—	(6.8)	2.88
PDA	HSA	POPC	500	1.0	5.4	3.13
	HSA	POPC	100	1.0	3.9	3.33
	an-HSA	POPC/$DNPS_2A$	85	1.0	5.9	3.33
	HSA	POPC/$DNPS_2A$	—	—	(20.2)	2.34
	HSA	DPPC-ether/$DNPS_2A$	—	—	(25.4)	2.20
	DPPC-ether/$DNPS_2A$	HSA	—	—	(11.1)	2.70
	FABP	POPC/$DNPS_2A$	240	0.2	10.0	2.76
	FABP	POPC/$DNPS_2A$	—	0.2	(7.2)	2.96
PHDA	HSA	POPC	500	1.0	12.5	2.63
	HSA	POPC	100	1.0	24.3	2.31
	HSA	POPC/$DNPS_2A$	100	1.0	33.9	2.03
	DPPC ether/$DNPS_2A$	HSA	—	—	(47.6)	1.83

*The donor refers to the protein or lipid matrix that was initially labeled with fatty acid.
#PC/ALB = molar ratio of phospholipid; FA/ALB = molar ratio of fatty acid to albumin; $\Delta G_a = -RT \ln K_p$.
§The partition coefficient (K_p) was determined by column chromatography and fluorescence titration (values in parentheses). K_p has the units of (mol FA/mol PC)/(mol FA/mol protein).

(Table 4). Arrhenius plots (not shown) were linear over the temperature range (10–50°C) studied. The activation energies, which varied from 14 to 28 kcal/mol, were a function of the structures of the transferring lipid and the donor matrix (Table 3). Activation energies do not always correlate with changes in the structure of the transferring species. Previous studies have shown that free energies of activation better reflect the effects of hydrophobicity on transfer kinetics because they include contributions from both entropic and enthalpic terms (McLean and Phillips, 1984; Pownall et al., 1983; Nichols, 1985). The correlation between the calculated free energies of activation for transfer and the acyl chain length and unsaturation are shown in Fig. 6. Even though there were not extensive measurements for all sets of carrier matrices, the differences in free energy of activation for a series of lipids within one matrix could be estimated. For saturated NEFA (Fig. 6 *A*), the incremental free energies of activation were 0.77, 0.70, and 0.59 kcal/mol/methylene unit for transfer from an-HSA, POPC SUV, and DPPC SUV, respectively. Zhang et al. (1996) observed a similar chain length dependence (740 cal/methylene unit) for a series of saturated fatty acids containing 14–26 carbon atoms. Although the chain length dependence of the free energies of activation for transfer of NEFA from DPPC and an-HSA was similar to that observed when POPC was the donor, the free energies of activation for a given NEFA were always higher when an-HSA or DPPC were substituted for POPC. In contrast, the free energy of activation for the transfer of *lyso*PCs from POPC SUV was similar to that of the corresponding NEFA, and characterized by an incremental change of 0.50 kcal/mol/methylene unit. Using a donor matrix composed of POPC SUV, each double bond added to a given acyl chain contributed a common decrement to the free energy of activation for three different acyl chain lengths (Fig. 6 *B*). These were −0.78, −0.75, and −0.78 kcal/mol/double bond for acyl chains containing 18, 20, and 22 carbons. Multiple linear regression on the data for the free energy of transfer of NEFAfrom POPC SUV gave the equation

$$\Delta G^{\ddagger} = 0.64n - 0.77m + 5.95 \quad (7)$$

Substitution of DPPC SUV for POPC SUV as the donor matrix revealed some differences in the energetics of transfer that are associated with the physical state of the donor particle. Whereas the free energies of transfer of oleic acid from DPPC SUV and POPC SUV are similar, the free energy of transfer of stearic acid from DPPC SUV is much higher than that observed from POPC (Fig. 6 *B*, *inverted triangles*).

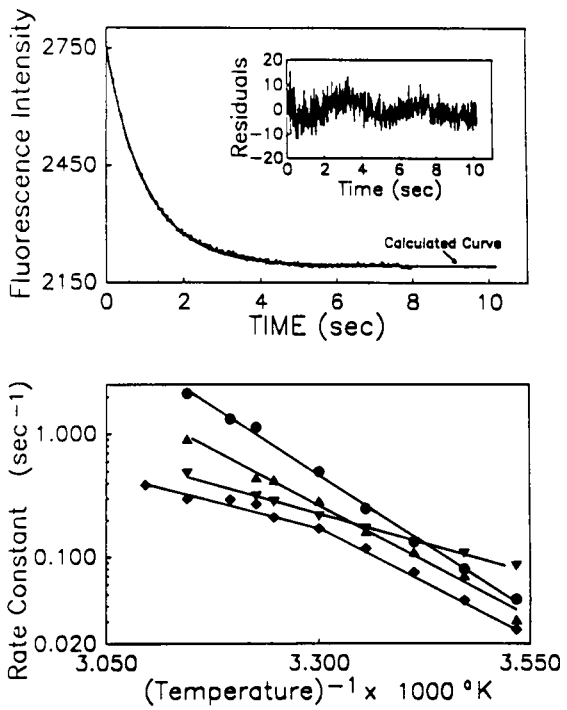

FIGURE 3 Representive kinetics for the transfer of PDA from HSA to POPC/DNPS$_2$A SUV. *Top:* Change in fluorescence intensity following the rapid mixing of PDA (1 μM) + HSA (1 μM) with POPC/DNPS$_2$A SUV (200 μM) at 37°C. Least-squares analysis according to first-order kinetics gave the rate constant for PDA transfer, $k = 0.96 \pm 0.00078\ s^{-1}$; the inset gives the residual difference between the observed and calculated fluorescence intensity. *Bottom:* Arrhenius plots derived from the temperature dependence of transfer of PDA from HSA (1 μM + 1 uM PDA) to POPC/DNPS$_2$A (200 μM) (●); HSA (1 μM + 2 μM PDA) to DPPC-ether/DNPS$_2$A (80 μM) (▲); POPC/DNPS$_2$A (50 μM and 0.05 μM PDA) to HSA (17 μM) (▼); and for DPPC-ether/DNPS$_2$A (70 μM + 0.5 μM PDA) to HSA (17 μM) (◆).

The effects of the physical state of the donor matrix on the thermodynamic parameters of the activated state were compared for stearate and PDA, which have similar rates of transfer (Table 5). For the transfer from liquid-crystalline phase, gel phase, and protein matrices, the activation free energies of transfer for the two fatty acids were essentially identical, whereas the activation enthalpies were 4.4–5.4 kcal/mol more positive for stearate. The thermodynamic parameters for stearate and PDA transfer from gel phase lipid and protein matrices were essentially the same. However, for both fatty acids the activation parameters for transfer from the liquid crystalline phase were markedly different from those found when a protein or a gel phase phospholipid donor was used (Table 5). The major differences were the much lower enthalpies of activation for transfer from liquid crystalline phase lipid and a compensatory increase in the entropy of activation. As a consequence, the two fatty acids have similar free energies of activation for transfer from the different donor matrices, and, thus, similar rates of transfer. However, the activation enthalpy varies in two different, independent ways. First, the activation enthalpy was ~4 kcal/mol more positive for stearate than for PDA. Second, for either fatty acid, the activation enthalpy was ~7–8 kcal/mol more positive for transfer from a gel phase phospholipid or a protein matrix.

TABLE 2 Concentration dependence of transfer of PDA from HSA to POPC/DNPS$_2$A vesicles

HSA (μM)	PDA/HSA Ratio	POPC/DNPS$_2$A SUV (mM)	(POPC/DNPS$_2$A)/ HSA*	k (s^{-2})#
10	1.0	1.0	100	0.22
5.0	1.0	0.5	100	0.24
1.0	1.0	0.1	100	0.21
1.0	1.0	1.0	1000	0.24
1.0	1.0	0.5	500	0.21
1.0	2.0	1.0	1000	0.19
1.0	3	1.0	1000	0.21
0.5	1.0	0.1	200	0.26
0.5	1.0	0.05	100	0.23

*Molar ratio of POPC/DNPS$_2$A SUV to HSA.
#The rate constants are the average value from analysis of six to eight kinetic traces at 25°C.

DISCUSSION

Much of our current understanding of NEFA transfer between surfaces is based on data obtained with fluorescent lipids (Doody et al., 1980; Pownall et al., 1983; Storch and Kleinfeld, 1986). Although these studies have helped identify the mechanism for NEFA transfer, they have failed to provide the rate constants for NEFA and fatty acid-binding proteins and particles that are physiologically relevant. In addition, the current body of data is too small to identify a quantitative relationship between structure of monoacyl amphiphiles and the thermodynamics of their transfer. In this study, the rates of transfer of physiological NEFA and *lyso*lecithins were determined by following the fluorescence changes that occurred in an anthraniloyl reporter group upon their association/dissociation with an-HSA. This method permits the measurement of rapid transfer kinetics in real time without interference from tryptophan, which is found in many fatty acid-binding proteins.

The physicochemical behavior of NEFA may regulate their metabolism, and one of the major criteria for the identification of valid fluorescent analogs of physiologic NEFA should be that they exhibit similar partitioning properties. The partitioning of oleic acid between POPC and HSA (K_p of 0.002, Table 1) and between DMPC vesicles and BSA (K_p = 0.006, Daniels et al., 1985) is similar. The partition coefficients for the fluorescent NEFA, PNA and PDA, are only 2 to 3 times higher than those of oleic acid, the physiologic NEFA with the greatest affinity for albumin (Table 1, Spector, 1986). These values and those previously reported for myristic acid (K_p = 0.0025) and stearic acid (K_p = 0.013; Daniels et al., 1985) are also similar. However, the partition coefficients for 7-an-stearic acid and PHDA are significantly higher, which may explain in part why their cellular metabolism does not emulate that of

TABLE 3 Summary of kinetic data for the transfer of pyrenyl fatty acids at 37°C

Fatty Acid	Donor Matrix	Acceptor Matrix	k (s^{-1})	E_a (kcal/mol)*	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta G^{\ddagger}$ (kcal/mol)
PDA	POPC/DNPS$_2$A	POPC	0.30	9.8	9.2	18.2
	POPC/DNPS$_2$A	HSA	0.27	10.5	9.9	18.3
	POPC	POPC/DNPS$_2$A	0.31	8.9	8.0	18.2
	HSA[#]	POPC/DNPS$_2$A	1.9	15.9	15.3	17.1
			1.1	19.8	19.2	17.4
	an-HSA	POPC/DNPS$_2$A	2.0	14.7	14.1	17.1
	DPPC-ether/DNPS$_2$A	HSA (T < 30)[§]	0.32	16.8	16.2	18.1
		HSA (T > 30)	0.23	7.8	7.2	18.3
	HSA (2)[¶]	DPPC-ether/DNPS$_2$A	0.53	16.3	15.7	17.9
	HSA (0.5)	DPPC-ether/DNPS$_2$A	0.48	16.3	15.7	17.9
	FABP	POPC/DNPS$_2$A	0.28	16.2	15.6	18.2
	VLDL	POPC/DNPS$_2$A	0.27	9.4	8.8	18.2
	LDL	POPC/DNPS$_2$A	0.35	9.7	9.1	18.1
	HDL	POPC/DNPS$_2$A	0.44	11.3	10.7	18.0
PHDA	DPPC-ether/DNPS$_2$A	HSA	0.00093	19.4	18.8	21.6

*Rates that were too fast to measure at 37°C were calculated from the temperature dependence of the data as described by the Arrhenius plots; the activation energies have an experimental error of ±5%.

[#]The two measurements are for two separate preparations of HSA and vesicles under similar conditions.

[§]Activation energies were calculated separately from data collected above and below 30°C.

[¶]The numbers in parentheses are the fatty acid to albumin molar ratio.

physiological NEFA (Naylor et al., 1991). In contrast, the partitioning behavior, transfer kinetics, and transfer kinetics of PDA are similar to those of physiological NEFA. In general, the partition coefficients indicate that the behavior of pyrenyl fatty acids is similar to that of physiologic NEFA. However, the relatively high affinity of PHDA for lipid surfaces may make this NEFA a good analog for long-chained NEFA, which are known to have a higher affinity for lipoproteins than for albumin (Shafrir et al., 1985).

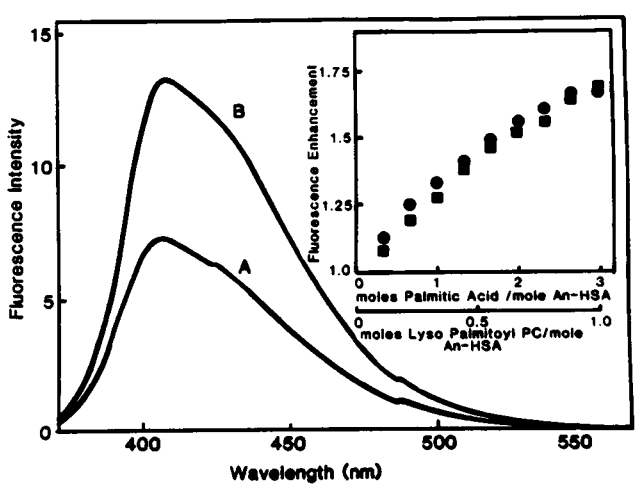

FIGURE 4 The fluorescence spectra of an-HSA (12 μM) recorded before (*A*) and after (*B*) incubation with palmitic acid (added as 3 mol % in POPC SUV). Final fatty acid to albumin molar = 3.2. The inset shows the fractional increase in fluorescence intensity of an-HSA (410 nm) as a function of the molar ratio of palmitic acid, added in POPC SUV, to an-HSA (■). Similarly, an-HSA was titrated with *lyso*palmitoyl PC (added as 1 mol % in POPC SUV) (●). Data were collected at room temperature (~23°C) with excitation at 355 nm.

Molecular determinants of transfer

The kinetics of NEFA and *lyso*lecithin transfer from vesicles to an-HSA were first-order, independent of the molar ratio of NEFA or *lyso*lecithin to albumin, and highly dependent upon the structure of the transferring amphiphile. For *lyso*lecithin, transfer probably only occurs from the outer monolayer since the transbilayer "flip-flop" rate is very slow (McLean and Phillips, 1984; Bhamidipati and Hamilton, 1989). In contrast, the "flip-flop" rate for NEFA is fast compared with the transfer between surfaces (Doody et al., 1980; Kamp et al., 1995) so that the observed rate corresponds to the rate-limiting step, desorption into the surrounding aqueous phase, which is followed by rapid association with acceptor particles or molecules. This mechanism has been shown to apply to cholesterol (Lund-Katz et al., 1982), NEFA (Doody et al., 1980; Pownall et al., 1983; Daniels et al., 1985), phospholipids (Massey et al., 1982; Pownall et al., 1991) and apolipoproteins (Hickson-Bick et al., 1988; McKeone et al., 1988). The major energetic barrier to transfer is the exposure of hydrophobic surfaces on the transferring amphiphile to the surrounding aqueous phase. For the transfer of single-chained pyrene-labeled amphihiles, $\Delta G^{\ddagger}$ increases by ~0.60–0.90 kcal/methylene unit, whereas for amphiphiles with two acyl chains, the increase is ~0.32 cal per methylene unit and 0.30 kcal per double bond (Pownall et al., 1983; 1991; Zhang et al., 1996). However, as the number of double bonds in the phospholipid acyl chains was increased, the incremental change in the free energy of activation per methylene unit increased dramatically. In this study, we observed respective changes in $\Delta G^{\ddagger}$ of 0.64 and −0.77 kcal/mol for the introduction of each methylene unit and double bond into the acyl chain. Our data show that each methylene unit and double bond contributes to the free energy of activation for

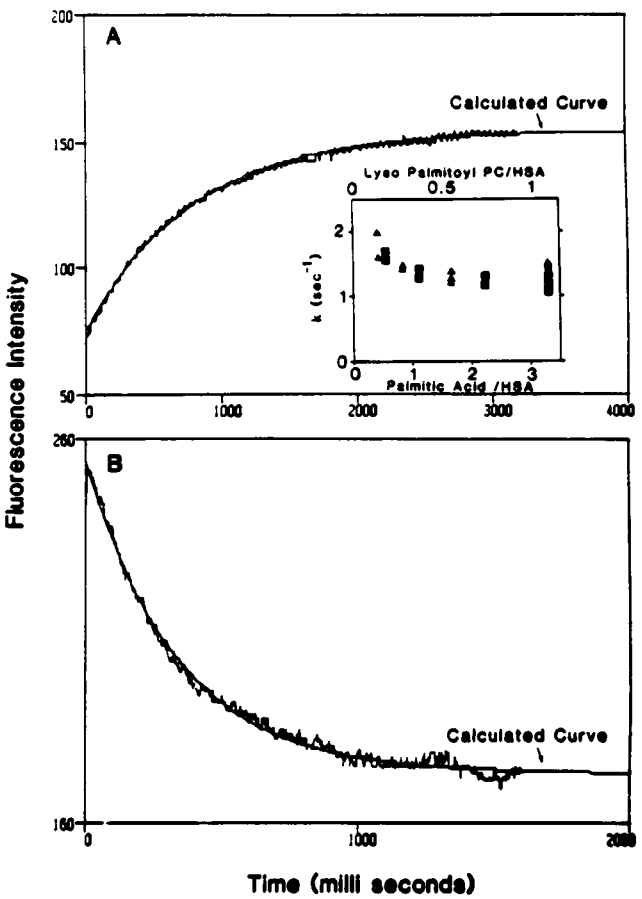

FIGURE 5 Kinetic traces recorded for the transfer of palmitic acid from POPC SUV to an-HSA (*A*) and from an-HSA to HSA (*B*). (*A*) A representative kinetic trace following the mixing of 12 μM an-HSA with 40 μM palmitic acid (3 mol % in POPC SUV) at 10°C. Analysis of the data according to first-order kinetics gave the solid line that is superimposed on the experimental data and a rate constant, $k = 1.25 \pm .01\ s^{-1}$. (*Inset*) The first order rate constants (k) as a function of the molar ratio of palmitic acid (▲) and *lyso*palmitoyl PC (■) to an-HSA. (*B*) Kinetics of transfer of palmitic acid from an-HSA (15 μM) + palmitic acid (45 μM) to HSA (7.5 μM) at 30°C monitored from the decrease in an-HSA fluorescence. Analysis according to (*A*) gave a rate constant, $k = 3.0 \pm 0.05\ s^{-1}$.

NEFA transfer in a predictable way that is independent of the chain length and the number of double bonds.

The linear relationship between the free energies of activation and the addition of double bonds or methylene units suggests that these two structural units contribute to the free energy of activation in a predictable way. Studies of other single-chain amphiphiles have yielded similar results (Pownall et al., 1983) suggesting that the effects of additional double bonds or methylene units on the transfer rates can be generalized. This would have an important consequence for the rates of transfer of oxidized *lyso*PCs. Oxidation of *lyso*PC increases its polarity. As a consequence, it is more water-soluble and should transfer more rapidly among surfaces than unoxidized analogs. *Lyso*PCs that have short chains or a large number of double bonds would be expected to transfer even faster and be preferentially released from the surfaces to which they are bound. Thus, these lipids would be expected to transfer to HDL faster than their unoxidized analogs.

Other determinants of the rates of NEFA transfer were small compared to the effects of additional double bonds and methylene units. Changing the matrix from fluid to solid by the substitution of DPPC-ether for POPC increased the ΔG^* for transfer of stearate, but not oleate (Fig. 6 (*B*)). Similarly, PDA transfer from DPPC-ether was associated with higher activation energies and ΔG^* at temperatures below the gel to liquid crystalline transition temperature (Table 3). These effects are probably due to the ability of straight-chained NEFA such as stearate and PDA to co-crystallize with the DPPC-ether, which exhibits a gel to liquid crystalline transition at 43°C (McKeone et al., 1986). Presumably, the additional free energy of activation is required to disrupt the enthalpy of crystallization. There was a relatively large difference (2.2 kcal) in the free energies of activation for the transfer of oleate and stearate from DPPC SUV. This is likely due to two effects. Oleate, which is a fluid lipid, does not readily co-crystallize with solid lipids so that there is a smaller contribution of the enthalpy of crystallization to the enthalpy of activation. Superimposed on this is the expected reduction of the free energy of activation by the additional double bond.

Although the activation energy for transfer from gel phase matrix is typically greater than that found for a liquid crystalline matrix, high activation energies do not always translate into high free energies of activation due to compensatory changes in the entropies of activation (Nichols, 1985; McLean and Phillips, 1984; Massey et al., 1982; Doody et al., 1980). This effect is clearly demonstrated in the transfer behavior of stearate and PDA (Table 5). In spite of the large differences between the enthalpies of activation for the transfer of PDA and stearate from gel phase lipid, liquid crystalline phase lipid and protein (an-HSA), the free energies of activation are very similar. This is due to the compensatory effects of the $T\Delta S^{\ddagger}$ term, which was negative for stearate in a protein and gel phase matrix. These data suggest that the structural changes that occur during the transfer of stearate from an-HSA are similar to those that occur during the NEFA transfer from gel phase lipid.

The free energy and attendant rates of transfer were not changed appreciably when donors of POPC SUV were replaced by plasma lipoproteins, HSA, or FABP, suggesting that the transfer rate is determined more by the structure of the transferring species than by that of the donor particle or matrix. As expected, on the basis of previous studies of cholesterol and phospholipid transfer (Lund-Katz et al., 1982; Pownall et al., 1991), the transfer rate increased when the donor particle was changed in the order VLDL, LDL, and HDL. The respective ratios of rate constants were 1:1.3:1.6. However, the difference was much smaller than that found for cholesterol and phospholipids, for which the corresponding ratios were 1:3:5. This again supports the hypothesis that the structure of the transferring species is the major determinant of NEFA transfer.

TABLE 4 Summary of kinetic parameters for the transfer of physiologic fatty acids and *lysolecithins* at 37°C

NEFA/*lyso*PC Transferred	Donor Particle*	k (s^{-1})#	$t_{1/2}$ (s)	E_a, kcal/mol	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta G^{\ddagger}$ (kcal/mol)
16:0	POPC	270.	0.0026	22.3	21.7	15.79
	an-HSA	26.	0.026	17.7	17.1	16.84
18:0	POPC	4.4	0.16	13.9	13.3	17.62
	DPPC	0.99	0.71	21.6	21.0	19.06
	an-HSA	0.60	1.2	21.9	21.3	18.37
18:1	POPC	41.	0.017	16.9	16.3	16.52
	DPPC	20.	0.035	17.4	16.8	16.98
18:2	POPC	570.	0.0012	25.8	25.2	15.61
18:3	POPC	1100.	0.00064	27.3	26.7	15.33
*lyso*palmitoyl PC	POPC	53.	0.013	21.5	20.9	15.69
*lyso*stearoyl PC	POPC	34.	0.021	17.6	17.0	16.68
	an-HSA	3.8	0.18	18.4	17.8	17.09
20:0	POPC	0.21	3.3	20.8	20.2	18.79
20:1	POPC	0.71	1.0	27.6	27.0	18.20
20:4	POPC	35.	0.02	24.4	23.8	15.83
22:0	POPC	0.024	30.	14.3	13.7	20.00
	DPPC	0.0034.	205	25.8	25.2	21.41
22:1	POPC	0.16	4.4	18.1	17.5	18.96
22:2	POPC	0.35	2.0	18.0	17.4	18.49
22:3	POPC	1.5	0.47	18.1	17.5	17.61
22:4	POPC	20.	0.035	22.5	21.9	16.20
22:6	POPC	77.	0.0091	26.4	25.8	15.48

*For transfer from SUV, the acceptor was an-HSA; for the transfer from an-HSA, the acceptor was HSA. POPC and DPPC were used as SUV.
#Values too fast to measure at 37°C were calculated from the temperature dependence of the rates using the Arrhenius equation; the calculated activation energies have an experimental error of ±5%.

The rate constants observed for transfer from SUV provide a benchmark by which transfer from other surfaces can be estimated. Spontaneous transfer of cholesterol from a small lipoprotein such as HDL is more than an order of magnitude faster than that observed from LDL (Lund-Katz et al., 1982), and the rate of phospholipid transfer over a spectrum of lipoprotein sizes increased with decreasing size (Massey et al., 1984). The relationship between lipid transfer rate is described by the Kelvin equation (Charlton and Smith, 1982), which states that the rate varies with the inverse of the radius (DeFay et al., 1966). Thus, rate constants would be expected to be much slower from surfaces such as real membranes, which have little curvature.

Lipid transfer from albumin and FABP

K_p values for an-HSA were ~4-fold higher than those of HSA. Thus, the chemical modification that produced the fluorescent albumin derivative is associated with a relatively small change in NEFA partitioning. The reported K_p (=0.05) for the partitioning of oleic acid between FABP and egg yolk PC vesicles (Cistola et al., 1988), is only slightly higher than what we found when PDA replaced oleic acid in a similar experiment, indicating again that PDA faithfully emulates physiological NEFA.

The mechanism and kinetics of transfer of *lyso*lecithin from albumin and NEFA from FABP have not been reported. The rates and free energies of activation for *lyso*PC transfer were similar to those of the corresponding NEFA, whereas the increase in the free energy of activation with chain length was very similar to that of the NEFA. These data suggest that acyl chain, but not the nature of the polar group, is an important determinant of the thermodynamics of transfer. Previous data have shown that the enthalpy of association of fatty acids with FABP is an important contribution to the free energy of association of NEFA with FABP (Richieri et al., 1995). Consistent with this, we observed a higher enthalpy of activation for the transfer of PDA from FABP than from POPC. However, when DPPC was the donor particle, the enthalpy increased to a value similar to that observed when FABP was the donor, suggesting that the interaction of PDA and other NEFA with FABP is similar to their interactions with crystalline lipids.

Physiological significance

The physicochemical regulation of NEFA transport is likely to be important to both intracellular and extracellular lipid metabolism. In plasma, the triglyceride-rich lipoproteins, which include the VLDL that are secreted by the liver and chylomicrons derived from the intestinal secretion, are substrates for lipoprotein lipase. The steady-state turnover number for lipoprotein lipase is ~100 NEFA/s (Clarke and Holbrook, 1985). It is expected that NEFA that have transfer rates that are faster than those of lipolysis will not accumulate on the surface of the substrate. However, for other NEFA such as stearic, arachidic, and behenic acids ($C_{18:0}$, $C_{20:0}$, and $C_{22:0}$, respectively) lipolysis rates are faster than those of NEFA transfer from the substrate surface. In addtion, transfer rates from very flat surfaces such as human plasma chylomicrons may be even slower due to the inverse relationship between transfer rate and radius.

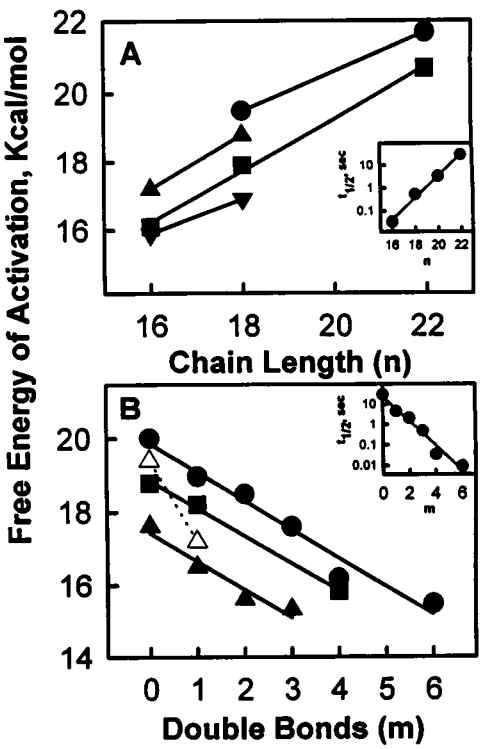

FIGURE 6 Effects of amphiphile structure on free energy of activation for transfer at 37°C. (*A*) Effect of acyl chain length on the free energy of activation for the transfer of saturated amphiphiles from DPPC SUV to an-HSA (●); POPC SUV to an-HSA (■); and an-HSA to HSA (▲); and of *lyso*PCs from POPC SUV (▼); to an-HSA. (*B*) Effect of acyl chain unsaturation on the free energy of activation for the transfer of fatty acids from POPC SUV to an-HSA, fatty acid chains contained 22 (●); 20 (■); and 18 carbon atoms (▲); or from DPPC SUV to an-HSA (▼).

Thus, during lipolysis, many of the long-chained NEFA are expected to accumulate on the surface of lipoproteins and alter the interactions of lipoproteins with other plasma proteins. This could be especially important during the lipolytic burst that occurs during the postprandial state. In vitro studies have indicated that the accumulation of NEFA in lipoproteins has a pronounced effect on lipid metabolism. Increased NEFA levels stimulate the CETP-mediated transfer of cholesteryl esters between VLDL and HDL (Sammet and Tall, 1985) and the transfer of VLDL phospholipids into HDL (Tall et al., 1985). The accumulation of NEFA also inhibits the activity of lipoprotein lipase through product inhibition and the desorption of lipoprotein lipase from its anchoring site on the glycosaminoglycans (Clark and Quarfordt, 1985; Saxena et al., 1989).

NEFA transport into cells is also probably controlled by their rates of desorption from lipid surfaces (Noy et al., 1986; Fleischer et al., 1986; Weisiger and Ma, 1988). At least four steps are involved in this process. These are 1) desorption of NEFA from HSA and lipoprotein surfaces within the plasma compartment; 2) translocation across the cell membrane; 3) desorption from the inner leaflet of the plasma membrane into the aqueous phase of the cytoplasm; 4) diffusion-controlled association with a carrier protein such as FABP or intracellular membranes. Steps 2 and 4 are much faster than steps 1 and 3 (Doody et al., 1980; Kamp and Hamilton, 1993), both of which are functions of the structure of the transferring species according to Eq. 6a. Although the rates of transfer of NEFA and *lyso*PC between lipid and protein surfaces are similar, the rates of translocation across a bilayer are much slower (McLean and Phillips, 1984; Bhamidipati and Hamilton, 1989). Therefore, the rate of *lyso*PC transport from the extracellular space to the cytoplasm is probably not a function of the chain length. Future investigations with fluorescent analogs may show whether cellular transport can be correlated with NEFA structure. These kinds of studies will be invaluable to the

TABLE 5 Summary of kinetic and thermodynamic parameters of the activated state for the transfer of stearic acid and PDA

Donor Matrix	Fatty Acid	k (sec^{-1})*	E_a (kcal/mol)	$\Delta G^{\ddagger\#}$ (kcal/mol)	$\Delta H^{\ddagger}$ (kcal/mol)	$T\Delta S^{\ddagger}$ (kcal/mol)
Liquid-Crystalline[§] Phase	Stearate	0.78	13.9	17.8	13.3	4.6
	PDA	0.22	9.5	18.7	8.9	9.7
	(Difference)[¶]		(4.4)	(−0.9)	(4.4)	(−5.1)
Gel Phase	Stearate	0.068	21.6	19.4	21.0	−1.6
	PDA	0.17	16.8	18.8	16.2	2.6
	(Difference)		(4.8)	(0.6)	(4.8)	(−4.2)
Protein Matrix	Stearate	0.22	21.9	18.7	21.3	−2.6
	PDA	0.56	16.5	18.1	15.9	2.3
	(Difference)		(5.4)	(0.6)	(5.4)	(−4.9)
Δ[(Liquid Crystalline Phase)	Stearate		−8.0	−0.9	−8.0	+7.2
− (Protein Matrix)]	PDA		−7.0	0.6	−7.0	+7.4

*The rate constants were determined at 30°C and are from Tables 2 and 3.

#The free energy ($\Delta G^{\ddagger}$), enthalpy ($\Delta H^{\ddagger}$), and entropy ($T\Delta S^{\ddagger}$) were calculated from the Eyring Activated Complex Theory as previously described (Pownall et al., 1983).

§For stearate, the liquid crystalline phase was POPC SUV, gel phase was DPPC vesicles, and protein matrix was an-HSA. The original data are in Table 3. For PDA, the values are for the average of transfer from POPC SUV, POPC/$DNPS_2$A SUV, VLDL, LDL, and HDL for liquid crystalline matrix, for transfer from DPPC-ether SUV for gel phase matrix, and for the average of the values for transfer from HSA, an-HSA, and FABP for a protein matrix (Table 2).

¶The difference is calculated for the $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $T\Delta S^{\ddagger}$ values between those for stearate minus the values for PDA.

development of a better understanding of how NEFA transport is regulated in vivo.

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